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REPORT

Reciprocal phenotypic plasticity in a predator–prey system: inducible offences against inducible defences?

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Abstract

We describe one of the first examples of reciprocal phenotypic plasticity in a predator–prey system: the interaction between an inducible defence and an inducible offence. When confronted with the predatory ciliate *Lembadion bullinum*, the hypotrichous ciliate *Euplotes octocarinatus* develops protective lateral wings, which inhibit ingestion by the predator. We show that *L. bullinum* reacts to this inducible defence by expressing an inducible offence – a plastic increase in cell size and gape size. This counteraction reduced the effect of the defence, but did not completely neutralize it. Therefore, the defence remained beneficial for *E. octocarinatus*. From *L. bullinum*'s point of view, the increase in feeding rate because of the offence was not larger than the increase in mean cell volume and apparently, did not increase the predator's fitness. Therefore, the inducible offence of *L. bullinum* does not seem to be an effective counter-adaptation to the inducible defence of *E. octocarinatus*.

Keywords

Ciliates, coevolution, *Euplotes octocarinatus*, gape-limited predator, inducible defence, inducible offence, *Lembadion bullinum*, trophic polyphenism, reciprocal phenotypic plasticity.

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INTRODUCTION

Phenotypic plasticity is wide-spread in predator–prey systems. Researchers have described numerous examples of inducible defences in prey (reviewed by Tollrian & Harvell 1999) and fewer cases of 'inducible offences' in predators (e.g. Gilbert 1980; Collins & Cheek 1983; Bernays 1986; Ricci & Banchetti 1993; Mittelbach *et al.* 1999; in the context of predation, the capacity to express an inducible offence is frequently referred to as a diet-induced or trophic polyphenism). However, the interplay between inducible defences and inducible offences has remained largely unstudied.

Recently, Agrawal (2001) pointed to the possibility of "reciprocal phenotypic changes in ecological time" and suggested that these might be more common than generally expected. Reciprocal phenotypic plasticity occurs when the inducible defence of a prey causes the predator to express an inducible counter-offence, or vice versa, potentially resulting in an 'ecological arms race'. If both the defence and the offence are adaptive, reciprocal phenotypic plasticity might be viewed as a result of predator–prey coevolution (Wicklow 1997; Agrawal 2001).

Very few cases of reciprocal phenotypic plasticity have been described to date. Notable examples are from plant–herbivore systems, where plants can induce chemical defences in response to herbivory (reviewed in Karban & Baldwin 1997) and herbivores may counter these defences by activating detoxification mechanisms (Snyder & Glendinning 1996; Bernays & Chapman 2000) or expressing alternative digestive enzymes (Bolter & Jongsma 1995; Jongsma & Bolter 1997). At the behavioural level, predator and prey may undergo patch-selection games, where the prey try to find patches with low predator density and the predators try to find patches with high prey density (Lima 2002 and references therein). Finally, in some intraspecific interactions, environmentally induced cannibalistic morphs elicit morphological (Wicklow 1988) or behavioural (Chivers *et al.* 1997 with further references) defences in their conspecific prey. In the present paper, reciprocal phenotypic plasticity is investigated in ciliated protozoa.

The hymenostome ciliate, *Lembadion bullinum* Perty 1849 is a raptorial feeding predator, which has its gape-size limited by the dimensions of a huge but inflexible peristome (cell mouth). In a previous paper, it has been shown that *L. bullinum* can express an inducible offence: when confronted with a large prey, it can delay cell division and increase its size (Kopp & Tollrian 2003, see also Kuhlmann 1993). The inducing cue is unknown, but most likely, *L. bullinum* mechanically recognizes prey size during the feeding process. As a result of a concomitant increase in gape-size, large morphs of *L. bullinum* are superior in feeding on large prey. Similar gape-size offences have been reported from other predatory ciliates, too (Ricci & Banchetti 1993 and references therein). With small prey, however, large morphs are less efficient and achieve lower cell division rates than small morphs. This is a cost of the inducible offence and explains why *L. bullinum* does not adopt the large size permanently. In summary, the inducible offence can be viewed as an adaptation to variation in the size of the dominant prey species.

In addition to its own plasticity, *L. bullinum* induces morphological defences in a considerable number of prey species (Kuhlmann & Heckmann 1985; for review see Wicklow 1997; Kuhlmann *et al.* 1999). Wicklow (1997) lists 11 inducible prey species from six genera, all of which exploit *L. bullinum*'s gape-limitation. For example, the hypotrichous ciliate *Euplotes octocarinatus* Carter 1972 can produce protective lateral wings, which increase its effective size and inhibit ingestion by gape-limited predators (Kuhlmann & Heckmann 1994). The large number of inducible prey suggests that the inducible offence of *L. bullinum* might serve as a counter-adaptation to inducible prey defences (in addition to being an adaptation to variation in the size between different prey species). In other words, the origin of the inducible offence might be partially explained by diffuse coevolution (i.e. coevolution between groups of species rather than individual species, see Janzen 1980).

In the present paper, we conduct a first step towards evaluating this hypothesis by focusing on the interaction between *L. bullinum* and *E. octocarinatus*. We show that *L. bullinum* activates its offence in response to the inducible defence of *E. octocarinatus*. However, this counteraction does not lead to a significant fitness benefit for the predator. We show that the defence of *E. octocarinatus* operates in a way that cannot easily be counteracted by *L. bullinum*, and we discuss the implications of these findings for the coevolution hypothesis.

METHODS

General methods

Initially, a clonal strain of *L. bullinum* was obtained from K. Wiackowsky (University of Krakow, Poland). Conjugation (sexual recombination) was infrequently observed in stock cultures, but never during experiments. Thus, while our *L. bullinum* were not strictly clonal, genetic diversity was arguably very low. All other cultures were obtained from H.-W. Kuhlmann (University of Münster, Germany).

All ciliates were kept in artificial SMB medium (Salt Medium for *biepharisma*; 1.5 mM NaCl, 0.05 mM KCl, 0.4 mM $CaCl_2$, 0.05 mM $MgCl_2$, 0.05 mM $MgSO_4$, 2.0 mM phosphate buffer, pH 6.8; Miyake 1981) at 20°C in the dark. Experiments were performed under similar conditions. Stock cultures of *L. bullinum* were fed *E. octocarinatus* or *E. aediculatus* Pierson 1943. Both *Euplotes* species received the unicellular green alga *Chlorogonium elongatum* Dangeard 1888, which was grown in SMC medium (Salt Medium for *Chlorogonium*; SMB + 1.25 mM NH_4NO_3, 15 mM $FeCl_3$, 0.8 mM $MnCl_2$; slightly modified after Miyake 1981) at 20°C under constant light and aeration.

The morphological defence of *E. octocarinatus* was induced by culturing *E. octocarinatus* together with the predatory turbellarian *Stenostomum sphagnetorum* Luther 1960 (raised with *Chlorogonium*) under conditions of abundant food. *S. sphagnetorum* induces the same morphological reaction in *E. octocarinatus* as *L. bullinum* (Kuhlmann & Heckmann 1985) but can be more easily separated from the ciliates after exposure. Although *S. sphagnetorum* also induces a behavioural defence mechanism in *E. octocarinatus*, the propensity to perform a characteristic escape reaction upon physical contact with the predator (Kuhlmann 1994), this reaction has never been observed with *L. bullinum*. The degree of induction of the morphological defence – which, in reality, depends on predator density (Kusch 1993), prey density (Wiackowski & Staronska 1999) and food availability (Wiackowski & Szkarlat 1996) – was controlled by selecting defended *E. octocarinatus* from cultures with a mean cell width of 80 μm (corresponding to strong but not extremely strong defence induction).

Measurements of cell dimensions were performed on fixed samples using a computer-based image-analysis system (AnalySIS, Soft Imaging Systems, Münster, Germany) connected to a Leitz Orthoplan microscope (Leica Microsystems, Wetzlar, Germany) at 160-fold magnification. Volume of *L. bullinum* was estimated as $\Pi/6 \times \text{length} \times \text{width}^2$, i.e. cells were assumed to be prolonged spheroids. Fixation was achieved by adding glutaraldehyde to the ciliate cultures, until a final concentration of approximately 0.25% was reached. However, in experiment 1, a final concentration of 2% (Sherr *et al.* 1989) preserved the anatomy of *L. bullinum*'s cell mouth better. Indeed, we recommend this concentration for future studies. The two concentrations of fixative caused slight differences in the length-to-width ratio of the preserved cells. Therefore, the biometrical data from experiment 1 vs. experiments 2 and 3 are not directly comparable.

Numerical results are presented as means ± SD. For biometrical results, such as cell length, we give the grand mean and SD (i.e. the mean and SD after pooling the data from all replicates of a treatment). Statistical analysis, in contrast, is performed on replicate means (except regression analysis of experiment 3), using SPSS for Windows 10.0 (SPSS Inc., Chicaco, IL, USA) and STATISTICA for Windows 5.1 (StatSoft Inc., Tulsa, OK, USA).

Response of *L. bullinum* to the inducible defence of *E. octocarinatus*

Experiment 1: The aim of the first experiment was to study the morphological reaction of *L. bullinum* to the inducible defence of *E. octocarinatus*. Six cultures of *L. bullinum* were fed daily with defended (induced) prey, whereas six control cultures received undefended prey. Prey density was not quantified, but was similar in both treatments and was sufficiently high to exclude food-limitation. Therefore, observed differences in cell size should not be because of differences in food supply.

Preliminary experiments had shown that it may take several generations for the size distribution of a *L . bullinum* culture to reach a stable state. Therefore, the inducing conditions were maintained for 2 weeks. After that period, 20 well-fed cells (containing visible food vacuoles) from each culture were starved for 24 h (for better standardization) and subsequently fixed and measured. Measurements included length and width of the cell and of the cell mouth (peristome).

Undefended prey employed in this experiment averaged 82.1 ± 6.61 μm in length and 50.0 ± 5.21 μm in width ($n = 60$), whereas defended prey were 102.4 ± 10.52 μm long and 78.2 ± 9.81 μm wide ($n = 60$; t-test on means of three replicates with 20 measurements each: d.f. = 4, $P < 0.001$ for both length and width).

In a previous paper (Kopp & Tollrian 2003), we have shown that *L. bullinum* raised on undefended *E. octocarinatus* display an intermediate expression of the inducible offence (i.e. they are of intermediate size). However, as these predators represent the smallest (least induced) morph in the present study, we will refer to them as the non-induced morph and to *L. bullinum* raised on defended *E. octocarinatus* as the induced morph. While this terminology is slightly inaccurate, it greatly simplifies discussion.

Effect of the inducible offence on predation rate

Two short-term feeding experiments were designed to test whether the inducible offence of *L. bullinum* is effective in overcoming the induced defence of *E. octocarinatus*.

As raising *L. bullinum* with induced *E. octocarinatus* (as in experiment 1) is very laborious, we instead used non-induced *E. aediculatus* (i.e. a close relative of *E. octocarinatus*) as a substitute food to obtain induced predators. This procedure is justified, because non-induced *E. aediculatus* are similar in size to induced *E. octocarinatus* and *L. bullinum* most likely reacts only to prey size, not to any particular prey species (Kopp & Tollrian 2003). Furthermore, preliminary experiments had shown that the morphological reactions of *L. bullinum* to these two types of prey are very similar (size of *L. bullinum* receiving *E. aediculatus* as food: length 143.0 ± 11.87 μm, width 82.5 ± 14.77 μm ($n = 360$); with defended *E. octocarinatus* as food: length 140.5 ± 11.16 μm, width 84.8 ± 14.05 μm ($n = 360$); MANOVA on means of four replicates: $P > 0.13$). Therefore, *L. bullinum* raised on non-induced *E. aediculatus* (mimicking induced *E. octocarinatus*) will also be called induced.

Lembadion bullinum were obtained from independent stock cultures for each replicate. A culture was assumed to be independent from its parent culture 1 week after inoculation. Well-fed cells with visible food vacuoles were selected 24 h before the experiments and starved in food free medium until exposure to the prey. Subsamples of both predators and prey were measured before the experiments. The duration of the feeding trials was chosen such that the predators did not become satiated (i.e. the vast majority did not consume more than one prey item, although, given enough time, they can easily ingest several; personal observation).

Experiment 2: The first feeding experiment had a 2 × 2 factorial design with two types of prey (induced vs. non-induced), two types of predator (induced vs. non-induced) and eight replicates per treatment. In each trial, 100 *E. octocarinatus* were offered to 100 *L. bullinum* in an individual well of a 12-well tissue culture plate containing 1 mL of medium. After 20 min, feeding was stopped by adding glutaraldehyde and the remaining *E. octocarinatus* were counted. We estimated both the absolute feeding rate (number of prey consumed per predator per hour) and the volume-specific feeding rate (absolute feeding rate divided by mean predator volume).

Undefended *E. octocarinatus* employed in the experiment had a mean length of 80.2 ± 9.78 μm and a mean width of 54.0 ± 7.82 μm ($n = 250$), whereas the mean length of defended *E. octocarinatus* was 98.4 ± 9.31 μm and their mean width 79.2 ± 9.97 μm ($n = 200$).

Experiment 3: In the second feeding experiment, we used only defended prey and offered them to either induced or non-induced *L. bullinum*. In addition to comparing the mean feeding rates of the two predator morphs, this experiment had the aim of assessing how the feeding rate of individual predators is influenced by predator size (cell length and width). This was achieved by using fluorescently labelled prey.

Fluorescent live-staining of prey was obtained by exposing defended *E. octocarinatus* to 1 μg mL^{-1} DAPI

(4′,6-diamidino-2-phenylindole) for 2 h (see Lessard *et al.* 1996; Pfister & Arndt 1998; Kopp & Tollrian 2003). This resulted in a brightly fluorescent nucleus that is easily detected even inside the predator's food vacuoles. In order to allow the prey to recover from the exposure, we started the experiments 2 h after the removal of the stain (achieved by filtration over a 15 μm gauze).

In an attempt to further standardize initial conditions, both predator morphs were fed approximately 1300 non-induced *E. octocarinatus* per mL during the last 48 h leading up to the starvation period preceding the trials. This caused a reduction in the size of the induced morph, but the difference to the non-induced morph remained highly significant (see results).

Each treatment was replicated 11 times. We stained 11 (independently raised) cultures of induced *E. octocarinatus*, divided them into two aliquots each and used each pair of aliquots for one pair of replicates (induced and non-induced predator). In each trial, approximately 4000 *E. octocarinatus* were offered to approximately 100 *L. bullinum* in 1 mL of medium using 6-well tissue culture plates. For each predator, we recorded length, width and the number of fluorescent food vacuoles. The induced prey employed in the experiment averaged 98.5 ± 9.74 μm in length and 78.9 ± 7.70 μm in width ($n = 1100$).

In order to analyse the relationship between cell dimensions and individual feeding rate we used multiple logistic regression. For each analysis, the individual data from all replicates were pooled. Length and width were entered as independent variables. The logistic regression model then predicts the probability that a cell of given dimensions will consume one or more prey items during the experiment. Pooling the classes with one or more consumed prey is a negligible simplification, because less than 4% of the predators consumed more than one prey.

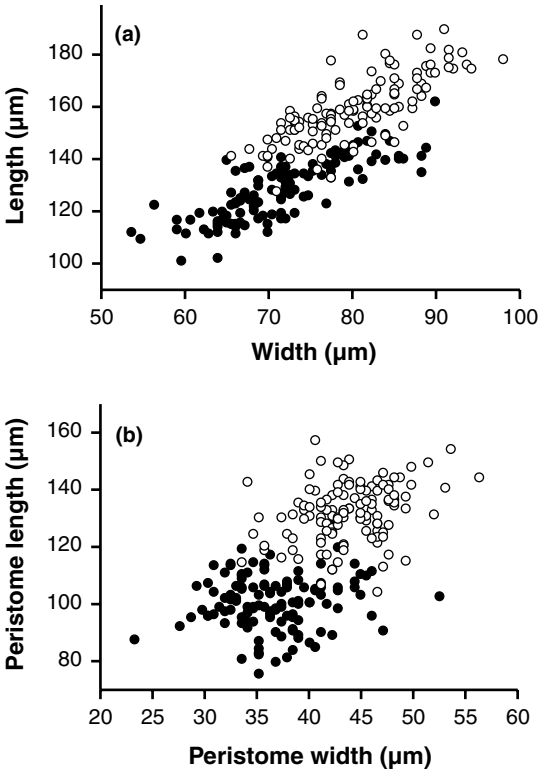

Figure 1 The morphological reaction of *Lembadion bullinum* to the inducible defence of its prey *Euplotes octocarinatus* in experiment 1. The inducible defence of *E. octocarinatus* induces an increase in the cell size of *L. bullinum* (i.e. *L. bullinum* expresses an inducible offence). The figures show morphometric data of two *L. bullinum* morphs: (a) cell dimensions and (b) dimensions of the cell mouth (peristome). The non-induced *L. bullinum* morph (filled circles) was raised on undefended prey, whereas the induced morph (open circles) was raised on defended prey.

RESULTS

Response of *L. bullinum* to the inducible defence of *E. octocarinatus*

Experiment 1: Feeding on induced prey in turn induced *L. bullinum* to increase in size: *L. bullinum* raised on defended *E. octocarinatus* were significantly larger and had a larger peristome than *L. bullinum* raised on undefended prey (MANOVA on replicate means: $P < 0.001$; *post hoc* Student–Neumann–Keuls tests for each of the individual variables – length, width, peristome length and peristome width: $P < 0.001$; Fig. 1, Table 1). In both predator morphs, cell length was positively correlated with peristome length (non-induced morph: $R^2 = 0.81$, induced morph: $R^2 = 0.88$, $P < 0.001$ for both), and cell width was positively correlated with peristome width (non-induced morph: $R^2 = 0.47$, induced morph: $R^2 = 0.39$, $P < 0.001$ for both).

Effect of the inducible offence on predation rate

Experiment 2: Both the defence of the prey and the offence of the predator had a significant effect on absolute feeding rate of *L. bullinum,* with no significant interaction between the two factors (Fig. 2a, Table 2). Undefended *E. octocarinatus* were more vulnerable to predation than defended ones, and induced *L. bullinum* consumed more prey than non-induced ones. However, the offence of *L. bullinum* only partially offset the defence of *E. octocarinatus*. The feeding rate of the induced morph on defended prey was lower than the feeding rate of the non-induced morph on undefended prey. Volume-specific feeding rate was influenced significantly only by the defence of the prey, not by the offence of the predator (Fig. 2b, Table 2). In other words, the effect of the offence on absolute feeding rate was not large enough to

Table 1 Morphometric data for the non-induced and induced morph of *Lembadion bullinum* in experiment 1. The non-induced morph was raised with undefended *Euplotes octocarinatus* as prey and the induced morph was raised with defended *E. octocarinatus*

	Non-induced morph	Induced morph
Length	128.5 ± 11.77	159.1 ± 12.59
Width	71.7 ± 7.63	80.3 ± 6.64
Peristome length	100.6 ± 9.52	132.1 ± 10.12
Peristome width	36.8 ± 4.59	43.9 ± 4.09

Data are means ± SD from the pooled data sets in μm ($n = 120$). For statistical analysis, see text.

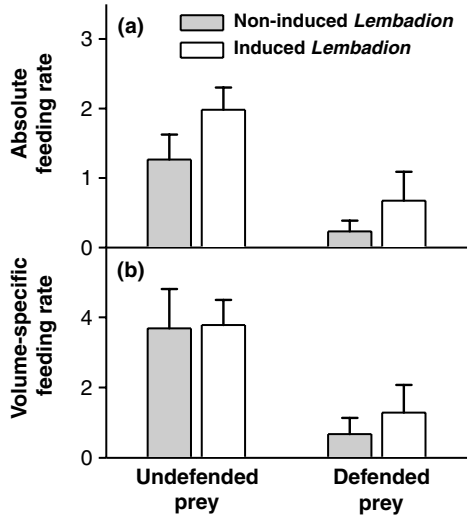

Figure 2 Effect of *Lembadion bullinum*'s counteraction to the inducible prey defence: feeding rates of non-induced and induced *L. bullinum* preying upon defended and undefended *Euplotes octocarinatus* in experiment 2 (means ± SD of eight replicates): (a) absolute feeding rates (ingested prey per predator per hour) and (b) volume-specific feeding rates (ingested prey per 10^6 μm^3 predator volume per hour). The offence of *L. bullinum* increased absolute feeding rate, but had no significant influence on volume-specific feeding rate. In contrast, the defence of *E. octocarinatus* decreased both absolute and volume-specific feeding rate. See Table 2 for statistical analysis.

overcompensate for the increase in predator volume. However, the low *P*-value for the interaction effect (Table 2) might suggest that the inducible offence tends to increase volume-specific feeding rate if the prey is defended. Non-induced *L. bullinum* were on average 120.0 ± 9.78 μm long and 73.4 ± 7.52 μm wide ($n = 240$). The corresponding values for the induced morph were 139.6 ± 10.91 for length and 84.1 ± 8.66 μm for width ($n = 240$).

Experiment 3: In contrast to the defended-prey treatments of the previous experiment, experiment 3 showed hardly any difference between absolute feeding rates of the two morphs. The non-induced predators consumed on average 0.21 ± 0.080 prey per hour, whereas the induced predators achieved a value of 0.23 ± 0.120 (paired *t*-tests on the replicate means, d.f. = 10, $P > 0.45$). Similarly, there was no significant difference for volume-specific feeding rates (non-induced morph 4.51 ± 1.774 ingested prey per 10^6 μm^3 predator volume per hour, induced morph 4.21 ± 1.821, paired *t*-test, d.f. = 10, $P > 0.45$). Compared with the respective treatments of experiment 2, mean numbers of prey captured per predator and time were lower in experiment 3, although prey density was 40-fold higher. Therefore, success rate (number of prey captured per available prey) in both morphs was considerably decreased.

For both morphs, individual feeding rate was positively affected by cell width and negatively affected by cell length (Table 3). The size difference between the two morphs was smaller than in the previous experiments (probably because of the initial feeding of both morphs with non-induced

Table 2 Results of ANOVA on the effects of predator and prey type on absolute and volume-specific feeding rate in experiment 2

	d.f.	MS	*F*	*P*
Absolute feeding rate				
Prey defence	1	5.556	102.048	<0.001
Predator offence	1	1.242	22.805	<0.001
Interaction	1	0.007	0.135	0.716
Error	28	0.054		
Volume-specific feeding rate				
Prey defence	1	31.678	95.206	<0.001
Predator offence	1	0.134	0.402	0.531
Interaction	1	0.946	2.842	0.103
Error	28	0.333		

Post hoc comparisons for the absolute feeding rates using the Student–Neumann–Keuls test indicate significant pairwise differences between all treatments.

Table 3 Results of multiple logistic regression on the effect of predator length and width on absolute feeding rate in experiment 3

	B	SE of *B*	Wald statistic (1 d.f.)	*P*
Non-induced morph ($n = 910$)				
Length	−0.080	0.014	31.069	<0.001
Width	0.197	0.019	106.208	<0.001
Intercept	−6.748	1.441	21.939	<0.001
Induced morph ($n = 1051$)				
Length	−0.052	0.010	27.178	<0.001
Width	0.118	0.014	74.663	<0.001
Intercept	−4.095	1.013	16.344	<0.001

E. octocarinatus, see material and methods), but still highly significant. The non-induced cells averaged 129.6 ± 8.54 μm in length and 78.1 ± 7.66 μm in width, whereas the induced predators reached a mean length of 140.1 ± 11.22 μm and a mean width of 83.5 ± 8.76 μm.

DISCUSSION

Among the current challenges in the study of phenotypic plasticity, Agrawal (2001) has identified the search for reciprocal phenotypic changes in ecological time. Here, we present evidence for this kind of reciprocity in the predator–prey system *L. bullinum–E. octocarinatus*: *L. bullinum* reacts to the inducible defence of *E. octocarinatus* by expressing an inducible offence (or, more precisely, by increasing the degree of induction, see material and methods, experiment 1), that is a plastic increase in cell size and gape size (Fig. 1). This counteraction is the best evidence so far from a predator–prey system of reciprocal phenotypic plasticity in predation-related morphological traits. Wicklow (1997) probably observed a similar response in *L. magnum,* a close relative of *L. bullinum*. In a vernal succession pool, he described a temporal correlation between the occurrence of an enlarged morph of *L. magnum* and inducibly defended *Sterkiella* spec. We suggest that more examples of this kind can be found in systems where phenotypic plasticity is widespread, such as the microbial or metazoan plankton.

Despite the significant morphological effect, the counteraction of *L. bullinum* proved comparatively ineffective against the defence of *E. octocarinatus*. In experiment 2, it lead to an increase in absolute feeding rate of predators feeding on defended prey, but this effect was not strong enough to fully neutralize the protective effect of the prey's defence. Therefore, the defence remained beneficial for the prey, despite the predator's counteraction.

In experiment 3, the inducible offence of *L. bullinum* had no significant effect on absolute feeding rate at all. The difference between the latter two experiments is most likely explained by the extremely low success rate observed in experiment 3 (see results). Feeding by *L. bullinum* may have been inhibited by the staining of the prey or by the extremely high prey densities (i.e. a swarming effect, see Bertram 1978). As the experimental setup was certainly more realistic in experiment 2 (no staining, lower prey density), it seems safe to conclude that the offence of the predator increases absolute feeding rate at least under some conditions.

Most importantly, however, in neither experiment was the effect of the offence strong enough to more than compensate for the increase in mean predator volume, i.e. the offence had no significant influence on volume-specific feeding rate (despite a non-significant trend in experiment 2). Volume-specific feeding rate is an approximate measure of fitness, because it should be roughly proportional to cell division rate. This is because, in order to divide, large cells must assimilate more biomass than small cells. Our results, therefore, imply that the inducible offence, when employed against the defence of *E. octocarinatus*, does not lead to a significant increase in *L. bullinum*'s fitness. Therefore, expressing the offence in this situation does not seem to be adaptive (though not truly maladaptive, either).

The low efficiency of *L. bullinum*'s counteraction might be explained mechanistically by the biometrical results from experiment 3. These results show that (within morphs) feeding rate of *L. bullinum* increases with cell width (supporting the notion of gape-limitation, as cell width is correlated with peristome width) but, unexpectedly, decreases with cell length. As for an explanation of this finding, we can only speculate. Possibly, long cells are less agile and therefore handicapped in some step of the predation process. In any case, the negative effect of length imposes a trade-off on *L. bullinum* that limits its ability to counter the defence of *E. octocarinatus* by an inducible increase in width. Such an increase in width will only lead to an increase in (absolute) feeding rate if it is sufficiently large compared with the correlated increase in length (see Fig. 1). In summary, the defence of *E. octocarinatus* appears to operate in a way that makes it particularly difficult for *L. bullinum* to develop an effective counter-adaptation.

Predator–prey coevolution?

Agrawal (2001) and Wicklow (1997) have suggested that reciprocal phenotypic plasticity might be a result of (potentially diffuse) coevolution. Here, we have shown that the inducible offence of *L. bullinum* may, to some extent, neutralize the defence of *E. octocarinatus*, but this effect is comparatively small and does not lead to a significant increase in the predator's fitness. Therefore, our data do not support the hypothesis that the inducible offence of *L. bullinum* is a coevolutionary adaptation to inducible prey defences.

However, it is too early to reject the hypothesis of diffuse coevolution completely. First, no data are available concerning the effect of the offence against the inducible defences of other prey species. Second, in a truly coevolutionary situation, the counteraction of *L. bullinum* might in fact have been adaptive in the past. In the meantime, however, *E. octocarinatus* could have improved its defence, for example by investing primarily in cell width, which poses the greatest problems for *L. bullinum* (see above). In other words, *E. octocarinatus* might be currently one step ahead of *L. bullinum* in a coevolutionary arms race. Such arms races, however, cannot be detected by simple feeding experiments.

In summary, although the present study does not yield positive evidence for the coevolution hypothesis, more data are needed for a definite assessment. Future work should focus on the selection pressures exerted on *L. bullinum* and on

the relative contribution by inducible defences. For example, it would be desirable to test the benefits the inducible offence provides against other inducibly defended prey and to analyse prey size variability and its sources in the field. The study of reciprocal phenotypic plasticity promises to yield new insights into the ecology and evolution of predator–prey interactions (see Adler & Grünbaum 1999; Lima 2002).

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