Ecology, 85(8), 2004, pp. 2302–2311

INDUCIBLE DEFENSES IN MULTIPREDATOR ENVIRONMENTS: CYCLOMORPHOSIS IN *DAPHNIA CUCULLATA*

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Abstract. Phenotypically plastic defenses are strategies to reduce predation risk in variable environments. Predator-induced formations of protective devices in cladocerans are prominent examples of phenotypically plastic defenses. To understand the adaptive value of a defense, it is better to investigate its effects in a multipredator context, instead of merely concentrating on a two-species interaction. Small cladocerans such as *Daphnia cucullata* are prey items for many aquatic invertebrate predators and thus comprise a useful model system to investigate defenses and their effects. In this study, we tested the hypothesis that the helmets of *Daphnia cucullata* are inducible with chemical cues from different kinds of predators and that they act as a generalized defense offering protection against several predators, each using a different hunting strategy.

Results from our induction experiment show that chemical cues released from *Chaoborus flavicans*, *Leptodora kindtii*, and *Cyclops* sp. induce significantly longer helmets and tail spines and thus act as proximate factors for cyclomorphosis in *Daphnia cucullata*. Our predation experiments revealed that the induced morphological changes offered protection against each of the predators tested. Interestingly, the protective mechanisms and the prey size classes which were protected differed between predator systems.

Our results suggest that phenotypic plasticity in *Daphnia cucullata* evolved as a ‘‘diffuse’’ coevolution against different invertebrate predators which selectively feed on small prey items. The additive benefits may increase the adaptive value and thus facilitate the evolution and persistence of this generalized defense.

Key words: cyclomorphosis; diffuse coevolution; functional morphology; inducible defenses; multipredator environments; phenotypic plasticity.

INTRODUCTION

Predation is a key factor in the evolution of prey species and the dynamics of prey communities. Changes in the predator environment can have tremendous effects on prey species and the related communities (Sih 1987, Lima and Dill 1990). In nature, most prey organisms are exposed to a variety of predators, which differ in their search and capture characteristics. Thus it is astonishing that most defense systems have been studied only in a two-species context.

While defensive effects against multiple attackers have been studied in some plant systems (e.g., Rausher et al. 1993, Berenbaum and Zangerl 1999), corresponding work on animal prey in multipredator environments is sparse (e.g., Stemberger and Gilbert 1987, Kuhlmann et al. 1999, Iyengar and Harvell 2002) and mainly restricted to behavioral traits (Sih et al. 1998). Defenses may be especially advantageous if they protect against a variety of attackers, and thus function as a multipurpose tool against predators. Tollrian and Harvell (1999*a*) suggested that multipredator environments are important for the evolution of inducible defenses. These phenotypically plastic responses have been observed in a variety of different taxa, including bryozoans (Harvell 1984) and barnacles (Lively 1986) in marine environments, and in algae (Van Donk et al. 1999), protozoans (Kuhlmann et al. 1999), rotifers (Gilbert 1999), cladocerans (Tollrian and Dodson 1999), amphibians (McCollum and Leimberger 1997), and fish (Brönmark et al. 1999) in freshwater environments.

A major difficulty inherent in devising methodology for estimating the protective effect of a specific defense is overcome with inducible defenses because the responses of predators to defended and undefended forms can be directly compared.

We used the predator-induced morphology in the water flea *Daphnia cucullata* to test the effectiveness of defenses in a multipredator context. *D. cucullata* (Fig. 1) is a common species in the epilimnion of eutrophic lakes across Europe. As an adaptation against visually hunting fish, which form a common selective force in large lakes, *D. cucullata* maintains a small, transparent body and a small clutch size (Hrbácek 1962). This renders *D. cucullata* an inferior competitor against larger cladoceran species when fish are absent (Weider and Wolf 1991), but a superior competitor when fish are present. Thus, population growth in *D. cucullata* is

Manuscript received 28 April 2003; revised 24 October 2003; accepted 2 December 2003; final version received 15 January 2004. Corresponding Editor: J. E. Havel.

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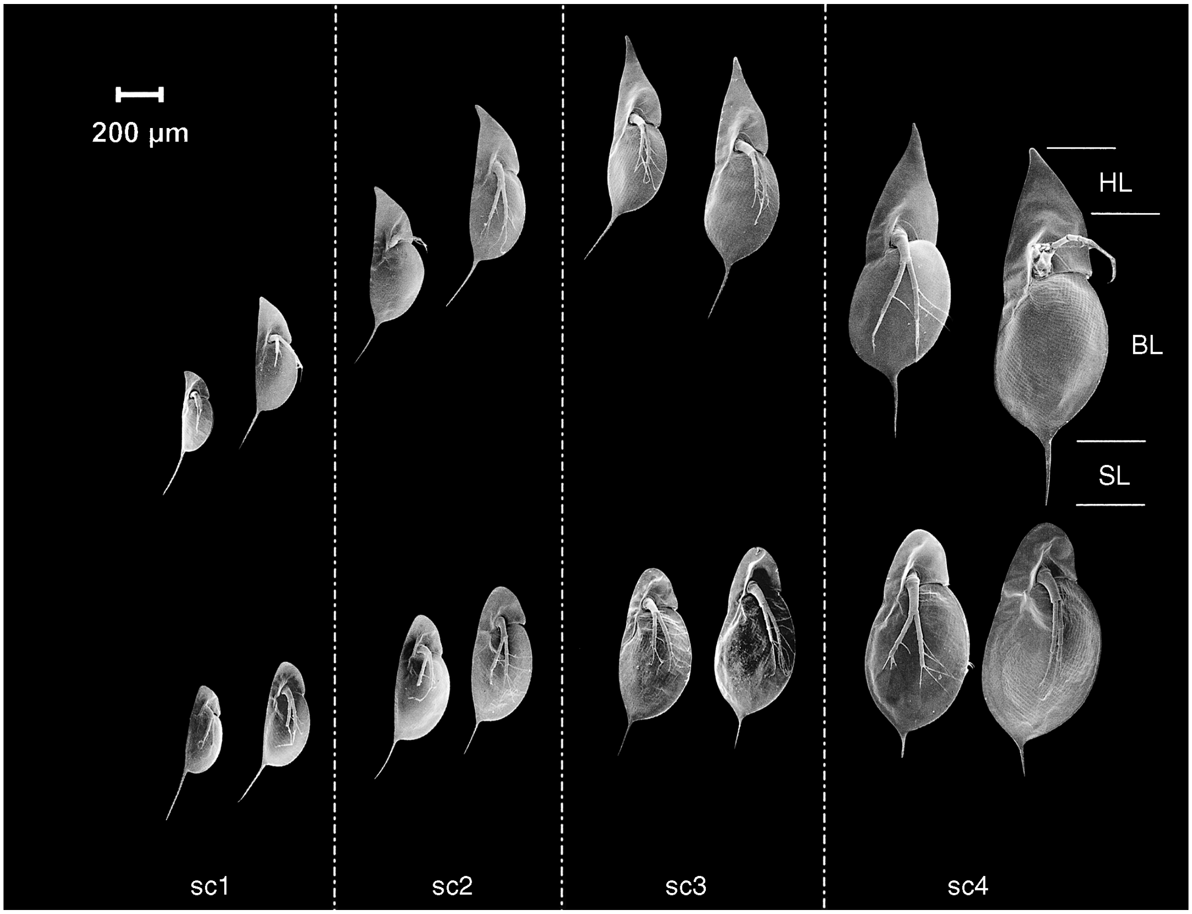

FIG. 1. Comparative scanning electron micrographs (for method see Laforsch and Tollrian [2000]) of the first eight instars of typical (bottom row) and helmeted (laboratory-induced; top row) *Daphnia cucullata*. The grouping size classes (sc) in our study (sc1–sc4) are separated by vertical lines. The arrangement of *Daphnia* follows the developmental pattern of the relative values of the plastic traits during these life stages. The morphological parameters recorded from *Daphnia cucullata* in our experiments were the helmet length (HL), the body length (BL), and the tail spine length (SL).

dependent on a moderate degree of fish predation (Hrbácek 1962). However, many invertebrate predators, while being large zooplankters, are adapted to fish predation and therefore coexist with fish. For example, the phantom midge *Chaoborus flavicans* avoids fish predation by an induced vertical migration (Dawidowicz 1993) and the predatory cladoceran, *Leptodora kindtii*, is highly transparent. Hence, *D. cucullata* is potential prey for many invertebrate predators and should possess the ability to react flexibly to a variable predation regime. *D. cucullata* shows a distinct seasonal polymorphism (cyclomorphosis) with short helmeted morphs from autumn to spring and long helmeted morphs in summer (Wesenberg-Lund 1908, Lampert and Wolf 1986). Although *D. cucullata* is used as a textbook example for phenotypic plasticity (Jacobs 1987, Lampert and Sommer 1993), the conditions under which these changes arise and are beneficial remain poorly understood. In *D. cucullata*, induction of increased helmet length by predatory larvae of the phantom midge *Chaoborus* has been shown (Tollrian 1990). However, an advantage of the helmets has not yet been proven.

In this study, we tested whether helmets and tail spines are inducible by chemical cues from several invertebrate predators. In addition, we studied whether the induced traits of *D. cucullata* act as defense against those invertebrate predators and explored the functional mechanism by which these induced traits offer protection.

MATERIAL AND METHODS

Induction experiment

A standard clone of *D. cucullata* isolated from Lake Thalersee, Germany, was used for the experiment. All predators in our study coexist with *D. cucullata* in Lake Thalersee. However, for the experiment, we isolated them from adjacent lakes where they occurred in higher densities. Fourth-instar larvae of *C. flavicans* were taken from Lake Klostersee, *L. kindtii* from lakes Klostersee and Eschenauersee, and the crustacean copepod *Cyclops* sp. from Lake Hartsee.

TABLE 1. Relative helmet length (%; mean and standard error) of *Daphnia cucullata* raised with (kairomone) and without (control) chemical cues released from different invertebrate predators.

Daphnia size class	Control			Kairomone			
				Chaoborus			
	Mean	SE	*N*	Mean	SE	*N*	*P*
1	15.12	0.41	62	30.19	0.47	94	<0.001
2	14.28	0.21	136	29.79	0.32	100	<0.001
3	13.03	0.25	62	25.84	0.34	74	<0.001
4	12.29	0.26	41	20.51	0.44	35	<0.001

Notes: Only one control treatment was carried out, because all experiments were conducted at the same time. *Daphnia* were divided into four body length size classes (1, <600 μm; 2, 600–800 μm; 3, 800–1000 μm; 4, >1000 μm) for analysis. *N* indicates the number of individual *Daphnia* pooled from three replicate beakers per treatment. Size classes were compared for treatment effects using a nested ANOVA with three replicates per treatment as random factor.

The experiment was conducted in the laboratory under constant conditions at 20°C and fluorescent light (14 h day:8 h night) in 1.5-L glass beakers. The animals were reared in an artificial medium based on ultrapure water, trace elements, and phosphate buffer (Jeschke and Tollrian 2000) with unlimited food (*Scenedesmus obliquus*). The medium was changed weekly. At the beginning of the experiment, 20 age-synchronized nonhelmeted animals with freshly deposited eggs were placed into each beaker in 1 L of medium. All beakers contained a cylindrical cage made of acryl (10 cm diameter and 12 cm length) and 100-μm nylon mesh that insured separation between predators and *D. cucullata* while guaranteeing the exchange of chemical cues. The predators were placed into these cages and fed daily with adjusted amounts of prey according to their selectivity to ensure the release of kairomones. Ten *Chaoborus* per cage received a mixture of 30 juvenile *D. cucullata* and *D. pulex* each day. Three *Leptodora kindtii* per cage received daily 30–40 *D. cucullata, Bosmina* sp., and *Ceriodaphnia* sp. In the copepod treatment, 25 *Cyclops* sp. per cage were fed daily with 30 *D. cucullata* and *Bosmina* sp. Pupate *Chaoborus* larvae and dead predators were replaced and the cages were cleaned daily. Beakers without predators served as a control. Pilot experiments had shown that chemical cues released by the prey species without predators had no inductive effect on *D. cucullata*. All treatments had three replicate beakers. The experiment with *L. kindtii* was conducted in the dark because the animals tend to swim against the walls of the beaker under light conditions in the laboratory.

All induction experiments ran simultaneously and lasted three to five weeks to include transgenerational induction effects (Agrawal et al. 1999). After this period, ~100 randomly selected *Daphnia* per beaker were measured under a dissecting microscope with a digital image-analysis system (Soft Imaging System, Analysis Pro, Münster, Germany). The morphological parameters recorded from *D. cucullata* were helmet length, body length, and tail spine length (Fig. 1). For analysis, *Daphnia* were divided into four body length classes (size class 1, <600 μm; size class 2, 600–799 μm; size class 3, 800–1000 μm; size class 4, >1000 μm). *D. cucullata* become mature at the end of the third body-length size class. To compensate for size-dependent changes in helmet and spine length within the classes, the relative helmet length ([helmet length/body length] × 100) and the relative tail spine length ([tail spine length/body length] × 100) were calculated. The relative values were arcsine-square-root transformed prior to analysis (Sokal and Rohlf 1995). Data were tested for normal distribution and homogeneity of variances. In body length size class 4 in the *Leptodora* treatment, an additional exponential transformation was applied to achieve homogeneity of variances (Winer et al. 1991). A nested ANOVA was performed to analyze for treatment effects between induced and control animals with treatment as fixed factor and three replicates per treatment as random factor.

Predation experiments

Three different predators, each showing a different hunting strategy, were used to test whether the morphological changes of *D. cucullata* act as a protection against invertebrate predation. The predators *C. flavicans*, *L. kindtii*, and *Cyclops* sp. were isolated as described in *Materials and methods: Induction experiment.* For the predation trials, *Chaoborus* kairomone-induced and noninduced cultures of *D. cucullata* were age synchronized in order to reduce the variation within size classes and to ensure that induced and noninduced animals of the same size class were of equal age. *D. cucullata* were grouped into three body-length size classes before the experiment (size class 1, <600 μm; size class 2, 600–800 μm; size class 3, >800 μm) using the digital image-analysis system.

The predation trials with *Cyclops* sp. were conducted in 20 mL petri dishes filled with artificial medium. The average size of the copepods was 1.6 ± 0.1 mm (mean ± 1 SE), measured from the tip of the head to the base of the caudal furca. Only the first and second body-length size classes of *D. cucullata* were used in the experiment because the copepods did not succeed in grasping larger prey items. The predators were starved for 24 h before the start of the experiment. One *Cyclops*

TABLE 1. Extended.

Kairomone							
Cyclops				*Leptodora*			
Mean	SE	*N*	*P*	Mean	SE	*N*	*P*
27.96	0.46	101	0.001	30.62	1.97	10	<0.001
27.70	0.41	75	<0.001	33.98	0.52	85	<0.001
23.07	0.29	99	<0.001	28.02	0.54	72	0.001
20.10	0.44	25	<0.001	19.70	0.92	25	<0.001

and either 10 induced or 10 noninduced *D. cucullata* were exposed to the test conditions (20°C) for 2 h in the dark. The trials were replicated 15 times for each size class. Three petri dishes without *Cyclops* served as controls to identify mortality unrelated to predation. The numbers of killed, completely eaten, and surviving animals were recorded.

The predation treatment with *L. kindtii* was also conducted in the dark in 200 mL of artificial medium at 20°C. The body length of the predators was measured from the top of the head to the caudal part of the body (Manca and Comoli 1995). The average body length of *Leptodora* in the experiment was 8.4 ± 0.1 mm. The average trap basket length calculated according to Manca and Comoli (1995) and Branstrator (1998) was 1.4 mm. A single nonstarved predator was placed into a glass beaker with both induced and noninduced *D. cucullata* (10 each) of the same size class. After 24 h, the number of killed and surviving *Daphnia* was recorded. The trials were replicated 7–12 times in each *Daphnia* size class. Once again, three beakers without predators served as controls.

We used 5 cm diameter petri dishes filled with 20 mL artificial medium for the predation experiments with the phantom midge, *C. flavicans*. A single, starved, fourth-instar larva of *C. flavicans* was placed into each petri dish with either 20 induced or 20 noninduced *D. cucullata*. We chose the high number of *Daphnia* to increase the encounter rate between predators and prey. The larvae were directly observed under artificial light at 20°C for 30 min. Those *Chaoborus* larvae that failed to strike within 10 min were removed from the experiment. Eaten *D. cucullata* were replaced immediately. The predation trials were replicated 10 times in the smallest size class and 14 times in the other size classes.

To shed light on the protective mechanisms, we directly observed the *Chaoborus* larvae to clarify on which steps of the predation cycle (search, attack, capture, or ingestion) the defense acts. We defined an attack as a jerky movement of the *Chaoborus* larvae directed towards a nearby prey, followed by a grasp. This could result in either an evasion or a capture, the latter of which we defined as the moment when the larvae had a hold on the *Daphnia*. Finally, ingestion was defined as the moment a *Daphnia* was swallowed into the pharynx of the *Chaoborus*. The number of attacks, contacts, evasions and captures which resulted in either escapes or ingestions was noted. From the data we calculated the following efficiencies:

1) The attack efficiency, which is an inverse measure of the protective effect of the induced defenses, was defined as the ratio of the number of ingestions to the total number of attacks. We used the attack efficiency, instead of a prey-oriented defense efficiency (1 − attack efficiency), because it is commonly used in functional response models (for review, see Jeschke et al. 2002).
2) The evasion efficiency, which is a measurement of the ability to escape without being caught, was defined as the ratio of the number of evasions to the total number of attacks.
3) The escape efficiency, which is a measure of the ability to escape after capture, was defined as the ratio of the number of escapes to the total number of captures.

The effects of induced *Daphnia* phenotypes on predation were analyzed by using Mann-Whitney *U* tests for *Chaoborus* and *Cyclops*. We used paired Wilcoxon tests for related samples for the *Leptodora* treatment. Efficiency data in the *Chaoborus* treatment were arcsine-square-root transformed prior to analysis (Sokal and Rohlf 1995).

RESULTS

Chemically induced traits

All predators induced longer absolute helmets and tail spines in the prey animals compared to the control animals in all size classes, with the only exception being the spine length in the first size class in the *Leptodora* treatment.

Similarly, the difference in the relative helmet length was highly significant between each of the predator kairomone treatments and controls in each of the four body length categories (Table 1). We always found the largest difference between kairomone and control treatments in the second *Daphnia* size class.

Likewise, the relative tail spine length in each size class of *Daphnia* induced with *C. flavicans* or *Cyclops* sp. was significantly longer than in the control *Daphnia*

TABLE 2. Relative tail spine length (%; mean and standard error) of *Daphnia cucullata* raised with (kairomone) and without (control) chemical cues released from different invertebrate predators. For details, see Table 1.

Daphnia size class	Control			Kairomone			
				Chaoborus			
	Mean	SE	*N*	Mean	SE	*N*	*P*
1	38.40	0.86	62	54.05	1.01	94	0.001
2	26.91	0.41	136	39.05	0.74	100	0.001
3	22.51	0.35	62	29.21	0.43	74	0.001
4	12.36	0.39	41	22.89	0.58	35	<0.001

(Table 2). In the *Leptodora* treatment the relative tail spine length was not different in the smallest size category. However, as in the previous two predator treatments, the induced *Daphnia* in each of the larger size categories had longer tail spines (Table 2).

Predation experiments

We found protective effects of the induced traits against each of the predators tested. However, the magnitude of protection differed between predators and between prey size classes.

Direct observation of C. flavicans *predation on* D. cucullata.—The evaluation of the total number of attacks (Table 3), yielded a significantly higher attack rate on helmeted compared to nonhelmeted morphs in the second and third size category. Only in juvenile instars with body length <600 μm did phantom midge larvae show no significant difference in total number of attacks. None of the examined efficiencies (attack, escape, evasion) showed a significant difference between morphs in the smallest size class. However, there was a significant advantage of the induced morph in the remaining size categories (Table 3). In the second and third size class, the attack efficiency decreased relative to the first *Daphnia* size class. This decrease was caused by both an increase in evasion efficiency and an increase in escape efficiency in the helmeted morph (Table 3).

Predation experiment with Cyclops *sp.*—Only the juvenile instars of *D. cucullata* are susceptible to predation by *Cyclops*. The helmeted morph had a higher survival rate (mean ± 1 SE: size class 1, typical, 7.93 ± 0.30, helmeted, 8.47 ± 0.25; size class 2, typical, 7.33 ± 0.39, helmeted, 9.53 ± 0.16) and was less often eaten (size class 1, typical, 1.53 ± 0.25, helmeted, 0.53 ± 0.13; size class 2, typical, 0.67 ± 0.28, helmeted, 0.13 ± 0.09). We found significant differences between both morphs in the second size category of the surviving animals and in the first size class of the eaten animals (Mann-Whitney *U*: survived animals, size class 1, $P = 0.205$, size class 2, $P < 0.001$; eaten animals, size class 1, $P = 0.004$, size class 2, $P = 0.086$). *Daphnia* were killed but not completely eaten significantly more often in the second size category of the nonhelmeted morph (size class 1, typical, 0.53 ± 0.21, helmeted, 1.00 ± 0.30, size class 2, typical, 2.00 ± 0.37, helmeted, 0.33 ± 0.16; Mann-Whitney *U*: size class 1, $P = 0.256$; size class 2, $P < 0.001$).

Predation experiment with L. kindtii.—In the first size class, there was no significant difference between the two morphs of *D. cucullata* in the number of prey items eaten by *Leptodora* (paired Wilcoxon test for related samples, $P = 0.546$; Fig. 2). Starting from a body length of 600 μm, there was a distinctly significant advantage for the induced *Daphnia* compared to the noninduced ones (size class 2, $P = 0.018$; size class 3, $P = 0.009$).

DISCUSSION

Defenses of prey organisms are frequently studied in a simplified two-species context. While this approach provides important information about defensive mechanisms and protective effects, studies focusing on two-species interactions offer only limited insights into understanding the ecological relevance of defenses. The impact of multiple predators on prey is poorly understood, especially in systems where different predators vary temporally and spatially in their abundance and importance. In situations where predators with different prey selectivity or modes of predation vary in their impact or abundance, prey species should evolve phenotypic plasticity, given that trade-offs have to be assumed (Tollrian and Harvell 1999*b*). In situations where a single kind of defense is effective against a variety of important predators (which may occur concurrently or alternately) a diffuse coevolution (sensu Janzen 1980) could be the consequence.

Our study revealed that helmet and tail spine formation in *Daphnia cucullata*, a well-known example of cyclomorphosis, offers protection against different types of invertebrate predators and is thus a likely example of diffuse coevolution against multiple predators. We showed that this particular prey defense is beneficial against predators with diverse foraging tactics, ranging from cruising predators (*Leptodora*), to ambush predators (phantom midge larvae) and active searching predators (copepods), although we did not explore possible interaction effects between predators exposed simultaneously to *D. cucullata*. In the natural habitat of *D. cucullata*, where predators vary in time and space, prey organisms must evolve defense strategies to respond flexibly to this changing multipredator

TABLE 2. Extended.

Kairomone							
Cyclops				*Leptodora*			
Mean	SE	*N*	*P*	Mean	SE	*N*	*P*
50.62	0.62	101	0.002	44.07	2.01	10	NS
38.20	0.59	75	0.001	33.11	0.49	85	0.045
30.12	0.23	99	0.001	26.64	0.36	72	0.029
23.45	0.72	25	<0.001	19.40	1.15	25	<0.001

environment. We might speculate that in *Daphnia* populations where the defense was originally induced by one predator, the individual benefit would increase with the abundance of other invertebrate predators. The additional defensive effect would further contribute to the total benefit of the inducible trait. It could thus become evolutionarily stable and establish as a predator-induced general defense.

Induction experiment

Tollrian and Dodson (1999) pointed out that *Daphnia* live in an ''olfactory sea'' and are able to discriminate between many chemical odors. Water-soluble substances released by predators provide reliable cues for prey species if they indicate the current predation pressure of a single predator. The so-called ''kairomones'' (Brown et al. 1970) are exclusively advantageous to the receiver in an interspecific information-transfer context (Dicke and Sabelis 1988). Kairomones have been shown to cause plastic responses in cladocerans, but most scientists have focused their studies on kairomones released from *Chaoborus*, *Notonecta*, or fish (for review, see Havel 1987, Tollrian and Dodson 1999).

Our investigation reveals that *D. cucullata* have morphologically plastic responses to cues from each of the predators tested. The result from our *Chaoborus* treatment concurs with the data published by Tollrian (1990). He showed that *D. cucullata* of intermediate and large size in the presence of the predator developed significantly longer helmets than those in controls. We achieved a similar result in the induction experiment with other invertebrate predators, although the extent of helmet formation as observed in nature could not be induced in the laboratory. For instance, the relative helmet length in *D. cucullata* of size class 2 in Lake Thalersee during summer is 45% on average, whereas helmet extension in our induction experiment was 30% on average. Predatory copepods are reported to affect phenotypic plasticity in *Bosmina* and *Daphnia* (e.g.,

TABLE 3. Direct observation of *Chaoborus flavicans* predation on typical vs. helmeted *Daphnia cucullata*.

Parameter, by *Daphnia* size class†	Predation on typical (%)			Predation on helmeted (%)			
	N	Mean	SE	*N*	Mean	SE	*P*
No. attacks							
1	10	8.50	0.72	10	6.30	0.79	0.072
2	14	6.29	0.67	14	8.71	0.79	0.039
3	14	4.71	0.57	14	10.57	1.24	<0.001
Attack efficiency							
1	10	78.60	3.47	10	75.97	4.92	0.543
2	14	62.76	4.59	14	31.52	5.00	<0.001
3	14	47.02	8.87	14	7.10	2.21	<0.001
Evasion efficiency							
1	10	18.42	3.56	10	19.02	4.51	0.790
2	14	20.73	4.72	14	46.23	5.27	0.002
3	14	35.47	7.03	14	61.29	3.99	0.009
Escape efficiency							
1	10	3.44	1.82	10	5.00	5.00	0.358
2	14	19.65	5.01	14	36.45	8.79	0.239
3	14	33.33	9.56	14	85.64	4.50	<0.001

Notes: Attack efficiency was calculated as (no. eaten *Daphnia*)/(total no. attacks) × 100; evasion efficiency as (no. evasions without capture)/(total no. attacks) × 100; and escape efficiency as (no. escaped *Daphnia* after capture)/(total no. captures) × 100. Significance levels for comparisons between the helmeted and typical morphs were calculated using Mann-Whitney *U* tests.

† Body length: 1, <600 μm; 2, 600–800 μm; 3, >800 μm.

FIG. 2. The number of animals eaten in the predation experiment with *Leptodora kindtii*. Means (+1 SE) of two different morphs of *Daphnia cucullata* in three size classes (body length: 1, <600 μm; 2, 600–800 μm; 3, >800 μm) are shown. Asterisks indicate highly significant differences ($P < 0.001$).

Dodson 1984, Post et al. 1995), but our study revealed for the first time that *Daphnia* change their morphology in the presence of *Leptodora*.

D. cucullata is a very small and transparent species and can be found in ponds with a high density of fish (Hrbácek 1958). Fish feed on larger prey items and the induced structures in *D. cucullata* should have no effect on fish predation. Hence, it is not surprising that helmets in *D. cucullata* are not inducible with fish kairomones (Pawlowski 2000). Similarly, Dodson (1988) revealed that *D. retrocurva* and *D. galeata* developed helmets in the presence of *Chaoborus* and *Notonecta*, but not in the presence of fish. Field studies indicate that plastic responses in *Cladocera* are strongest when invertebrate predators are abundant (Mort 1989, Pijanowska 1991). Together with these observations, our results imply that cyclomorphosis in *D. cucullata* in the wild is at least partly induced by predator kairomones.

Our three predator treatments differed in the magnitude of induction (see Tables 1 and 2). This difference could be due to variation in kairomone concentration in our study. Such a dose–response relationship was demonstrated for neckteeth formation in *D. pulex* (e.g., Havel 1985*a*, Tollrian 1993). On the other hand, predator-specific kairomones could be the cause for the variable degree of response to the different predator environments. For example, Barry and Bayly (1985) demonstrated that *D. carinata* is only inducible with notonectids. Laforsch and Tollrian (*unpublished manuscript*) revealed that *Leptodora* could induce helmet formation in *D. cucullata* but not neckteeth development in *D. pulex*. Accordingly, it seems likely that *D. cucullata* can distinguish between different predators in a multipredator environment. However, these speculations are beyond the scope of our study because we controlled neither for equal amounts of predator biomass nor for equal amounts of prey consumed.

Interestingly, the maximum induction of the traits in our study is found in the size range of *Daphnia* with the strongest threat of invertebrate predators (Swift and Fedorenko 1975, Pastorok 1981), consistent with the hypothesis that selection has shaped the defense to be most active during the most vulnerable life stages (Dodson 1974).

Predation experiments

Our predation experiments, with three invertebrate predators that coexist with *D. cucullata* in the wild, show for the first time a distinct advantage of the induced morph. So far only a few studies have shown protective effects of helmets in other species (Havel 1985*b*, Mort 1986).

Spitze and Sadler (1996) showed that tail spines also act as a protection against *Chaoborus* predation. Similarly, an elongated tail spine in other *Daphnia* species was reported to be protective against fish (e.g., Kolar and Wahl 1998) and copepods (e.g., Balseiro and Vega 1994). Invertebrate predators, which usually locate their prey with mechanoreceptors, are often restricted in their ability to catch, handle, or ingest their prey items. The phantom midge larva is the best-documented example of a size-selective predator with a preference for smaller prey organisms (e.g., Tollrian 1995). This selectivity is based on a size-dependent interaction between the encounter probability and strike efficiency of the ambush predator (Pastorok 1981). Similarly, *Leptodora* are limited by the morphology of their feeding basket and consequently can only catch prey up to a certain size (e.g., Branstrator 1998). Size selectivity in copepods is not universally agreed upon. Williamson (1983) suggested that body size of prey items is not crucial to a successful strike, whereas Gliwicz and Umana (1994) demonstrated that smaller cladocerans such as *D. cucullata* remained vulnerable to *Acanthocyclops* predation until maturation and larger animals were safe from copepod predation. The latter is in accordance with the result of our predation experiment, where only the first two size classes were susceptible to *Cyclops* predation. Copepods often attack their prey dorsally, and thus the tail spine is discussed as the predominant defense against this predator (Balseiro and Vega 1994). We show that the plastic traits act as protective devices against *Cyclops* predation, even in the smallest neonates. Similarly, *Leptodora* predation was significantly less successful on longer-helmeted and spined specimens in our trials (Fig. 2). Induced animals larger than 600 μm had a distinct advantage in both treatments.

In general, two different sorts of defense strategies can be distinguished (Tollrian and Dodson 1999). The first are precapture defenses that make prey less conspicuous or harder to detect, and the second are postcapture defenses that impede handling or ingestion of the prey. Induced morphological defenses belong predominantly to the second category as already suggested in Dodson's (1974) ''anti-lock-and-key'' hypothesis. For example, neckteeth in juvenile *D. pulex* offer only a postcapture advantage (Havel and Dodson 1984). We showed that cyclomorphic features of *D. cucullata* offer advantages at different steps of the *Chaoborus* predation cycle. We did not find an advantage before an attack, (i.e., a ''stealth'' mechanism that would allow the prey to pass a tactile predator without being attacked), since the attack rate on helmeted individuals was not lower. Induced individuals were actually attacked more often in size categories 2 and 3. However, these relatively high attack rates can be offset by the low attack efficiency for the induced morphs (Table 3). Induced *Daphnia* escaped and the predator remained hungry and continued to attack.

In our experiment, induced *D. cucullata* of intermediate sizes had a significant advantage between the steps of attack and capture (Table 3). It was not possible to clarify whether *D. cucullata* was harder to grasp or simply able to escape faster. Hebert (1978) suggested that helmets may supply more area for antenna muscle attachment that in turn would allow the animals to escape faster. However, in the case of *Chaoborus*, this scenario seems unlikely, because *Chaoborus* attacks take less than 1/100 second (R. Tollrian, *unpublished data*), certainly faster than *Daphnia* escape-swimming speed. Alternatively, helmets and tail spines could lead to a misjudgment of prey distance by the predator.

Finally, our study revealed that induced *Daphnia* of the largest size had an additional advantage between the steps of capture and ingestion. The induced traits render *D. cucullata* difficult to swallow. Phantom midge larvae are gape-limited predators (Pastorok 1981, Swift 1992) and enlarged structures such as helmets and tail spines can interfere with their ability to ingest prey.

Interestingly, the induced traits in *D. cucullata* acted during different life stages, from the smallest neonates that have been protected against copepods to the adults that escaped *Chaoborus* captures. This result may resolve the question of why some cladocerans form helmets and others neckteeth (Tollrian and Dodson 1999). Neckteeth have been shown to defend early instars of larger cladocerans against *Chaoborus* larvae (Tollrian 1995), while our study showed that smaller species require protection from several invertebrate predators and helmets act as general multipurpose defenses.

CONCLUSIONS

Phenotypically plastic organisms are adapted to heterogeneous environments. Multipredator regimes are common and the evolution of inducible defenses is a suitable agent to cope with the sometimes unpredictable hazard of a variety of predators, each foraging in a different way. *D. cucullata*, a very small cladoceran, can coexist with fish and is threatened by several predaceous invertebrates. Chemical cues released by each of these predators may indicate their temporal impact and so induce cyclomorphosis in the field. Finally, after one century of research in this ''classical'' system, our study provides evidence that predator kairomones act as proximate cues and a defensive effect is an ultimate reason for cyclomorphosis in *D. cucullata*. In freshwater habitats, there is a strong pressure to adapt to a variety of predator's foraging strategies and the combination of an elongated helmet and tail spine seems to be a perfect device to reduce the predation risk caused by multiple predators, especially since the same defensive traits can act at different stages of the predation cycle. The combined benefits of defenses against different predators may be the result of a ''diffuse'' coevolution and possibly the key factor for evolution and persistence of these inducible defenses in multipredator environments.

ACKNOWLEDGMENTS

We thank W. Gabriel for encouragement and discussion, M. Kopp and J. M. Jeschke for comments, M. Kredler and E. Hochmuth for help during experiments, John Baines and Scott Stevens for linguistic improvements, and the editor John Havel and two anonymous reviewers for comments on the manuscript.

LITERATURE CITED

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defences in animals and plants. Nature **401**:60–63.

Balseiro, E. G., and M. Vega. 1994. Vulnerability of *Daphnia middendorffiana* to *Parabroteas sarsi* predation: the role of the tail spine. Journal of Plankton Research **16**:783–793.

Barry, M. J., and I. A. E. Bayly. 1985. Further studies on predator induction of crests in Australian *Daphnia* and the effects of crests on predation. Australian Journal of Marine and Freshwater Research **36**:519–535.

Berenbaum, M., and A. Zangerl. 1999. Coping with life as a menu option: inducible defenses of the wild parsnip. Pages 10–32 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Branstrator, D. K. 1998. Predicting diet composition from body length in the zooplankton predator *Leptodora kindtii*. Limnology and Oceanography **43**:530–535.

Brönmark, C., L. B. Pettersson, and P. A. Nilsson. 1999. Predator-induced defense in crucian carp. Pages 203–217 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Brown, W. L., Jr., T. Eisner, and R. H. Whittaker. 1970. Allomones and kairomones: transspecific chemical messengers. BioScience **20**:21–22.

Dawidowicz, P. 1993. Diel vertical migration in *Chaoborus flavicans*: population patterns vs. individual tracks. Archiv für Hydrobiologie, Beihefte Ergebnisse der Limnologie **39**: 19–28.

Dicke, M., and M. W. Sabelis. 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? Functional Ecology **2**:131–139.

Dodson, S. I. 1974. Adaptive change in plankton morphology in response to size-selective predation: a new hypothesis of cyclomorphosis. Limnology and Oceanography **19**:721–729.

Dodson, S. I. 1984. Predation of *Heterocope septentrionalis* on two species of *Daphnia*: morphological defenses and their cost. Ecology **65**:1249–1257.

Dodson, S. I. 1988. Cyclomorphosis in *Daphnia galeata mendotae* Birge and *Daphnia retrocurva* Forbes as a predator-induced response. Freshwater Biology **19**:109–114.

Gilbert, J. J. 1999. Kairomone-induced morphological defenses in rotifers. Pages 127–141 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Gliwicz, M. Z., and G. Umana. 1994. Cladoceran body size and vulnerability to copepod predation. Limnology and Oceanography **39**:419–424.

Harvell, C. D. 1984. Predator-induced defenses in a marine bryozoan. Science **224**:1357–1359.

Havel, J. E. 1985*a*. Cyclomorphosis of *Daphnia pulex* spined morphs. Limnology and Oceanography **30**:853–861.

Havel, J. E. 1985*b*. Predation of common invertebrate predators on long-featured and short featured *Daphnia retrocurva*. Hydrobiologia **124**:141–150.

Havel, J. E. 1987. Predator-induced defenses: a review. Pages 263–278 *in* W. C. Kerfoot and A. Sih, editors. Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, New Hampshire, USA.

Havel, J. E., and S. I. Dodson. 1984. *Chaoborus* predation on typical and spined morphs of *Daphnia pulex*: behavioral observations. Limnology and Oceanography **29**:487–494.

Hebert, P. D. N. 1978. Cyclomorphosis in natural populations of *Daphnia cephalata* King. Freshwater Biology **8**:79–90.

Hrbácek, J. 1958. Typologie und Produktivität der teichartigen Gewässer. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie **13**: 394–399.

Hrbácek, J. 1962. Species composition and the amount of zooplankton in relation to fish stock. Rozpravy Ceskoslovenske Akademie Ved, Rada matematickych a prirodnich ved **72**:1–117.

Iyengar, E. V., and C. D. Harvell. 2002. Specificity of cues inducing spines in the bryozoan (*Membranipora membranacea*). Marine Ecology Progress Series **225**:205–218.

Jacobs, J. 1987. Cyclomorphosis in *Daphnia*. Pages 325–352 *in* R. H. Peters and R. de Bernardi, editors. Daphnia. Memorie dell'Istituto Italiano di Idrobiologia **45**.

Janzen, D. H. 1980. When is it coevolution? Evolution **34**: 611–612.

Jeschke, J. M., M. Kopp, and R. Tollrian. 2002. Predator functional responses: discriminating between handling and digesting prey. Ecological Monographs **72**:95–112.

Jeschke, J. M., and R. Tollrian. 2000. Density-dependent effects of prey defences. Oecologia **123**:391–396.

Kolar, C. S., and D. H. Wahl. 1998. Daphnid morphology deters fish predators. Oecologia **116**:556–564.

Kuhlmann, H. W., J. Kusch, and K. Heckmann. 1999. Predator-induced defenses in ciliated protozoa. Pages 142–159 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Laforsch, C., and R. Tollrian. 2000. A new preparation technique of daphnids for scanning electron microscopy using hexamethyldisilazane. Archiv für Hydrobiologie **149**:587–596.

Lampert, W., and U. Sommer. 1993. Limnoökologie. Thieme, Stuttgart, Germany.

Lampert, W., and H. G. Wolf. 1986. Cyclomorphosis in *Daphnia cucullata*: morphometric and population genetic analyses. Journal of Plankton Research **8**:289–303.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology **68**:619–640.

Lively, C. M. 1986. Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*. Evolution **40**: 232–242.

Manca, M., and P. Comoli. 1995. Seasonal changes in size of the feeding basket of *Leptodora kindtii* (Focke) in Lago Maggiore as related to variations in prey size selection. Limnology and Oceanography **40**:834–838.

McCollum, S. A., and J. D. Leimberger. 1997. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. Oecologia **109**:615–621.

Mort, M. A. 1986. *Chaoborus* predation and the function of phenotypic variability in *Daphnia*. Hydrobiologia **133**:39–44.

Mort, M. A. 1989. Cyclomorphosis in *Daphnia galeata mendotae*: variation and stability in phenotypic cycles. Hydrobiologia **171**:159–170.

Pastorok, R. A. 1981. Prey vulnerability and size selection by *Chaoborus* larvae. Ecology **62**:1311–1324.

Pawlowski, M. 2000. Variabilität in Morphologie, Verhalten und genetischer Zusammensetzung einer natürlichen *Daphnia cucullata* population. Ausmaß—Ursachen—Konsequenzen. Shaker, Aachen, Germany.

Pijanowska, J. 1991. Seasonal changes in morphology of *Daphnia cucullata*. Archiv für Hydrobiologie **121**:79–86.

Post, D. M., T. M. Frost, and J. F. Kitchell. 1995. Morphological responses by *Bosmina longirostris* and *Eubosmina tubicen* to changes in copepod predator populations during an whole-lake acidification experiment. Journal of Plankton Research **17**:1621–1632.

Rausher, M. D., K. Iwao, E. L. Simms, N. Ohsaki, and D. Hall. 1993. Induced resistance in *Ipomea purpurea*. Ecology **74**:20–29.

Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. Pages 203–223 *in* W. C. Kerfoot and A. Sih, editors. Predation: direct and indirect impacts on aquatic communities. University Press of New England, Lebanon, New Hampshire, USA.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impact of multiple predators on prey. Trends in Ecology and Evolution **13**:350–355.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. Freeman, New York, New York, USA.

Spitze, K., and T. D. Sadler. 1996. Evolution of a generalist genotype: multivariate analysis of the adaptiveness of phenotypic plasticity. American Naturalist **139**:229–247.

Stemberger, R. S., and J. J. Gilbert. 1987. Multiple-species induction of morphological defenses in the rotifer *Keratella testudo*. Ecology **68**:370–378.

Swift, M. C. 1992. Prey capture by the four larval instars of *Chaoborus crystallinus*. Limnology and Oceanography **37**: 14–24.

Swift, M. C., and A. Fedorenko. 1975. Some aspects of prey capture by *Chaoborus* larvae. Limnology and Oceanography **20**:418–425.

Tollrian, R. 1990. Predator-induced helmet formation in *Daphnia cucullata* (Sars). Archiv für Hydrobiologie **119**: 191–196.

Tollrian, R. 1993. Neckteeth formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: morphological effects of *Chaoborus* kairomone concentration and their quantification. Journal of Plankton Research **15**:1309–1318.

Tollrian, R. 1995. *Chaoborus crystallinus* predation on *Daphnia pulex*: can induced morphological changes balance effects of body size on vulnerability? Oecologia **101**:151–155.

Tollrian, R., and S. I. Dodson. 1999. Inducible defenses in *Cladocera*. Pages 177–202 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Tollrian, R., and C. D. Harvell. 1999*a*. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Tollrian, R., and C. D. Harvell. 1999*b*. The evolution of inducible defenses: current ideas. Pages 306–321 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Van Donk, E., M. Lürling, and W. Lampert. 1999. Consumer-induced changes in phytoplankton: inducibility, costs, benefits, and the impact on grazers. Pages 89–103 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Weider, L. J., and H. G. Wolf. 1991. Life-history variation in a hybrid species complex of *Daphnia*. Oecologia **87**: 506–513.

Wesenberg-Lund, C. 1908. Plankton investigations of Danish Lakes. General part. Danish Freshwater Biology Laboratory, No. 5. Nordisk Vorlag, Copenhagen, Denmark.

Williamson, C. E. 1983. Behavioral interactions between a cyclopoid copepod predator and its prey. Journal of Plankton Research **5**:701–712.

Winer, B. J., D. R. Brown, and K. M. Michels. 1991. Statistical principles in experimental design. Third edition. McGraw-Hill, New York, New York, USA.