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An investigation of predator-induced defence responses in ciliated protozoa

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Abstract
Predator-induced defence responses have been reported in 15 species of ciliates representing three subclasses: Hypotrichia, Stichotrichia and Hymenostomia. However, the discovery of this phenomenon in such distantly related groups suggests that it could be more widespread within the Ciliophora. In laboratory experiments, we tested 23 potential prey species with a range of potential predators including other ciliates (12 spp.), amoebae (two spp.), rotifers (two spp.), a turbellarian worm (*Stenostomum sphagnetorum*), and an oligochaete worm (*Chaetogaster* sp.). In each experiment, one predator species was incubated for 24 h with one potential prey species and the latter was examined for evidence of a defence response. One new example of predator-induced morphological change was recorded (*Euplotes viridis*) and detailed observations were made for one poorly known example (*E. eurystomus*). Both species significantly increased their width (by about 35% and 23%, respectively) in the presence of the turbellarian worm *S. sphagnetorum*. An induced life cycle change was recorded for the first time among hypotrichs with *E. muscorum* exhibiting significantly increased rates of encystation in the presence of the ciliate predators *Dileptus anser* and *Spathidium* sp. Finally, *Euplotes patella*, *Euplotes* sp. and *Stylonychia pustulata*, which are usually regarded as omnivorous rather than predatory ciliates, all induced morphological change in *Colpidium kleini*, the *C. kleini* cells becoming significantly shorter and wider. No examples of induced defence response were found among groups other than hypotrichs and hymenostomes.

Keywords: *Ciliates, induced morphological defence, predator, prey*

Introduction

Predator-induced defences caused by chemical or mechanical stimuli are known to occur in a variety of different aquatic prey organisms. The reaction to the threat of predation can consist of morphological, chemical or behavioural changes or can be related to life history. Morphological changes have been recorded in a wide range of aquatic organisms from a variety of different ecosystems, e.g. amphibians (Smith and Van Buskirk 1995), fishes (Brönmark and Miner 1992), invertebrates such as bryozoans, corals, cladocerans and rotifers (reviewed by Havel 1987; Dodson 1989; Harvell 1990), pelagic algae (Van Donk

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and Lüring 1995), cyanobacteria (Fiałkowska and Pajdak-Stós 1997), and ciliate protozoa (for reviews see Wicklow 1997; Kuhlmann et al. 1999).

Ciliated protozoa are useful models for testing different aspects of the inducible defences because of their short life cycles, the possibility of carrying out many experiments and repetitions in a short time and the relatively low cost of culture maintenance. Until now, predator-induced defence responses have been reported in 15 ciliate species representing six genera and three subclasses: Hypotrichia (two genera), Stichotrichia (two genera) and Hymenostomatia (two genera). The aim of this study was to determine whether defence responses can be induced in a wider variety of ciliates and/or by a wider variety of predators than is currently recognized. The types of defence responses investigated were morphological, behavioural and those related to life history.

Material and methods

Cultures—general conditions

The organisms used in the study, which comprised ciliates (35 spp.), amoebae (two spp.), rotifers (two spp.), a turbellarian worm (one sp.), and an oligochaete worm (one sp.), were obtained either from Sciento (Manchester, UK) or from the culture collection maintained by the Department of Hydrobiology (Jagiellonian University, Poland). A complete list of the organisms, their origin and the food with which they were maintained is given in Table I.

All organisms were cultivated separately in 5-cm diameter Petri dishes using Żywiec mineral water (Żywiec Zdrój S.A, Węgierska Górka, Poland) as the growth medium. All cultures were fed with an appropriate food species every 2 days. Cultures were kept in the dark at 20°C ($\pm$1°C) in a Versatile Environmental Test Chamber (Sanyo MLR-350, Japan).

Preliminary experiments

Because certain combinations of predator and prey were being tested for the first time, and since the predatory threat was unknown for certain prey, preliminary experiments were carried out in order to determine appropriate predator/prey ratios for use in the main experiments. The aim was to find ratios that give the maximum chance of inducing defence responses while allowing at least 50 prey cells to survive after 24 h of incubation with the predator. The densities of prey and predators varied depending on species, efficiency of predator and the real predatory threat. The densities of predators and prey were sometimes higher than those observed in nature in order to maximize the speed and magnitude of the defence response.

A total of 35 ciliate species was used in the experiments. Eleven of these were the potential prey for eight predatory ciliates as well for turbellarian and oligochaete worms in the main experiment. *Euplotes patella* was treated once as prey and once as predator in independent sets of experiments. The other 17 species of ciliates were confronted only with predatory ciliates, amoebae, rotifers, turbellarian, and oligochaete worms in an additional experiment.

Main experiment

Predators were starved for 2 days prior to each experiment. Prey organisms were fed the day before, but not during, the experiments. Therefore, no alternative food source was available to the predators during the experiments. All experiments were carried out using

Table I. List of ciliates and other organisms used in predator/prey investigations.

Species	Origin	Fed on
Ciliates		
Amphileptus pleurosigma	Sciento (Manchester, UK)	c
Aspidisca sp.	Sciento (Manchester, UK)	b
Bursaria truncatella	Sciento (Manchester, UK)	f, c
Chilodonella uncinata	Jagiellonian University	b
Coleps hirtus	Jagiellonian University	f
Colpidium colpoda	Jagiellonian University	b
Colpidium kleini	Jagiellonian University	b
Colpidium striatum	Sciento (Manchester, UK)	b
Colpoda cucullus	Sciento (Manchester, UK)	b
Colpoda steinii	Sciento (Manchester, UK)	b
Dexiostoma (Colpidium) campylum	Jagiellonian University	b
Dileptus anser	Sciento (Manchester, UK)	c
Euplotes eurystomus	Jagiellonian University	f
Euplotes muscorum	Jagiellonian University	f
Euplotes octocarinatus	Jagiellonian University	f
Euplotes patella*	Sciento (Manchester, UK)	f
Euplotes viridis	Sciento (Manchester, UK)	f
Euplotes sp.	Jagiellonian University	f
Frontonia leucas	Sciento (Manchester, UK)	b, f
Furgasonia sp.	Jagiellonian University	cy
Glaucoma sp.	Sciento (Manchester, UK)	b
Homalozoon vermiculare	Jagiellonian University	c
Lacrymaria olor	Sciento (Manchester, UK)	c
Lembadion bullinum	Jagiellonian University	c
Oxytricha sp.	Sciento (Manchester, UK)	f
Paramecium bursaria	Sciento (Manchester, UK)	b
Podophyra collini	Sciento (Manchester, UK)	c
Pseudomicrothorax dubius	Jagiellonian University	cy
***Spathidium* sp.**	Sciento (Manchester, UK)	b
Stentor coeruleus	Sciento (Manchester, UK)	b, c, f
Stylonychia mytilus	Jagiellonian University	f
Stylonychia pustulata	Jagiellonian University	f
Tachysoma sp.	Sciento (Manchester, UK)	f
Tetrahymena pyriformis	Sciento (Manchester, UK)	b
Vorticella sp.	Sciento (Manchester, UK)	b
Amoebae		
Amoeba proteus	Sciento (Manchester, UK)	c
Chaos carolinense	Sciento (Manchester, UK)	c
Worms		
Stenostomum sphagnetorum (turbellarian worm)	Jagiellonian University	c
Chaetogaster sp. (oligochaete worm)	Jagiellonian University	c
Rotifers		
Brachionus plicatilis	Sciento (Manchester, UK)	c
Philodina sp.	Sciento (Manchester, UK)	c

Predators are in bold; *predator and prey. b, bacteria; c, ciliate; cy, cyanobacteria; f, flagellate.

24-well, flat-bottom tissue culture plates (Renner GmbH, Germany). Each well was 16 mm in diameter and had a maximum volume of 2.5 ml.

For each experiment a 1 ml aliquot of prey ciliate culture was added to a randomly chosen well. The prey ciliate concentration was adjusted, the optimal concentration having

been determined during the preliminary experiments. A single predator species was added to the well, the number of predator individuals being calculated according to the predator/prey ratios determined in the preliminary experiments. Control wells contained prey organisms only. The numbers of predators and prey used in each well are shown in Table II. Three replicates were prepared for each treatment. The plates were incubated for 24 h at 20°C (±1°C) in the dark.

After 24 h acid Lugol's was added to each well to a final concentration of about 1%. After a few hours the lengths and widths of 50 well-fixed prey cells were measured using an inverted microscope (Olympus IMT-2) and computer scanning system for image analysis (Computer Scanning Systems Ltd, Warsaw, Poland). Total numbers of predators and prey in each well were also counted in order to assess the predation pressure for each combination.

Statistical analysis

The mean length and width of the prey ciliates was calculated for each treatment. The data were analysed using the one-way ANOVA and Tukey's a posteriori test from the Statistica packet for IBM computers. In the case of heterogeneity of variances the probability P was estimated using a randomization program (RT version 1.02; Manly 1994). Treatments were compared with the control using the Student's t test. In the case of multiple repetitions the Bonferroni correction was taken. The cell size distribution of the prey ciliates was calculated for each treatment in order to determine whether predators were selecting small (versus large) prey cells.

Results

The results of those predation experiments for which detailed analysis of the prey could be performed are shown in Tables II–IV. The main inducible effect observed in the prey was a change in cell morphology. In the case of *Euplotes muscorum*, however, an increase in encystation rate was recorded when incubated with either *Dileptus* or *Spathidium* (Figure 1). Instances of statistically significant changes in prey morphology and life history are indicated in Table II.

The principal predator-induced morphological response observed during these experiments were changes in prey cell length and width. From Table III it can be seen that for most prey species significant changes in cell dimensions were induced by at least one predator. Four prey species, however, failed to exhibit any change in length or width in any of the treatments, namely *Chilodonella uncinata*, *Coleps hirtus*, *Paramecium bursaria*, and *Tachysoma* sp. Among the predators, only *Spathidium* and *Dileptus* failed to induce a change in cell length or width in any of the prey.

The results of the experiment to evaluate the level of predation pressure within each treatment, i.e. the ability of each predator to significantly reduce prey numbers, are shown in Table IV. With the exception of *Euplotes eurystomus*, numbers of each prey species were significantly reduced by at least one predator. Similarly, every predator was able to significantly reduce the numbers of at least one prey species.

The cell size distributions of the prey species were analysed for each treatment. The histograms in Figure 2 show an example of typical size distributions for a prey ciliate *C. kleini*. By comparing the cell size distribution of the control population with that of the population exposed to predation by *Euplotes* sp., it can be seen that the two distributions are

Table II. Experimental design and ANOVA results for prey width changes. Statistically significant effects were not observed with any treatment except those indicated.

Prey	No. of prey in 1 ml	Predator	No. of predator in 1 ml	Statistically significant effect
Euplotes eurystomus	200	*Stenostomum sphagnetorum*	3	$F_{[4,10]}=12{,}03$; $P=0.017$
		Homalozoon vermiculare	5	
		Stylonychia mytilus	5	
		Lembadion bullinum	50	
Euplotes muscorum	200	*Stenostomum sphagnetorum*	2	
		Homalozoon vermiculare	5	
		Stylonychia mytilus	15	
		Lembadion bullinum	15	
		Euplotes sp.	30	
		Spathidium sp.	20	(many cells were encysted)
		Dileptus anser	5	(many cells were encysted)
Euplotes viridis	120	*Stenostomum sphagnetorum*	2	$F_{[5,12]}=57{,}09$; $P<0.0001$
		Homalozoon vermiculare	2	$F_{[5,12]}=21{,}96$; $P<0.0001$
		Stylonychia mytilus	5	
		Spathidium sp.	15	
		Dileptus anser	30	
Colpidium colpoda	600	*Stenostomum sphagnetorum*	2	
		Homalozoon vermiculare	2	
		Stylonychia mytilus	5	
		Lembadion bullinum	30	$F_{[5,12]}=7{,}27$; $P<0.002$
		Euplotes sp.	30	
Colpidium kleini	1000	*Stenostomum sphagnetorum*	5	
		Homalozoon vermiculare	5	
		Stylonychia mytilus	2	
		Lembadion bullinum	30	$F_{[4,10]}=137{,}87$; $P<0.0001$
		Euplotes sp.	30	$F_{[4,10]}=137{,}87$; $P<0.0001$
		Euplotes patella	30	$F_{[3,8]}=56{,}2$; $P<0.0001$
		Stylonychia pustulata	25	$F_{[3,8]}=56{,}2$; $P<0.0001$
Colpidium campylum	1000	*Stenostomum sphagnetorum*	5	
		Homalozoon vermiculare	5	
		Stylonychia mytilus	2	
		Lembadion bullinum	30	
		Euplotes sp.	30	$F_{[5,12]}=5{,}89$; $P<0.006$
		Euplotes patella	30	$F_{[2,6]}=14{,}45$; $P<0.005$
		Stylonychia pustulata	5	$F_{[1,4]}=84{,}74$; $P<0.0008$
Chilodonella uncinata	500	*Stenostomum sphagnetorum*	2	
		Homalozoon vermiculare	2	
		Lembadion bullinum	2	
		Stylonychia pustulata	2	
Coleps hirtus	200	*Stenostomum sphagnetorum*	2	
		Homalozoon vermiculare	5	
		Stylonychia mytilus	3	
		Lembadion bullinum	20	
		Euplotes sp.	10	
		Stylonychia pustulata	20	
Paramecium bursaria	150	*Stenostomum* sp.	5	
		Homalozoon vermiculare	5	
		Stylonychia mytilus	15	
		Lembadion bullinum	15	
Tachysoma sp.	200	*Stenostomum* sp.	3	
		Homalozoon vermiculare	5	
		Stylonychia mytilus	3	
		Lembadion bullinum	50	

Table II. *(Continued)*.

Prey	No. of prey in 1 ml	Predator	No. of predator in 1 ml	Statistically significant effect
Euplotes patella	200	*Stenostomum* sp.	3	
		Homalozoon vermiculare	5	
		Stylonychia mytilus	3	
		Lembadion bullinum	50	

Each treatment contained a different prey/predator combination as indicated; three independent replicates per treatment were made.

essentially similar in shape although the latter is displaced to the right indicating the cells within this population are generally larger than those of the control population. Similar results were obtained for all other predator/prey combinations investigated.

Discussion

Among the four *Euplotes* species examined, one (*E. patella*) remained unaffected by the presence of predators, two (*E. eurystomus* and *E. viridis*) showed a predator-induced morphological defence, and one (*E. muscorum*) had a behavioural response. Although the morphological defence response has been reported previously for *E. eurystomus* (Kuhlmann and Heckmann 1985), that for *E. viridis* is reported here for the first time. Three predators induced the morphological response in *E. viridis*: *Lembadion bullinum*, *Stylonychia mytilus* and *Stenostomum sphagnetorum*. The changes observed were similar to those that occur in other *Euplotes* species and included the development of extended lateral wings and dorsal projections (Kuhlmann and Heckmann 1985). It was noted, however, that the extent of the induced defence in *E. viridis* was at least in part dependent on its nutritional state. Well-fed specimens of *E. viridis* changed both the length and the width of the cell, whereas width changes only were observed in 48 h starved cells. These results are similar to those reported by Wiąckowski and Szkarłat (1996) for *E. octocarinatus*. The length changes in well-fed forms of *Euplotes* may be a result of the large amount of potential energy involved in reconstruction of cell ultrastructure (Jerka-Dziadosz et al. 1987) or be caused by the fact that many more cells in well-fed cultures are likely to be in the synthesis (S) phase or at the beginning of division (G2) phase of the cell cycle. Such cells might be capable of a more flexible morphological change, including increasing their length.

In the case of *E. eurystomus*, only *Stenostomum* induced the morphological changes. This could be explained by the fact that *E. eurystomus* is one of the largest freshwater *Euplotes* species and, of the predators tested, only *Stenostomum* could engulf it easily. Kuhlmann and Heckmann (1985) investigated morphological defence reponses in five *Euplotes* spp. and found that the two largest species (*E. woodruffi* and *E. eurystomus*) either did not change or changed to a markedly less extent than the other species. The hypothesis that predators other than *Stenostomum* failed to induce a defence response in *E. eurystomus* because their small and/or insufficiently flexible mouth structure prevented them from engulfing this particular prey organism needs to be tested using other predators.

It is also noteworthy that although *Stenostomum* induced morphological changes in *E. eurystomus* and *E. viridis*, it did not significantly reduce numbers of either of them. A similar observation was made by Kusch (1995) for *E. daidaleos*. The most likely explanation is that *Stenostomum* produces significantly more of its karimone (S-factor) than other predators thus inducing a stronger morphological change in the prey. Previous research has shown

Table III. Probability of significant changes in prey cell length and width after 24 h exposure to various predators.

		Predator species						
Prey species	Parameter	*Stenostomum sphagnetorum*	*Homalozoon vermiculare*	*Lembadion bullinum*	*Euplotes* sp.	*Euplotes patella*	*Stylonychia mytilus*	*Stylonychia pustulata*
Euplotes eurystomus	Length	–	–	–	NT	NT	–	NT
	Width	$P=0.0001$(*)	–	–	NT	NT	–	NT
Euplotes viridis	Length	$P<0.0001$	–	NT	NT	NT	–	NT
	Width	$P<0.0001$	–	NT	NT	NT	$P<0.0001$	NT
Euplotes patella	Length	$P=0.0013$	–	–	NT	NT	–	NT
	Width	$P<0.0001$	$P<0.0001$	–	NT	NT	–	NT
Colpidium colpoda	Length	–	–	$P=0.025$(*)	–	NT	–	NT
	Width	–	–	$P=0.002$	–	NT	–	NT
Colpidium kleini	Length	–	–	$P=0.001$	–	$P=0.003$(*)	–	$P=0.003$(*)
	Width	–	–	$P<0.0001$	$P<0.0001$	$P<0.0001$	–	$P<0.0001$
Colpidium campylum	Length	–	–	–	–	–	–	–
	Width	–	–	–	$P=0.005$	$P=0.005$	–	$P=0.0008$

In the case of heterogeneity of variance probability (P) was estimated using a randomization method (*); –, no significant changes; NT, not tested. Only those predators which induced significant differences between treatment and control (using Tukey's test or Student's t test with Bonferroni correction) are listed. Other predator/prey combinations were either not tested or failed to produce significant differences.

Table IV. ANOVA results for the number of different prey cells alive after 24 h exposure to various predators compared to controls incubated in the absence of predators.

Prey species	Exp.	ANOVA results/ probability	Predator species								
			Stenostomum	*Homalozoon vermiculare*	*Stylonychia mytilus*	*Lembadion bullinum*	*Euplotes* sp.	*Euplotes patella*	*Stylonychia pustulata*	*Spathidium* sp.	*Dileptus anser*
Euplotes eurystomus	1	$F_{[4,10]}=0.62$; $P=0.66$	−	−	−	−	NT	NT	NT	NT	NT
Euplotes viridis	2	$F_{[5,12]}=19.65$; $P<0.0001$	−	+	+	NT	NT	NT	NT	+	−
Euplotes muscorum	3	$F_{[7,16]}=26.53$[a]; $P<0.0001$	+	−	+	−	−	NT	NT	+	+
		$F_{[7,16]}=19.07$[b]; $P<0.0001$	−	−	−	−	−	NT	NT	+	+
Colpidium colpoda	4	$F_{[5,12]}=15.19$; $P=0.0001$	+	+	+	+	−	NT	NT	NT	NT
Colpidium kleini	5	$F_{[4,10]}=32.11$; $P<0.0001$	+	+	+[c]	+	+	+[c]	+[c]	NT	NT
Colpidium campylum	6	$F_{[5,12]}=116.97$; $P<0.0001$	+	+	+	+	+[d]	+[d]	NT	NT	NT
Chilodonella uncinata	7	$F_{[4,10]}=12.69$; $P=0.0006$	−	−	NT	+	NT	NT	−	NT	NT
Coleps hirtus	8	$F_{[6,14]}=25.06$; $P<0.0001$	+	−	+	−	+	NT	NT	−	NT
Paramecium bursaria	9	$F_{[4,10]}=14.53$; $P=0.0004$	−	+	−	−	NT	NT	NT	NT	NT
Tachysoma	10	$F_{[4,10]}=75.07$; $P=0.0001$	+	−	+	+	NT	NT	NT	NT	NT
Euplotes patella	11	$F_{[4,10]}=5.55$; $P=0.0128$	−	−	+	−	NT	NT	NT	NT	NT

Exp., experiment; NT, not tested; +, significant reduction in prey cell number; −, no significant reduction in prey cell number. [a]Results concerning the free living cells; [b]results concerning the number of cysts; [c]probability: $F_{[3,8]}=41.76$, $P<0.0001$; [d]probability: $F_{[2,6]}=50.46$, $P=0.0002$.

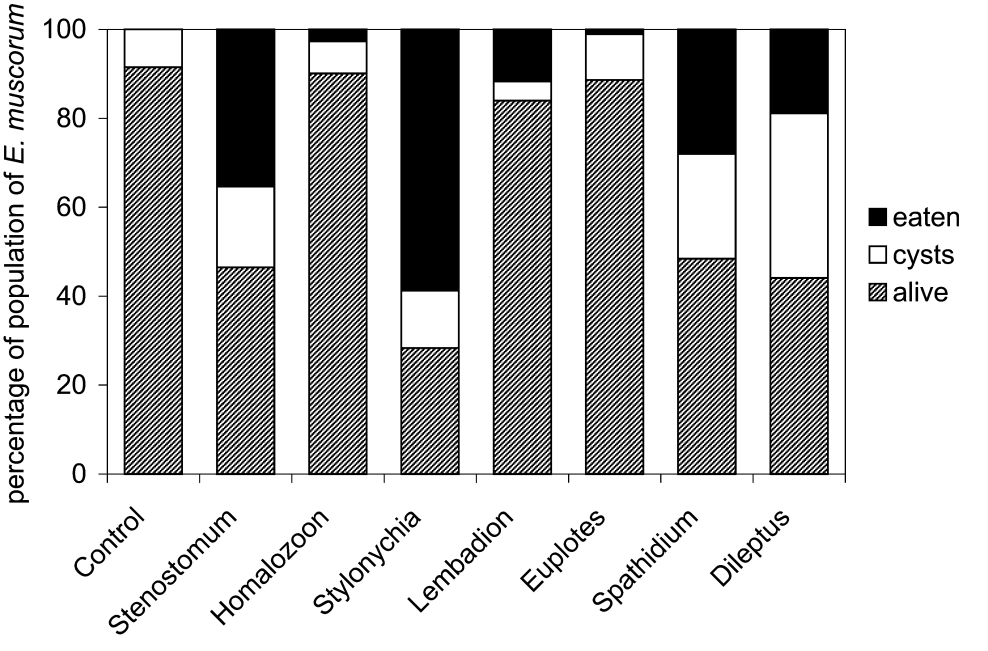

Figure 1. Effect of different predators on *Euplotes muscorum*. Results are means of three independent replicates for each treatment; "eaten", *E. muscorum* cells ingested by predators; "cysts", encysted *E. muscorum* cells; "alive", uningested, trophic *E. muscorum* cells.

that *Stenostomum* produces up to 10 times more of its karimone than, for example, the ciliate *Lembadion* (Kusch and Heckmann 1992; Kusch 1993).

The fourth species of *Euplotes* investigated, *E. muscorum*, did not exhibit induced morphological change. However, in treatments with *Dileptus* and *Spathidium*, significantly higher rates of encystation of *E. muscorum* were recorded compared both to other treatments and to the control. Since the natural habitat for *E. muscorum* is wet mosses rather than open freshwater, the high propensity for encystation in this species may be due to its relatively unstable habitat (Fauré-Fremiet et al. 1954). Furthermore, it should be noted that encystation is sometimes caused by factors other than temperature extremes and lack of moisture. The amoeba *Chaos carolinense* and the ciliate *Didinium nasutum*, for example, are known to form cysts when population densities, and therefore metabolite concentrations, are high (Jackson and Berger 1985). However, this was not the case in the current experiments. The hypothesis that cyst formation is induced by predators and has defensive significance is supported by the fact that various predacious *Spathidium* and *Dileptus* species often occur in the same habitats as *E. muscorum*. Furthermore, the cysts adhere strongly to surfaces so they are probably unavailable to these types of predator, and their cyst wall may make them resistant to digestion. Further research is required in order to confirm these two suggestions.

Morphological changes in *Colpidium kleini* induced by *Lembadion bullinum* have previously been reported (Fyda 1998; Fyda and Wiąckowski 1998). In the current experiments two other ciliates were found to induce similar changes in *Colpidium*, namely *Euplotes* sp. and *Stylonychia pustulata*, both of which are omnivorous rather than typical predatory ciliates. Both species, as well as *Stylonychia mytilus*, significantly decreased the numbers of *C. kleini* cells, but only *Euplotes* sp. and *S. pustulata* induced effective shape changes.

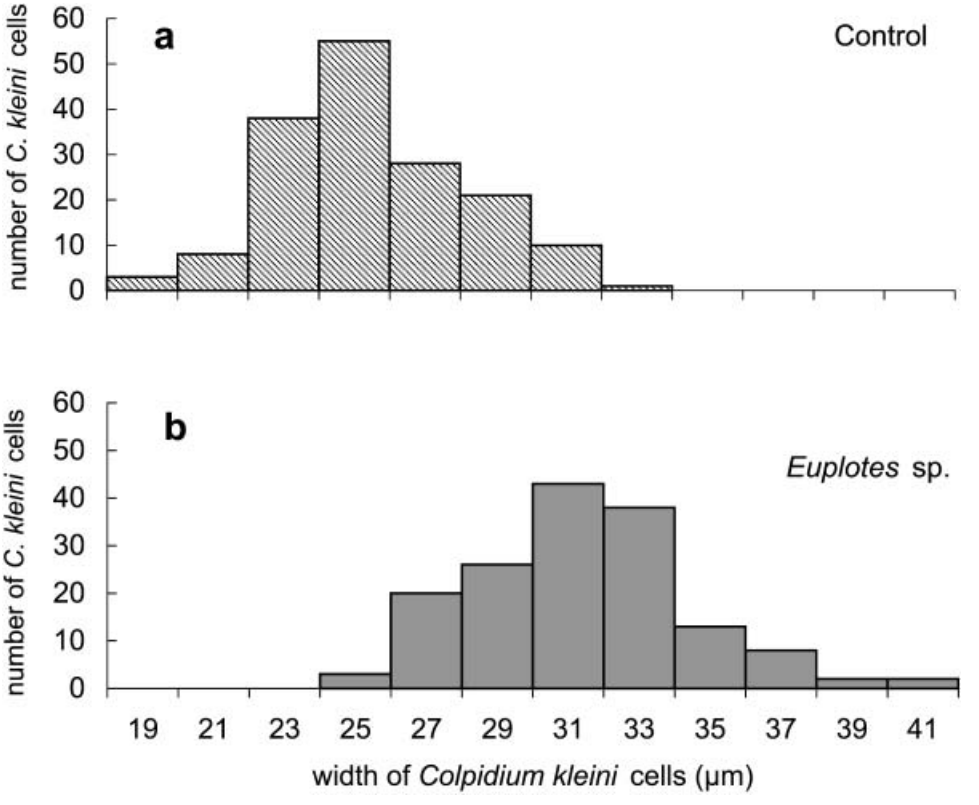

Figure 2. Prey width distributions of *Colpidium kleini* from the experiment described in Table II, i.e. (a) in the absence of predators; (b) in the presence of *Euplotes* sp. Each distribution was calculated after 24 h and represents the mean of three replicates (n=150).

Coleps hirtus is an unusual prey ciliate. Its body is covered in calcified armoured plates and it has three or four prominent spines at the posterior end. It would therefore seem to be well protected against predation. In our experiments we did not observe any induced morphological changes in *C. hirtus*, although in the presence of *Stylonychia mytilus*, *Euplotes* sp. and *Stenostomum* its density was reduced. *Coleps* is sometimes observed in very high densities in natural habitats but is eaten by very few protists or metazoans, one exception being the crustacean *Daphnia pulex* (Jack and Gilbert 1993, 1997; Sanders and Wickham 1993; Mohr and Adrian 2000). It is thought unlikely that the possession of armour plating and spines is the only reason for the resistance to predation in *Coleps* and it has been suggested that chemical defences might also be involved (Dodson 1989). The production of defensive toxins, however, is often energetically very costly so it is unusual for this phenomenon to occur together with morphological defences. Schwartz et al. (1983), for example, found only one small cladoceran, *Scapholeberis*, which uses both chemical and morphological defences. Among ciliates, chemical defences are often associated with those which have pigmented granules as such as *Stentor coeruleus* (Miyake et al. 2001) or pigmented extrusomes such as *Blepharisma japonicum* (Harumoto et al. 1998). It has been suggested that telotrochs of the peritrich ciliate *Campanella umbellaria* may also employ chemical defences since, compared to other potential prey of similar size, they are far less

likely to be captured and eaten by predators such as *Daphnia*, *Mesocyclops* or *Asplanchna* (Jack and Gilbert 1993, 1997).

None of the litostomes in the current experiments, i.e. *Homalozoon*, *Dileptu*s and *Spathidium*, induced morphological changes in any of the prey ciliates tested. This can be explained by the mode of prey capture used by these direct interceptor predators. The cytostome of prostomatids is furnished with toxicysts that paralyse their prey. The benefits to the predator include the fact that the prey is less likely to escape and, if necessary, can be eaten in parts (Seshachar et al. 1971; Harumoto and Miyake 1991). Consequently *Dileptus*, for example, can feed on a wide range of metazoan as well as protistan prey (Brown and Jenkins 1962) while *Homalozoon* can feed on *Stentor* and rotifers (Weinreb 1955). In such cases, induced morphological changes would be seemingly useless for the prey. Our findings contrast with those of Kuhlmann and Heckmann (1985, 1994) who reported morphological changes in *Euplotes* spp. induced by *Dilept*us. The predator: prey ratio in their experiments, however, was 1:1 whereas in ours it was 1:40 for *E. muscorum* and 1:4 for *E. viridis*. This suggests that the prey response for predators armed with toxicysts is weak and that a very high predator threat is necessary in order to induce a morphological response.

Predator-induced defences are ecologically interesting feedback mechanisms that result in damping population oscillations of both prey and predators (Heckmann 1995) and their development could cause the coevolution of predators. Our results support the suggestion of Görtz et al. (1999) that predator-induced phenotypes, which are known for several ciliate species, may be more common than previously supposed among some groups, namely hypotrichs, stichotrichs and hymenostomes. However, we failed to find evidence of induced defence responses among any of the other groups investigated.

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