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Genotype × genotype interactions between the toxic cyanobacterium *Microcystis* and its grazer, the waterflea *Daphnia*

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cyanobacteria toxins, eco-evolutionary dynamics, genotype × genotype interactions, geographic mosaic of coevolution, harmful algal blooms, top–down control.

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Abstract

Toxic algal blooms are an important problem worldwide. The literature on toxic cyanobacteria blooms in inland waters reports widely divergent results on whether zooplankton can control cyanobacteria blooms or cyanobacteria suppress zooplankton by their toxins. Here we test whether this may be due to genotype × genotype interactions, in which interactions between the large-bodied and efficient grazer *Daphnia* and the widespread cyanobacterium *Microcystis* are not only dependent on *Microcystis* strain or *Daphnia* genotype but are specific to genotype × genotype combinations. We show that genotype × genotype interactions are important in explaining mortality in short-time exposures of *Daphnia* to *Microcystis*. These genotype × genotype interactions may result in local coadaptation and a geographic mosaic of coevolution. Genotype × genotype interactions can explain why the literature on zooplankton–cyanobacteria interactions is seemingly inconsistent, and provide hope that zooplankton can contribute to the suppression of cyanobacteria blooms in restoration projects.

Introduction

Cyanobacteria are an ancient group of autotrophic bacteria that are found in both freshwater and marine environments and are an important component of the primary producers (Huisman et al. 2005). Cyanobacteria dominate at high nutrient concentrations and high temperatures, and in inland standing waters throughout the world, there is an increasing incidence of dense cyanobacteria blooms fuelled by eutrophication and climate change (Kardinaal and Visser 2005; Zurawell et al. 2005; Jöhnk et al. 2008; Paerl and Huisman 2008; Kosten et al. 2011). Many cyanobacteria species produce a diverse range of toxic metabolites and bioactive compounds such as hepato-, neuro-, cyto- and endotoxins (Sivonen and Jones 1999; Codd et al. 2005) that are hazardous to both human and livestock health (Kuiper-Goodman et al. 1999; Codd et al. 2005). Cyanobacteria blooms can cause major problems both in terms of ecological structure and functioning of aquatic systems (Ghadouani et al. 2003; Dao et al. 2010) as well as public health, livestock health and recreation (Bell and Codd 1994; Jochimsen et al. 1998; Kuiper-Goodman et al. 1999; Ouellette and Wilhelm 2003; Zimba et al. 2006; Stewart et al. 2008; Martínez Hernandez et al. 2009). Much effort is therefore invested in preventing or controlling cyanobacteria blooms (Chorus and Mur 1999; Codd 2000; Paerl et al. 2001). The most effective management is to avoid cyanobacteria blooms by reducing nutrient loads and restoring water quality (Chorus and Mur 1999; Paerl et al. 2001; Anderson et al. 2002). Hence, most applications with respect to the control of nuisance cyanobacteria blooms take a bottom-up approach. They often involve profound interference with the physical or chemical structure of water bodies, such as artificial mixing or flushing (e.g. Huisman et al. 2004, 2005; Maier et al. 2004) or

precipitation and fixation of phosphorus in the sediments with La- or Al-rich clays (Douglas et al. 1999; Robb et al. 2003; van Oosterhout and Lürling 2011). A reduction of nutrient loads is, however, sometimes hard to achieve, especially when sources of nutrient input are diffuse or when nutrient enrichment is partly because of atmospheric deposition. And even when successful, most of these approaches are expensive and work only in relatively small water bodies and in systems for which a heavy investment is counterbalanced by strong added value, such as public swimming waters. Another much advocated strategy to improve the ecological quality of nutrient-enriched water bodies and prevent the occurrence of cyanobacterial blooms is to combine control of external nutrient inputs with food-web manipulation (Moss et al. 1996; Madgwick 1999; Jeppesen et al. 2007; Kasprzak et al. 2007). Several recent studies have focused on potential agents of biological control for the prevention of cyanobacterial blooms, using bacteria, viruses and unicellular grazers (e.g. Sigee et al. 1999; Nishibe et al. 2004; Choi et al. 2005; Tucker and Pollard 2005; Honjo et al. 2006; Zhang et al. 2008; Van Wichelen et al. 2010), exploiting allelopathic interactions (Wu et al. 2011) or manipulating fish stocks (Madgwick 1999; Jeppesen et al. 2007; Kasprzak et al. 2007). An important asset of biological control of cyanobacteria blooms resides in the fact that the controlling agent through its population growth may exert its impact throughout larger water bodies and for longer periods of time. Top–down control may also be more powerful in shallow water bodies where internal eutrophication through resuspension of sediments reduces the strength of bottom-up control (Moss 2010). There are many known cases of successful biomanipulation, including a few cases in which bloom-forming cyanobacteria were kept under control by zooplankton grazing (Peretyatko et al. 2010).

Zooplankton–cyanobacteria interactions have been discussed extensively over the years, yet the literature yields a highly inconsistent picture (see also Zurawell et al. 2005). Several studies indicate that *Daphnia* may control the development of *Microcystis* blooms depending on initial conditions and history (e.g. Christoffersen et al. 1993; Matveev et al. 1994; Sarnelle 2007; Dejenie et al. 2009; Peretyatko et al. 2010). Other studies, however, reported that toxic *Microcystis* could not be controlled by zooplankton grazing, as *Microcystis* suppressed *Daphnia* population growth and resulted in a decrease in zooplankton biomass and a shift in zooplankton community structure towards smaller species and individuals (e.g. Ghadouani et al. 2003). In line with these observations, there are several cases where biomanipulation failed when cyanobacteria were present (Gliwicz 1990; Gulati et al. 2008). Large-bodied zooplankton species are claimed to be particularly vulnerable as they can ingest the cyanobacteria and thus get intoxicated (Gliwicz and Siedlar 1980). *Microcystis*, a commonly occurring cyanobacterium genus, can suppress zooplankton in several ways. First, through the formation of colonies, they may reduce ingestion and interfere with filtering activity in *Daphnia* (Lampert 1981, 1982). Secondly, cyanobacteria tend to be poor food. They feature low levels of highly unsaturated fatty acids and low sterol contents (Brett and Müller-Navarra 1997; von Elert et al. 2003), and their membrane and mucilage layers are not readily digestible (Kurmayer and Jüttner 1999), which renders them nutritionally unfavourable for zooplankton compared with, for example, green algae. Thirdly, *Microcystis* strains produce a wide range of secondary metabolites. Examples of cyanotoxins that are deleterious to *Daphnia* are microcystins (Chen et al. 2005), protease inhibitors (Schwarzenberger et al. 2010), microviridin peptides (Kaebernick et al. 2001), and the polyunsaturated fatty acid gamma-linolenic acid (Reinikainen et al. 2001), among others (Nizan et al. 1986; Jungmann and Benndorf 1994). Other studies, however, did not find any deleterious effect of cyanobacteria on *Daphnia* (De Bernardi et al. 1981; Matveev et al. 1994).

An important finding in the debate on *Microcystis*–zooplankton interactions is the observation that there are genetic differences both in the grazer and the prey in their mutual responses (Kurmayer et al. 2001; Wilson et al. 2005). For example, the ability to form colonies in the presence of grazers (e.g. van Gremberghe et al. 2009a), the fatty acid composition (e.g. Martin-Creuzburg et al. 2008), and secondary metabolites differs among *Microcystis* strains, thus potentially inducing a very diverse response in zooplankton (Jungmann 1992; Czarnecki et al. 2006). Likewise, differences in responses of *Daphnia* when exposed to cyanobacteria have been reported (Hietala et al. 1995; Hairston et al. 2001; Schwarzenberger et al. 2010). In recent years, evidence has accumulated that *Daphnia* may develop tolerance against toxic cyanobacteria (Gustafsson and Hansson 2004; Sarnelle and Wilson 2005; Blom et al. 2006; Wilson et al. 2006; Sarnelle et al. 2010) and may genetically adapt to better cope with cyanotoxins (Hairston et al. 1999, 2001; Gustafsson et al. 2005). Sarnelle and Wilson (2005) and Blom et al. (2006) compared *Daphnia* clones isolated from lakes with low and high prevalence of bloom-forming cyanobacteria and concluded that populations exposed to high cyanobacterial levels over long periods of time can genetically adapt to being more tolerant to toxic cyanobacteria in the diet. Hairston et al. (1999, 2001) similarly showed genetic adaptation of *Daphnia* in Lake Constance to increased abundances of cyanobacteria over time using a resurrection ecology approach, hatching *Daphnia* clones from different time periods corresponding

to different eutrophication periods of the dormant egg bank. Gustafsson and Hansson (2004) and Gustafsson et al. (2005) demonstrated induced and maternally transferred tolerance in *Daphnia* when pre-exposed to *Microcystis*. They observed a higher survival probability, accelerated maturation and early first reproduction as well as a higher number of offspring when comparing animals born from *Microcystis*-exposed mothers compared to naive *Daphnia*. Sarnelle et al. (2010) observed that *Daphnia* populations with prior experience with toxic cyanobacteria may show positive population growth even at high concentrations of cyanobacterial toxins. Acclimation and genetic adaptation likely play a significant role in determining *Microcystis–Daphnia* interactions.

Microcystis and *Daphnia* may strongly interact with each other, as *Microcystis* may intoxicate *Daphnia,* whereas *Daphnia* may feed on *Microcystis*. The high amount of genetic variation in defence mechanisms in *Microcystis* strains and in resistance to *Microcystis* toxins in *Daphnia* then raises the question to what extent populations of both species may coevolve in response to each other, leading to local coadaptation (Thompson 2005). The occurrence of genotype × genotype interactions is a prerequisite for the development of local adaptation in a dynamic, geographic mosaic of coevolution (Thompson 2005). As a first test of this idea, we designed an experiment to quantify to what extent susceptibility to *Microcystis* in *Daphnia* is not only dependent on *Daphnia* genotype and *Microcystis* strain, but also on genotype × genotype interactions, similar as in, for example, host–parasite interactions (e.g. Carius et al. 2001). Genotype × genotype interactions would explain why in some studies *Daphnia* seem to be able to control *Microcystis,* whereas in other systems, *Microcystis* seem to control *Daphnia*. Using a meta-analysis approach, Wilson et al. (2006) also concluded that toxicity induced by cyanobacteria on growth rate and survival is strongly dependent on the cyanobacterium and zooplankton strains used, and not as much on the presence or absence of microcystins, as is generally accepted. Here, we experimentally test for genotype × genotype interactions in a systematic way by confronting 10 different genotypes of the water flea *Daphnia* with 10 different strains of the cyanobacterium *Microcystis* in a full factorial design. Getting a better grip on genotype × genotype interactions and potential coadaptation between daphnia and toxic cyanobacteria might help to develop successful strategies for top–down control of toxic blooms by zooplankton grazers.

Material and methods

Experimental organisms

We worked with 10 *Microcystis* strains (Table 1) isolated from three different populations in Belgium: strains ML76, ML50, ML49, ML14 were isolated from a 7-ha lake in the natural reserve of Leeuwenhof at Drongen (Ghent, September 2004), strains MT50, MT45, MT38, MT6 were isolated from a pond in the natural reserve of Tiens Broek at Tienen (August 2005), and strains MW24 and MW31 were isolated from a pond in Westveld Park at Sint-Amandsberg (Ghent, MW24 in July 2007, MW31 in July 2008). All strains have been genotyped using 16S and 23S rDNA internal transcribed spacer sequences (Janse et al. 2004). They all belong to the species *Microcystis aeruginosa,* except for MW24, which belongs to *Microcystis viridis* (I. van Gremberghe personal observation; Van Wichelen et al. 2010). All strains have also been analysed for their microcystin content using ELISA (Enzyme Linked Immuno-Sorbent Assay; van Gremberghe et al. 2009a; Van Wichelen et al. 2010). ELISA revealed the presence of microcystin in strains ML76, ML50, MT50, MT45, MW24 and MW31, but not in the remaining four strains. These strains are known not to contain the *mcy* genes A and E (van Gremberghe et al. 2009a). All strains

Table 1. Characterization of the *Microcystis* strains used in the experiment.

Name	Origin	Isolation date	Microcystin concentration (in pg per ng C)	Growth rate	Tendency to form colonies
ML76	Leeuwenhof	9/2004	17.9	0.478	Never
ML50	Leeuwenhof	9/2004	2.8	0.388	Always
ML49	Leeuwenhof	9/2004	0	0.409	Never
ML14	Leeuwenhof	9/2004	0	0.453	Sometimes
MT45	Tiens Broek	8/2005	4.00	0.468	Sometimes
MT50	Tiens Broek	8/2005	10.61	0.376	Sometimes
MT38	Tiens Broek	8/2005	0	0.435	Always
MT6	Tiens Broek	8/2005	0	0.500	Sometimes
MW24	Park Westveld	7/2007	5.56	0.427	Sometimes
MW31	Park Westveld	7/2008	13.88	0.517	Sometimes

Data from I. van Gremberghe, personal observation; van Gremberghe et al. 2009a; Van Wichelen et al. 2010.

were cultured in WC-medium (Guillard and Lorenzen 1972 without pH adjustment) in 750-mL tissue culture flasks with filter caps. They were incubated in a light-chamber with light intensity of ca. 35 μmol photons m^{-2} s^{-1}, temperature of 20 ± 1°C, and a light/dark cycle of 16 h:8 h. The batch cultures were harvested every 2 weeks by centrifugation of the cultures at 1812 *g*, 20°C for 10 min, discarding the supernatant, and resuspending the cells with fresh autoclaved WC-medium.

We used six *Daphnia magna* Straus, 1820 clones from Belgium and four *Daphnia similis* Claus, 1876 clones from Ethiopia in our experiment (Table 2). Two of the Belgian *D. magna* clones, DWEST_02 and DWEST_04, were isolated from the same pond (Westveld Park) as *Microcystis* strains MW24 and MW31. This pond was recently drained in an effort to control cyanobacteria blooms, and while the *Microcystis* strains were isolated from the pond before this event (in July 2007 and 2008), the *Daphnia* clones were collected after pond restoration (in September 2009). The other four Belgian clones were hatched in fall 2009 from the upper 2–3 cm of four different pond sediments collected in winter 2007: clone DBLAIN_NF6 was hatched from the sediments of a small pond in the nature reserve De Blankaart (Woumen); DTER1_12 was derived from Tersaert, a pond in Neerijse; DLRV_F2 was hatched from Langerodevijver, a 21-ha shallow lake in the natural reserve of Doode Bemde (Korbeek-Dijle); DMO_F15 came from a pond in Moorsel (Tervuren); with the exception of Langerodevijver, all these ponds are also referred to in the study by Jansen et al. (2010a,b) and have been characterized for their abiotic conditions (Rousseaux 2011). In addition to the six *D. magna* clones, we also worked with four *D. similis* clones that were collected in 2009 from artificial reservoirs in the semiarid highlands of Tigray, Northern Ethiopia (see Dejenie et al. 2008). The four clones were isolated from three different but neighbouring populations: DMG1_01 from the 14.7 ha reservoir Mai Gassa I; DMG2_01 and DMG2_02 from the 9.1 ha reservoir Mai Gassa II; and DGM2_05 from the reservoir Gereb Mihiz (17.7 ha). These reservoirs are described in the study by Dejenie et al. (2008). Owing to exceptionally dry weather in 2008–2009, Mai Gassa I and II dried up completely in May–August 2009. Mai Gassa I and II normally contain intense *Microcystis* blooms. In 2009, however, probably associated with the fish kill induced by the drystands, *Microcystis* densities were relatively low and *Daphnia* densities were relatively high. We included Ethiopian *D. similis* in our analysis because the Ethiopian reservoirs suffer from far more intense *Microcystis* blooms than most ponds in Belgium. Also, in an enclosure experiment carried out in two reservoirs, we obtained clear indications that local *Daphnia* populations may contribute to a suppression of the growth of *Microcystis* (Dejenie et al. 2009). All *Daphnia* clones were kept for multiple generations in the laboratory before using them in experiments. Prior to the start of the experiment, *Daphnia* clones were kept under optimal conditions for two generations to reduce the interference of maternal effects with our results and to obtain enough individuals for the experiment. Animals were cultured individually in 210-mL jars in a climatic room at 20 ± 1°C and a light/dark cycle of 16 h:8 h; food levels were restored daily to 5×10^4 *Scenedesmus obliquus* cells mL^{-1} and their medium, consisting of 24 h aged tap water, was refreshed twice weekly.

For convenience, in the remainder of the paper, we use the term 'strain' when we refer to a *Microcystis* genotype and the term 'clone' when we refer to a *Daphnia* genotype.

Table 2. Characterization of the *Daphnia* clones used in the experiment.

Name	*Daphnia spp.*	Origin	Sampling year
DWEST_02	*D. magna*	Park Westveld (Belgium)	2009
DWEST_04	*D. magna*	Park Westveld (Belgium)	2009
DBLAIN_NF6	*D. magna*	De Blankaart (Belgium)	2007
DTER1_12	*D. magna*	Tersaert 1 (Belgium)	2007
DLRV_F2	*D. magna*	Langerode (Belgium)	2007
DMO_F15	*D. magna*	Moorsel (Belgium)	2007
DMG1_01	*D. similis*	Mai Gassa I (Ethiopia)	2009
DMG2_01	*D. similis*	Mai Gassa II (Ethiopia)	2009
DMG2_02	*D. similis*	Mai Gassa II (Ethiopia)	2009
DGM2_05	*D. similis*	Gereb Mihiz (Ethiopia)	2009

Experimental design and procedures

A full factorial design was used combining the 10 *Daphnia* clones with the 10 *Microcystis* strains in a food gradient of *Microcystis*: *Scenedesmus* in 0:100, 20:80, 40:60, 60:40, 80:20, 100:0 carbon ratios. *Scenedesmus obliquus* was used as a standard good-quality food to prepare food mixtures with *Microcystis*. During the experiment, total food concentration was restored daily to 1.0 mg C L^{-1} *Microcystis* + *Scenedesmus*. According to literature (Lampert 1977; Gustafsson and Hansson 2004), this is a sufficiently high food concentration for rapid growth and good reproduction of daphnids. For *Microcystis*, we estimated cell volumes using a sphere as an approximation of the coccoid form of *Microcystis* cells (Holm and Armstrong 1981). We converted the biovolume to amount of carbon using the formula 0.216·cell $volume^{0.939}$ = pgC·$cell^{-1}$ (Menden-Deuer and Lessard 2000). We harvested fresh *Microcystis* suspensions for all 10 strains every 2 days by centrifugation of exponentially

growing cultures. We established the concentration and average cell diameter of the resulting suspensions using a Coulter counter (Multisizer™ 3 COULTER COUNTER®; Beckman Coulter Inc., Brea, CA, USA), and diluted appropriate amounts in 200 mL dechlorinated water to obtain a total carbon content of 1.0 mg C L^{-1} food concentrations in all experimental jars. In treatments with a 100% *Microcystis* diet, cell concentrations ranged in between 150 000 and 200 000 cells mL^{-1}, depending on the cell size of the strain used. Such cell concentrations of *Microcystis* (and higher) often occur in nature (e.g. Kurmayer et al. 2003; Kann 2006); similarly, relative proportions of >80% *Microcystis* in the phytoplankton community have regularly been reported (e.g. Zurawell et al. 1999; Downing et al. 2001; Song et al. 2010). To count *Microcystis* in the Coulter counter, we first boiled subsamples of each strain for 6 min in a hot water bath, to disperse colonies and obtain single intact cells for counting and measuring (Joung et al. 2006). *Daphnia* were fed with suspensions of *Microcystis* that were at most 2 days old. Prior quantification of the total organic carbon levels in our *Scenedesmus* strain showed that 1.0 mg C L^{-1} corresponds to 118 000 cells mL^{-1}.

All treatments were replicated three times. In total, the experiment consisted of a combination of 10 *Daphnia* clones × 10 *Microcystis* strains × 6 *Microcystis* concentrations × 3 replicate units of 10 *Daphnia* individuals each, for a total of 1800 experimental units. For each unit, 10 2-day-old *Daphnia* juveniles were used. The animals were exposed to the different food treatments for 48 h; this is a standard period for acute aquatic ecotoxicity tests with *Daphnia* (OECD Adopted 2004). After this time, surviving individuals were counted and mortality was recorded. All experiments were conducted in a light chamber with light intensity of ca. 35 μmol photons m^{-2} s^{-1}, temperature of 20 ± 1°C, and a light/dark cycle of 16 h:8 h. Because of the size of the experiment, exposures were spread over a period of 27 days. The starting day for each clone (combined with all strains and concentrations) was randomized, and replicate units of a given clone were always started on at least two different days.

Statistical analysis

Mortality count data were analysed with generalized linear mixed models using 'R' (lmer function in package lme4, software version 2.12.1, R Development Core Team 2005). We used a model with binomial error distribution and logit link function to test for main effects and relevant interaction terms of *Microcystis* strain, *Daphnia* clone, and *Microcystis* concentration on the proportion of dead animals. As we were primarily interested in our *Microcystis* strains and *Daphnia* clones as representatives of the entire population of possible strains and clones, they were both considered random categorical factors in this analysis. *Microcystis* concentration was incorporated as a continuous variable. The day on which each jar entered the experiment was inserted into the model as a random categorical blocking factor, 'day', to correct for handling differences. We acknowledge that this first model does not include the full complexity of our design, as it ignores the origin of clones and strains in the analysis (but see below for an analysis that does take origin into account). This simplification was done because the full model resulted in a too high computational complexity.

As overall mortality was low (see Results), we constructed a second model on a data set omitting the treatments with low *Microcystis* concentrations (where mortality was almost zero). Only data corresponding to the 80% and 100% *Microcystis* diet for each clone × strain combination were included. We still included the % *Microcystis* in the model, but merely as (fixed) categorical blocking factor. In this second analysis, we included the *Daphnia* species as a fixed factor, with *Daphnia* clone nested in *Daphnia* species. The *Daphnia* clones used in our experiment indeed belong to two different species, *D. similis* and *D. magna*, originating from two different countries, Ethiopia and Belgium, respectively. Similarly, we included the lake where *Microcystis* was isolated from as a fixed factor, with *Microcystis* strain nested in lake. We acknowledge that the second model is to be preferred over the first model that ignores origin. We merely report the results of the first model to demonstrate there are no substantial differences between the results of an analysis of the whole range of *Microcystis* concentrations versus a subset including only the highest concentrations.

To take a closer look at the pairwise differences between clones and between strains and at genotype × genotype interactions, we performed *post hoc* Tukey's HSD tests following a linear model (ANOVA) in 'R' (R Development Core Team 2005). In this analysis, we included *Daphnia* clone and *Microcystis* strain as fixed independent variables, and the proportion of dead individuals in each jar in the 80% and 100% *Microcystis* treatments as dependent variable. Treatment of *Daphnia* clone and *Microcystis* strain in this analysis as fixed categorical variables is justified by the fact that here we are interested in the differences between specific pairs of clones or strains. Treatment was implemented as a fixed categorical blocking factor. We only included levels of 80% and 100% *Microcystis*, because the other levels induced very low levels of mortality. We are aware that our data do not fulfil the assumptions of ANOVA (arcsin transformations did not improve the fit) but are confident that the model is sufficiently robust for this analysis of pairwise comparisons. This is supported by the fact that the basic

model results of the ANOVA (See Table S4) do not differ substantially from the results generated by our generalized linear mixed models. We here resorted to a basic and simplified ANOVA as the complexity of the generalized linear model prevents a straightforward analysis of *post hoc* comparisons.

Finally, to analyse whether mortality induced by *Microcystis* strains is related to their microcystin concentration, the Pearson's correlation coefficient was calculated between the average mortality imposed by the different *Microcystis* strains (considering 80% and 100% *Microcystis* treatments only) and the microcystin concentration of those strains.

Results

Daphnia mortality induced by *Microcystis*

In general, the mortality we observed was rather low (mean <5%; $n = 1782$) but increased with the concentration of *Microcystis* (Fig. 1). At the lowest concentrations (20–40%) of *Microcystis*, mortality was around 3.6% and at the highest concentrations (80–100%), mortality was on average 7% (Fig. 1) with values ranging from 0% to 18.5% depending on the *Daphnia* clone and *Microcystis* strain combination (Fig. 2). All factors and interaction terms that were included in our analysis have a significant impact on *Daphnia* mortality in our experiment (Table 3). There is a significant *Microcystis* concentration effect confirming that mortality in *Daphnia* increases with increasing *Microcystis* concentrations in their food (Fig. 1). The significant interactions of clone and strain with concentration (including the three-way interaction, Table 3) are mainly caused by the fact that differences in mortality are especially pronounced at the higher concentrations, and indicate that the extent to which high *Microcystis* concentrations induce mortality is dependent on the *Daphnia* clone and *Microcystis* strain, or their combination. The significant main effect of *Daphnia* clone ($\chi^2_1 = 111.14$, $P < 0.001$, Table 3, Fig. 2A) confirms that *Daphnia* genotypes differ in overall susceptibility to *Microcystis*, while the significant main effect of *Microcystis* strain ($\chi^2_1 = 118.32$, $P < 0.001$, Table 3, Fig. 2B) indicates that *Microcystis* genotypes differ in overall toxicity to *Daphnia*. The *Daphnia* clone effect seems to attribute more to the total amount of explained variation than the *Microcystis* strain effect in our analysis (Table 3). A large part of the variation is, however, explained by a highly significant *Daphnia* clone × *Microcystis* strain interaction effect ($\chi^2_1 = 132.93$, $P < 0.001$, Table 3, Fig. 3).

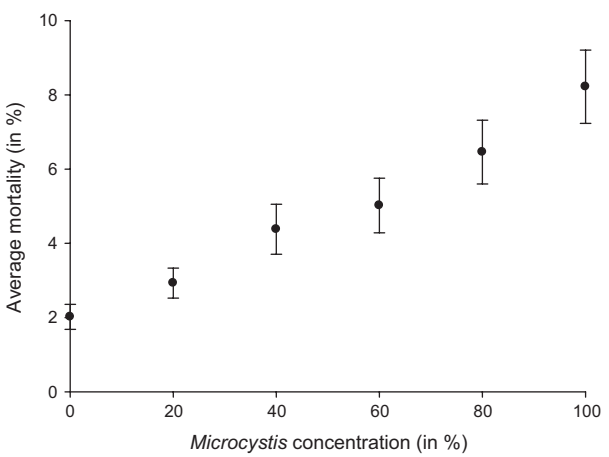

Figure 1 Average *Daphnia* mortality (%) when exposed to increasing *Microcystis* concentrations, showing a significant effect of dietary *Microcystis* concentration on the mortality of *Daphnia*. Error bars indicate the standard error of mean ($n = 297$).

Ranking of the *Daphnia* clones and pairwise comparisons of mortality in the 80–100% *Microcystis* concentrations (Fig. 2A,C) reveal that clones DMO_F15 and DWEST_02 are the most resistant clones, while DMG2_02 and DGM2_05 are the most sensitive to exposure to *Microcystis*. Ranking of the *Microcystis* strains (Fig. 2B,D) shows that MW31 and MT50 are the most toxic to *Daphnia,* while ML50 and ML49 induce the least mortality. The interaction between clone and strain is clearly visible by the patchiness of the grey shades in Fig. 3 where we plotted the mortality intensity for each *Daphnia* clone–*Microcystis* strain combination (See also Fig. S1 for the pairwise differences in mortality among *Daphnia* clone–*Microcystis* strain combinations). Three clone × strain combinations differ significantly from most of the others, namely DMG2_02-MW31, DGM2_05-MW31, and DGM2_05-MW24. These are combinations that result in particularly high mortalities (>30%, Fig. 3).

The generalized linear mixed model based on the truncated data set including only data from the highest two *Microcystis* concentrations (Table 4), which takes into account the nested design, confirms the significance of our main effects *Daphnia* clone ($\chi^2_1 = 96.91$, $P < 0.001$), *Microcystis* strain ($\chi^2_1 = 16.64$, $P < 0.001$) and their interaction ($\chi^2_1 = 44.47$, $P < 0.001$). The explained variance by the clone and strain effect is here even greater, and the high importance of the clone × strain interaction is confirmed. Furthermore, the interaction between *Microcystis* lake and *Daphnia* species proves to be significant ($\chi^2_2 = 13.25$, $P = 0.001$, Fig. 4C). Indeed, while there is a significant effect of the lake *Microcystis* was isolated from ($\chi^2_2 = 8.99$, $P = 0.011$, Fig. 4A), it is clear from Fig. 4C that this lake effect is strongly dependent on whether we consider Belgian or Ethiopian *Daphnia*. *Daphnia* mortality is highest when Ethiopian *D. similis* clones are exposed to *Microcystis* strains from Westveld park, followed by combinations of Ethiopian *D. similis* and Tiensbroek

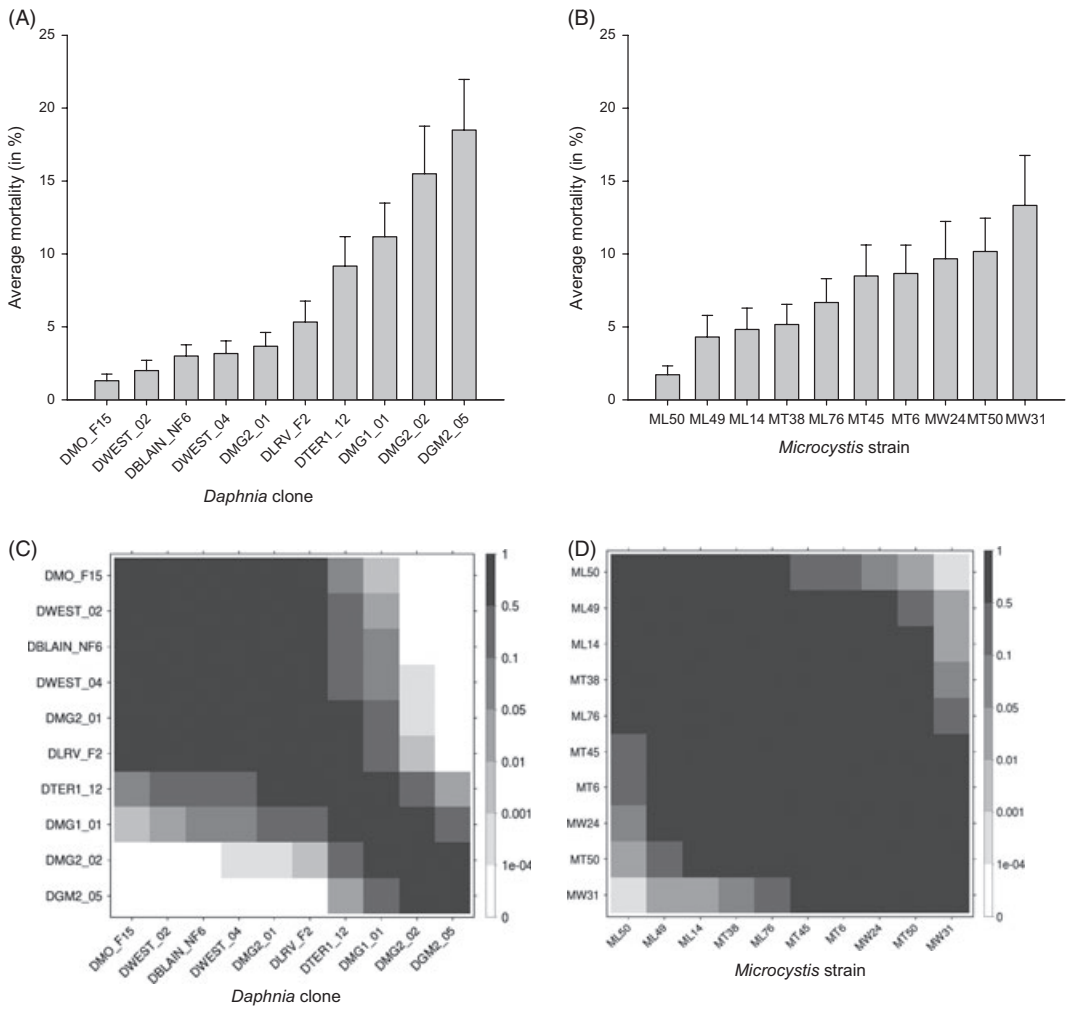

Figure 2 (A,B) The average percentage mortality of each of 10 *Daphnia* clones when exposed to *Microcystis* (A) and average mortality in *Daphnia* caused by each of 10 *Microcystis* strains (B) in the treatments with 80% and 100% *Microcystis*. The error bars indicate the standard error of mean (n = 60, except n_{DMO_F15} = 54, and $n_{ML14} = n_{ML49} = n_{ML50}$ = 58 due to missing values). (C,D) *P*-values of pairwise comparisons indicating significant differences in susceptibility between *Daphnia* clones (C) and in induced mortality between *Microcystis* strains (D).

Microcystis. The four remaining combinations are less lethal. Excluding the *Microcystis* strain belonging to the different morphospecies *M. viridis*, MW24, from the analysis presented in Table 4 does not change any of these results (see Table S1).

Mortality and microcystin-LR concentration

The Pearson's correlation coefficient between the average mortality induced by the different *Microcystis* strains (considering the 80% and 100% *Microcystis* concentrations only) and the actual microcystin concentration of those strains, as determined by ELISA, is not significant (r = 0.53, P = 0.12, Fig. 5). Figure 5 shows an overall tendency for a relationship between average *Daphnia* mortality and microcystin content of the *Microcystis* strains, but the pattern is rendered insignificant by the fact that some strains deviate from the general trend. *Microcystis* strain ML76 appears less toxic than expected by its microcystin content, while the four nonmicrocystin-producing strains induce some mortality in *Daphnia* nevertheless. This is particularly striking in strain MT6. If strain ML76 is excluded from the analysis, the Pearson's correlation coefficient becomes significant (r = 0.77, P = 0.015).

Discussion

Genotype by genotype interactions

Overall, our experiment confirms the results of earlier studies showing that exposure to *Microcystis* results in mortality in *Daphnia* (e.g. Lampert 1981; Nizan et al. 1986; Reinikainen et al. 1994; DeMott 1999). The

Table 3. Results of the generalized linear mixed model with binomial error distribution and logit link function on *Daphnia* mortality over the complete range of *Microcystis* concentrations.

	Effect	χ^2	χ df	% of Variance	*P*-value
Clone	Random	111.14	1	3.78	<0.001***
Strain	Random	118.32	1	0.44	<0.001***
Concentration	Continuous	184.32	1		<0.001***
Clone × Strain	Random	132.93	1	12.42	<0.001***
Clone × Concentration	Random	13.68	1	<0.01	0.002**
Strain × Concentration	Random	10.54	1	<0.01	0.001**
Clone × Strain × Concentration	Random	17.87	1	<0.01	<0.001***
Day effect	Random	447.98	1	38.80	<0.001***
Error				44.56	

P-values lower than 0.001 are marked with '***', between 0.001 and 0.01 with '**'.

relatively low mortality rates even at 100% *Microcystis* diet were unexpected, but may be related to the relatively short exposure time we used (2 days).

Our results confirm earlier work reporting genetic differences in toxicity among *Microcystis* strains (e.g. Nizan et al. 1986; Jungmann and Benndorf 1994; Czarnecki et al. 2006) and in resistance among *Daphnia* genotypes (Gustafsson et al. 2005; Sarnelle and Wilson 2005; Blom et al. 2006; Wilson et al. 2006). These genetic differences are well known and indicate that there is ample evolutionary potential for toxicity in *Microcystis* to evolve and for resistance in *Daphnia* populations to evolve in response to the occurrence and strain composition of *Microcystis* populations. Intriguingly, our results indicate that genetic variation in *Daphnia* resistance explains more variation in mortality than genetic variation in *Microcystis* toxicity. This is unexpected, as cyanobacteria are known to exhibit a wide variety of grazing avoidance mechanisms and substantial differences in mortality induction could be expected. Yet, the degree to which *Microcystis* induces mortality in *Daphnia* upon relatively short-term exposure seems to vary dramatically depending on the *Daphnia* genotype used. This brings a different perspective to cyanobacteria–zooplankton interactions, which builds further on the studies showing acclimation, maternal effects and genetic adaptation in *Daphnia* to *Microcystis* (Hairston et al. 2001; Gustafsson et al. 2005; Sarnelle et al. 2010; Schwarzenberger et al. 2010).

The key observation of our study is that there is an important genotype × genotype interaction effect on mortality in *Daphnia*: the degree to which *Daphnia* suffers from exposure to *Microcystis* does not only depend on the *Daphnia* and *Microcystis* genotype, but also on which genotypes interact with each other. While *Microcystis* strains MW31 and MT50 are overall the most toxic to *Daphnia*, some *Daphnia* genotypes suffer little from them, even though they experience significant mortality from exposure to some other *Microcystis* strains. These genotype × genotype interactions may explain the confusing picture that is provided by the literature on cyanobacteria–zooplankton interactions, in which widely different results are obtained depending on the study. For example, Christoffersen et al. (1993) and Sarnelle (2007) suggest *Daphnia* can control the development of *Microcystis*

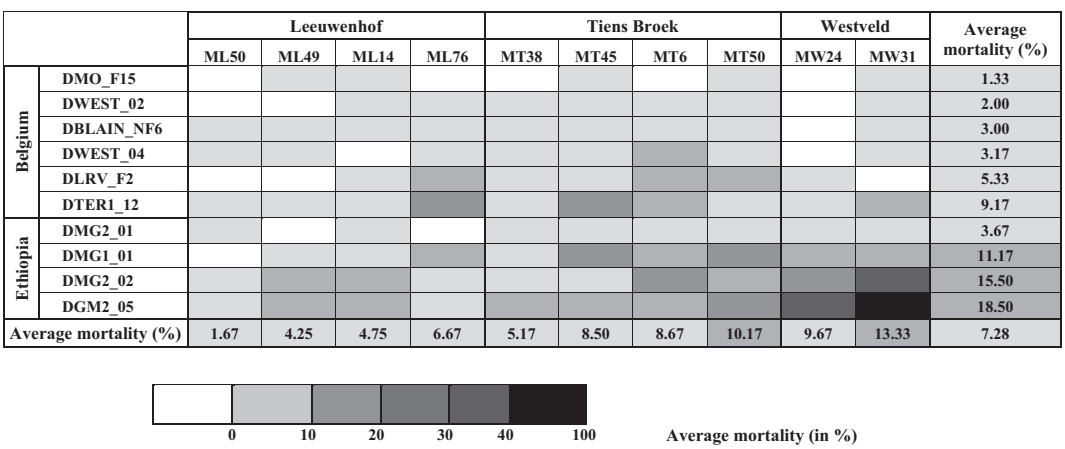

Figure 3 Interaction plot showing average mortalities of *Daphnia* in the 80% and 100% *Microcystis* concentrations in all 100 combinations of 10 different *Daphnia* clones (rows) and 10 different *Microcystis* strains (columns). Mortalities are coded as grey scales from 0–10%, 10–20%, 20–30%, 30–40% and >40% mortality. The genotypes of both interacting species are ordered along their overall susceptibility and toxicity, in addition to a grouping according to origin (*Daphnia*: country; *Microcystis*: lake). The resulting pattern shows, in addition to differences in susceptibility among *Daphnia* genotypes and in toxicity among *Microcystis* strains, strong genotype × genotype interactions (see also Table 4).

Table 4. Results of the generalized linear mixed model with binomial error distribution and logit link function on *Daphnia* mortality in the 80% and 100% *Microcystis* concentrations.

	Effect	χ^2	χ df	% of variance	*P*-value
Clone effect (nested in Species)	Random	96.91	1	18.83	<0.001***
Strain effect (nested in Lake)	Random	16.64	1	1.89	<0.001***
Clone × Strain	Random	44.47	1	13.46	<0.001***
Lake effect	Fixed	8.99	2		0.011*
Species effect	Fixed	1.823	1		0.177
Species × Lake	Fixed	13.25	2		0.001**
Concentration	Fixed	7.97	1		0.005**
Day effect	Random	258.56	1	35.98	<0.001***
Error				29.84	

P-values lower than 0.001 are marked with '***', between 0.001 and 0.01 with '**', between 0.01 and 0.05 with '*'.

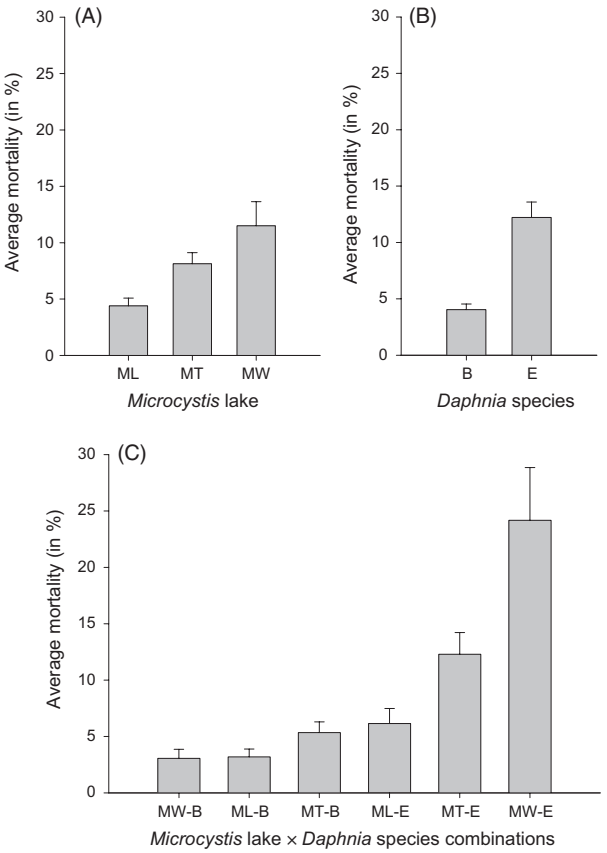

Figure 4 The average percentage mortality for (A) *Microcystis* strains from each lake. (B) *Daphnia* clones from each species/locality. (C) Each *Daphnia* species × *Microcystis* lake combination, in treatments with *Microcystis* concentrations, 80% and 100%. The error bars indicate the standard error of mean (n_{ML} = 234, n_{MT} = 240, n_{MW} = 120, n_B = 354, n_E = 240, $n_{MW\text{-}B}$ = 72, $n_{ML\text{-}B}$ = 138, $n_{MT\text{-}B}$ = 144, $n_{ML\text{-}E}$ = 96, $n_{MT\text{-}E}$ = 96, $n_{MW\text{-}E}$ = 48). ML refers to the lake Leeuwenhof, MT: Tiensbroek, and MW: Westveldpark. B denotes the Belgian species *Daphnia magna*. E refers to the Ethiopian *Daphnia similis*.

blooms depending on initial conditions and history, while other studies stress the highly deleterious influence that *Microcystis* exerts on *Daphnia*, reducing population growth and survival (Gliwicz and Siedlar 1980; Nizan et al. 1986; Ghadouani et al. 2003). Our results imply that there is no general resistance mechanism in *Daphnia* nor in *Microcystis*. Given the high diversity of secondary metabolites and the capacity for colony formation in different *Microcystis* strains, it is likely that there are trade-offs among defences. Similarly, there may be trade-offs against counter-defences in *Daphnia*. This has important implications for our view on how toxic *Microcystis* blooms develop, which may not so much be the result of zooplankton grazing in general but rather may reflect the outcome of interactions between defences and counter-defences in auto- and heterotrophs, comediated by the costs imposed by both the development of these defences and counter-defences. Importantly, genotype × genotype interactions provide the raw material for local co-adaptation and thus may lead to a geographic mosaic of coevolution between the cyanobacteria that protect themselves against grazing and their grazers that protect themselves against toxicity. The concept of the geographic mosaic of coevolution (Thompson 2005; or the concept of evolving metacommunities if one takes the broader community into account, see Urban et al. 2008) may offer a strong framework to investigate *Microcystis–Daphnia* interactions. Genotype × genotype interactions and associated coevolutionary dynamics are well studied in host–parasite systems (Carius et al. 2001; Ebert 2008). The strong interaction effect between *Microcystis* and *Daphnia* genotype in our experiment suggests a high potential for a coevolutionary arms race, similar to that among hosts and parasites. There is growing evidence that genetic diversity and evolutionary changes may strongly impact the dynamics of predator–prey interactions, as have been demonstrated by the seminal studies of Yoshida et al. (2003, 2007). The genotype × genotype interactions we report suggest that *Daphnia* may not develop generalized responses against *Microcystis* but rather may specifically adapt to local assemblages of cyanobacteria strains and vice versa. This localized coevolutionary arms race may also have practical consequences, as the capacity of zooplankton to genetically adapt to locally occurring *Microcystis* strains may increase the likelihood that the development of a bloom can be prevented by grazing.

Intriguingly, the Ethiopian *D. similis* clones tended to be more sensitive to *Microcystis* than the Belgian *D. magna* clones, even though the *D. similis* genotypes were isolated from reservoirs in Ethiopia that are usually

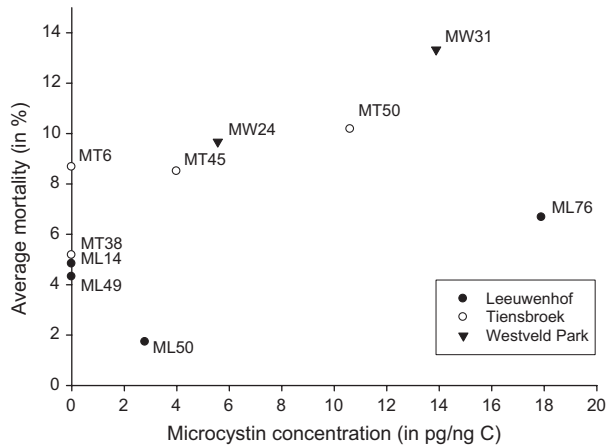

Figure 5 The mortality of the different *Microcystis* strains, averaged over all *Daphnia* clones exposed to 80% and 100% *Microcystis*, was plotted against the microcystin concentration of those strains. Symbols indicate the pond where the *Microcystis* strains were isolated from. Pearson's correlation coefficient $r = 0.53$ ($P = 0.12$).

heavily infected with *Microcystis* blooms. Although it is speculative, it is worth mentioning that all *Microcystis* strains used in our experiment were isolated from Belgium and not from Ethiopia. If there is a strong effect of localized coevolution in *Daphnia–Microcystis* interactions, it is conceivable that *Daphnia* species that were not exposed to specific *Microcystis* strains, not even at a regional level, may be more sensitive to the toxic compounds these strains produce than *Daphnia* that were exposed earlier to these strains. When we analyse our data building a model containing only Ethiopian *Daphnia* clones (data from all *Microcystis* strains and concentrations, see Table S2), the percentage of variance that can be attributed to *Microcystis* strain identity rises from 0.44% in the analysis containing all *Daphnia* clones (Table 3) to almost 10% (9.38%, $\chi^2_1 = 162.58$, $P < 0.001$), while the variance of the main effect of *Daphnia* clone (1.44%, $\chi^2_1 = 83.81$, $P < 0.001$) and the clone × strain interaction effect (3.70%, $\chi^2_1 = 32.26$, $P < 0.001$) are relatively low compared to 3.78% and 12.42%, respectively, in the analysis including the Belgian clones. This stronger impact of *Microcystis* strain identity is expected for first exposures of local grazer populations with novel types of defences. On the contrary, when we only analyse the Belgian data (see Table S3), we observe a relatively high (19.41%) contribution of genotype × genotype interactions in explaining variation in mortality ($\chi^2_1 = 43.36$, $P < 0.001$), while the amount of variation explained by *Microcystis* strain identity is reduced to almost 0% ($\chi^2_1 = 22.41$, $P < 0.001$) (*Daphnia* clone: 7.54%, $\chi^2_1 = 28.80$, $P < 0.001$). In a geographic mosaic of coevolution, we indeed expect overall differences between genotypes to be reduced and interaction effects to be important. It is noted that the difference that we observe between the *D. similis* and *D. magna* genotypes in terms of resistance may be either a species effect or related to geography, as geography and species identity are confounded in our experiment. Moreover, as we could not expose the *Daphnia* in our study to Ethiopian *Microcystis* strains, one should not interpret our results as indicating that Ethiopian *Daphnia* are less resistant against *Microcystis* toxins than Belgian *Daphnia*. First, this would be unexpected given that, overall, the incidence of *Microcystis* blooms in Ethiopia is much higher than in Belgium. Secondly, we actually observed in an earlier study that local *Daphnia* populations inhabiting two reservoirs in the highlands of Tigray were able to suppress a developing *Microcystis* population in enclosures (Dejenie et al. 2009).

Daphnia survival in our experiment is primarily because of physiological adaptations rather than to, for example, behavioural responses. With the short exposure times used, one way for the grazers to survive in principle might be to stop feeding so that exposure to toxins is minimized. However, in an additional experiment in which we monitored three of the here studied *Daphnia* genotypes and *Microcystis* strains during 5 days, we visually checked gut fullness of animals after 2 and 5 days of feeding on 100% *Microcystis*, and almost all animals ($n = 72$) had full guts and thus were actively feeding on *Microcystis* (V. Lemaire and L. De Meester, unpublished data). The observation that the mortality we observed occurred in a time span of only 2 days strongly points towards the effect of a toxin after ingestion. Indeed, mechanical difficulties in handling colonies or low nutritional value, although also defence strategies of cyanobacteria, are unlikely to lead to mortality within 48 h, as *Daphnia* juveniles can use a reserve of maternal lipids up until the age of 4 days (Reinikainen et al. 1994).

Even though we find strong genotype × genotype interactions, our data reveal a weak indication that microcystin may still play a role in overall toxicity. We observed a tendency for *Microcystis* strains to cause higher mortality in *Daphnia* when they contain higher microcystin levels, but the correlation was not significant ($r = 0.53$, $P = 0.12$, see also Fig. 5). Figure 5 shows that some strains showed lower toxicity than expected based on microcystin measurements. We need to interpret this with caution, however, as microcystin was not quantified directly on the stocks used as food in our experiments, but prior to the experiments as part of earlier research (van Gremberghe et al. 2009a,b,c; Van Wichelen et al. 2010). In addition, although ELISA is a sensitive method, it is not able to differentiate between microcystin types (e.g. microcystin-LR, -LY, -LW, -LF, and -RR), which are known to have different impacts on biota (Ibelings and

Havens 2008). More importantly, some of the supposedly nontoxic strains clearly induced mortality in *Daphnia*. These strains are known not to contain the *mcy* genes A and E (van Gremberghe et al. 2009a), and confirm that toxicity of *Microcystis* is not only dependent on microcystin but can be mediated by a variety of polypeptides (e.g. Kaebernick et al. 2001; Schwarzenberger et al. 2010). These results are consistent with the rejection of microcystins as a general determinant of toxicity by Wilson et al. (2006) in their meta-analysis. Our results thus sketch a subtle picture in which there are strong genotype × genotype interactions, but still certain defence systems may have more impact than others. Our results emphasize the complexity of toxicity mechanisms in *Microcystis*, without ignoring the role microcystin has to play. Future research might contribute to our understanding of the mechanistic basis of the genotype × genotype interactions we report in this study, by quantifying and differentiating among the different microcystin types and other polypeptides in the used *Microcystis* strains.

Our experiment is to our knowledge unique in combining a set of *Microcystis* strains and *Daphnia* genotypes in all pairwise combinations and provides strong evidence for genotype × genotype interactions shaping defences and counter-defences in auto- and heterotrophs. Yet there are a number of methodological limitations. First, although we document strong genotype × genotype interactions, the design of our study does not allow to directly test for local genetic adaptation by comparing sympatric and allopatric combinations and reciprocal exposures. We could not work with Ethiopian *Microcystis* strains, and we had only one habitat from which we had *Microcystis* strains and *Daphnia* genotypes (Westveld park pond), and these were isolated in different years. Intriguingly, while the Westveld *Microcystis* strains were among the most toxic to *Daphnia* among all *Microcystis* strains, the two *Daphnia* clones from that same pond showed very low levels of susceptibility to these two strains. Given that we only have one such data point, however, this remains an anecdotal observation. Thus, although in documenting genotype × genotype interactions we provide evidence for an important condition for a geographic mosaic of coevolution to develop, our results do not provide direct evidence for local genetic coadaptation between *Microcystis* and *Daphnia* in nature. Secondly, both of our statistical models attribute a substantial amount of the variance to the random factor 'day' (see Tables 3 and 4). We cannot explain this effect without some speculation, but the most obvious explanation is that there was some day-to-day variation in chemical composition of the *Microcystis* cultures (toxin concentrations or other). This might be due to the fact that batch cultures are intrinsically never fully standardized. It is known that chemical composition of *Microcystis* is impacted, for example, by population growth rates (Long et al. 2001). Yet this effect of day does not interfere with the conclusions of our study, because for each *Daphnia–Microcystis* combination treatment, there were three replicates that were by design spread over time. Furthermore, we used all of the *Microcystis* strains every day during the entire experimental period so that the day effect cannot hide an effect of strain identity.

Applications in the control of cyanobacteria blooms

The emerging picture from our work is that genotype × genotype interactions may be an important determinant of *Microcystis–Daphnia* interactions, which is expected to result in complex dynamics of coadaptation. Our results add to the evidence that genotype identity and genetic diversity may impact the dynamics of predator–prey interactions in zooplankton feeding on phytoplankton (Yoshida et al. 2003, 2007). Although we do not measure bio-control directly, our results are expected to have important implications for the prevention and control of cyanobacteria blooms.

As mentioned in the introduction, top–down control of cyanobacteria blooms by zooplankton has received relatively little attention in recent years, mainly because of the observation that cyanobacteria may intoxicate zooplankton so that the latter are not capable of suppressing an existing bloom (Gulati et al. 2008). Gulati et al. (2008) in their review on lake restorations in north-western Europe identified the incapability of daphnids to graze on filamentous or colonial cyanobacteria as one of key bottlenecks that can explain the failure of biomanipulation measures. Yet, our results on genotype by genotype interactions may explain why studies on *Daphnia*–cyanobacteria interactions have yielded contradicting results in the past and offer new perspectives to exploit the adaptive potential of *Daphnia* populations to control cyanobacterial blooms. Our results suggest that biomanipulation, involving a massive reduction in fish biomass to boost development of large-bodied *Daphnia*, might work even in lakes that are prone to cyanobacteria blooms if the genotype composition of both *Daphnia* and *Microcystis* is taken into account. Indeed, given the high population growth rate of *Daphnia* and associated high phytoplankton clearance rates, top–down control by *Daphnia* may be possible on the condition that one can boost the development of *Daphnia* populations that are adapted to the local strain composition of *Microcystis*. Experimental evolution in *Daphnia* has been shown to result in genetic shifts leading to adaptation to the stressor within a time period as short as a few months (Van Doorslaer et al. 2009; Jansen et al. 2011a,b). Yet, it is probably important that the phytoplankton community is not entirely

dominated by cyanobacteria when the *Daphnia* are expected to develop, as cyanobacteria are nutritionally poor food (Brett and Müller-Navarra 1997; von Elert et al. 2003) and may not support the rapid development of dense *Daphnia* populations when dominant. In practice, this implies that cyanobacteria control in lakes by top–down impact may only work after sufficiently strong winters, when the cyanobacteria fail to remain abundant year-round, creating a window of opportunity in the spring for *Daphnia* to develop to sufficiently high densities. In sufficiently small systems (e.g. garden ponds or open-air water reservoirs for horticulture), one could contemplate to inoculate *Daphnia* early in the season to boost population development and top–down control. In doing this, however, it will be important to carefully select the *Daphnia* population for its capacity to control the local set of *Microcystis* strains. One obvious way to do this would be to sample dormant egg banks of *Daphnia* from different water bodies in the region and use experimental evolution to select for genotypes that can cope with the local strains of *Microcystis* and culture them in the laboratory to sufficient densities for re-inoculation. Obviously, a targeted approach involving the inoculation of *Daphnia* would only work for small systems: if we accept that it is feasible to obtain 1×10^6 (juvenile) *Daphnia* in controlled outdoor mesocosm systems, this would yield a density of 0.1 *Daphnia* L^{-1} in a pond of 1 ha and 1 m deep. This might be sufficient to prevent a cyanobacteria bloom to develop, but the effort would be substantial.

Conclusions

The genotype × genotype interactions reported by the present study may be an important determinant of *Microcystis*–*Daphnia* interactions, which is expected to result in complex dynamics of coadaptation. These dynamics have implications both for the characteristics of cyanobacteria blooms that may develop in a given system, as these will also be influenced by the genetic characteristics of the grazer population, and with respect to the potential for top–down control of cyanobacteria blooms. Future studies should focus on confirming genotype × genotype interactions and further characterizing the potential occurrence of a geographic mosaic of coevolution between *Daphnia* and *Microcystis*, and should address both the dynamics through time at the local and regional scale as well as the potential of top–down control that is implied by our results.

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Data Archiving Statement: Data for this study are available as Online Supplementary Materials.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Results of pairwise comparisons (Tukey HSD, based on the ANOVA in Table S4) between all clone × strain combinations: The *P*-values (A) and the absolute differences in average mortality (B) observed in the 80% and 100% *Microcystis* concentrations.

Table S1. Results of the generalized linear mixed model with binomial error distribution and logit link function on *Daphnia* mortality in the 80% and 100% *Microcystis* concentrations, without combinations including the *Microcystis viridis* strain MW24.

Table S2. Results of the generalized linear mixed model with binomial error distribution and logit link function on *Daphnia* mortality of Ethiopian clones over the complete range of *Microcystis* concentrations.

Table S3. Results of the generalized linear mixed model with binomial error distribution and logit link function on *Daphnia* mortality of Belgian clones over the complete range of *Microcystis* concentrations.

Table S4. Results of an ANOVA on a linear model of the proportion *Daphnia* mortality in the 80% and 100% *Microcystis* concentration.

Table S5. Full data with the number of dead and surviving individuals (out of 10) for the three replicates of each combination of 10 Daphnia clones × 10 *Microcystis* strains × 6 concentrations of *Microcystis* (in %).

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