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Benefits of morphological defence demonstrated by direct manipulation in larval dragonflies

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ABSTRACT

Many prey species evolved morphological structures to hold off predators. As morphology and behaviour are frequently entwined, it is very difficult to demonstrate the assumed defence benefit of the morphological traits. Using a novel approach of directly manipulating morphological defence in larval dragonflies, we demonstrated that spines were an effective morphological defence against predatory fish. Our results showed that the survival probability of larval dragonflies being attacked from behind was four-fold higher in larvae possessing spines than in larvae without spines. However, spines were ineffective against attacks from the front. We discuss the relevance of our study for understanding inducible defence.

Keywords: benefits, inducible defence, morphological defence, plasticity, predator.

INTRODUCTION

Predation influences various aspects of the life history and ecology of prey (Kerfoot and Sih, 1987). Several defence mechanisms have evolved – for example, behavioural, chemical and morphological (Edmunds, 1974; Havel, 1987). Some anti-predator defences are induced only when required, because defence is costly and constitutive anti-predator defences have been paid for in advance (Harvell, 1990; DeWitt *et al.*, 1998). Therefore, in environments heterogeneous over time or space, prey have often evolved inducible – that is, phenotypic plastic – defences (see Lively *et al.*, 2000, and references therein). In contrast, permanent defence is predicted in more constant environments.

Morphological defences, including crypsis, armour and spines, are widespread and have been described mainly from protozoans, rotifers and cladocerans (Tollrian and Harvell, 1999). These morphological characteristics are often post-contact defences and are assumed to provide effective protection against predator attacks (Havel, 1987). In dragonflies, abdominal spines presumably offer a protective defence against predatory fishes (Johansson and Samuelsson, 1994; Mikolajewski and Johansson, in press).

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To our knowledge, morphological defences and their benefits have always been investigated by either (1) comparing distantly or closely related species (Rundle and Brönmark, 2001; Mikolajewski and Johansson, in press) or (2) within a species by inducing these defences (see Tollrian and Harvell, 1999). However, by inducing morphological defences, other traits like behaviour are often altered as well. Kusch (1993) and Lüning (1995) reported that differences found in prey vulnerability to predation are due to the induced behavioural modifications rather than to the induced morphological defences. This is also true for comparing related species. They might not only differ in morphology but also in behaviour (DeWitt *et al.*, 1999; Rundle and Brönmark, 2001; Mikolajewski and Johansson, in press). A way to solve this problem is to directly manipulate the trait under study. This can be done by removing the defensive structure and using an experimental design in which behavioural traits are excluded. This approach would decrease the probability of interacting traits, since prey organisms just differ in the modified morphological attribute. This new approach was taken here.

Many larval dragonflies develop prominent abdominal spines (Norling and Sahlén, 1997). In the presence of predatory fish, larval *Leucorrhinia dubia* develop longer lateral and dorsal abdominal spines (Arnquist and Johansson, 1998) that increase handling time by fish (Johansson and Samuelsson, 1994). However, eventually all larvae were eaten regardless of the handling time. By comparing larvae of three *Leucorrhinia* species expressing different spine lengths, Mikolajewski and Johansson (in press) demonstrated that survival probability increases with elongated spine length.

The aims of our study were: (1) to determine whether the back-pointing abdominal spines offer the same survival value independent of the direction of fish attacks; (2) to establish whether dorsal and lateral spines cooperate in protecting the larvae against fish attacks; and (3) to demonstrate that induced longer spines provide a benefit in terms of increased survival.

MATERIAL AND METHODS

We used *Leucorrhinia caudalis* (Charpentier, 1840) as the study organism, since it expresses the longest spines of all species in the genus *Leucorrhinia* (Norling and Sahlén, 1997). It possesses dorsal spines at abdominal segments 3–9 and lateral ones at segments 7–9 (Fig. 1).

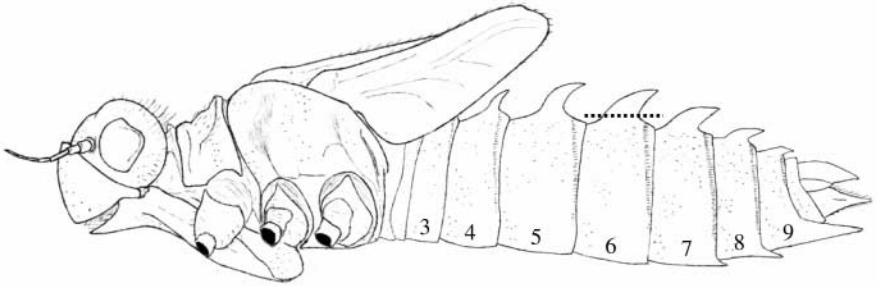

Fig. 1. Lateral view of a larval *Leucorrhinia caudalis*. Numbers mark the abdominal segments 3–9. The dotted line at dorsal abdominal spine 6 indicates where the spines were cut.

We sampled last instar larvae of *L. caudalis* from a lake in Brandenburg, Germany on 19 April 2002. The lake contained predatory fish species like perch, pike and roach. Larvae were brought to the laboratory and kept in aquaria (40 × 20 cm; height 25 cm) filled with 15 litres of non-chlorinated tap water and artificial vegetation. As predators we used perch (*Perca fluviatilis* L.) whose diet includes larval dragonflies (Rask, 1986). Odonate larvae were fed every other day with *Daphnia* and fish were given living chironomids bi-weekly.

In all larvae, head width and length of dorsal spines at abdominal segments 6 and 7 as well as lateral segments 8 and 9 were measured using a dissection microscope. Head width is the most reliable measurement for overall size in odonates (Benke, 1970). Spine length was measured from the tip of the spine to the base of the spine (Johansson and Samuelsson, 1994). Spines were cut at the basal end of the spine as close to the abdomen as possible (Fig. 1). Spines in larval dragonflies are evaginations of the integument (Richards and Richards, 1979). Even though spines are mostly made of cuticule (D.J. Mikolajewski, personal observation), very slight injuries can be caused by cutting spines. Injured larvae could potentially be more attractive to fish than uninjured ones. Therefore, we placed the larvae individually for one day in plastic tubs containing 100 ml tap water and a 3 × 3 cm piece of plastic gauze to ensure complete wound repair. In insects, wound repair by melanization is completed after 5 h (Lai-Fook, 1966). Additionally, we added 10 *Daphnia* as food and only larvae that had eaten at least half of them were used in the following predation experiment. All manipulated larvae did so. Control larvae were treated similarly. Injuries occur very frequently in Odonata in the wild (e.g. Baker and Dixon, 1986), and therefore the cutting of spines is not thought to have a negative impact on the larvae.

The experiment was split into two parts: (1) feeding larvae with lateral and dorsal spines to fish, which attacked them from the front or back; and (2) cutting either all lateral or all dorsal abdominal spines with micro-scissors, and feeding the larvae to perch. Experimental trials were performed in 50-litre aquaria each containing 40 litres of non-chlorinated tap water. After measuring their body length, two perch were placed in each aquarium where they were allowed to acclimatize for 4 days. A total of 30 perch were used in the experiment (mean length 11.8 cm, range 8.9–12.9 cm). Since fishes learn to avoid or elude protective traits after a long period (past experiences), all fishes were just used three times for each trial across all treatments. Additionally, there was always at least one week between re-use of the perch.

A trial started by dropping a larva in the middle of an aquarium. The following variables were recorded: (i) which fish caught the larva, (ii) from which direction the fish attacked (front or back) and (iii) whether the fish rejected the larva after the first attack. The fish attacked the larva immediately after it was dropped in the aquarium. The larva had no opportunity to display anti-predator behaviour or other traits that may be responsible for a successful escape. Larvae that were not attacked immediately by the fish were excluded from the analysis. We also excluded fish attacks towards larvae from the side, since sample size (5 repetitions) was too low. Perch reject prey by spitting it out forcefully over a distance of at least 10–15 cm (personal observation). By taking fish rejection of the larvae as the response variable, we can show directly whether survival probability is increased or decreased according to spine presence/absence.

The data on attacks by perch on larvae are dichotomous – rejected or devoured – and were analysed using generalized linear models with binomial error distributions and a logit link function (Crawley, 1993). The effects of attack direction, spine type, and the interactions with the continuous covariate fish size were examined using log-likelihood ratio tests comparing the deviance of a model including all factors with a model excluding the one being tested. The dichotomous data were analysed with GLIM 3.77, all other data with StatView 5.0.

RESULTS

(1) Since larval head width and the length of all four measured spines were uniformly distributed over the treatments, we did not include these factors in the model for the experiment (MANOVA; $\lambda = 0.864$, $F_{5,38} = 1.199$, $P = 0.3282$). (2) Larval head width and spine length did not differ between the three treatments – larvae possessing dorsal as well as lateral spines, only dorsal spines, and only lateral spines ($\text{MANOVA}_{(\text{all spines} \leftrightarrow \text{only dorsal spines})}$; $\lambda = 0.961$, $F_{3,72} = 0.965$, $P = 0.414$; $\text{MANOVA}_{(\text{all spines} \leftrightarrow \text{only lateral spines})}$; $\lambda = 0.925$, $F_{3,75} = 2.032$, $P = 0.116$). Therefore, we did not include these two factors in our model for the experiment.

In the first part of the study, we found a significant difference in survival probability after an attack from the front or behind (test of full model: $\chi^2 = 30.50$, d.f. = 3, $P < 0.001$) (Table 1). After an attack from behind, perch rejected larvae of *L. caudalis* more frequently (80.77%, $n = 26$) than after an attack from the front (16.67%, $n = 18$).

We included only attacks from behind in the second part of the study (see above). Larvae with just lateral or dorsal spines were rejected significantly less often (test of full model: $\chi^2 = 29.83$, d.f. = 5, $P < 0.001$) (Table 1). While ~80% of all larvae with both spine types were rejected, only ~20 % of the larvae possessing only one spine type were spat out (Fig. 2). No difference was found between larvae possessing only dorsal or only lateral spines (Fig. 2). In both analyses, larger fish had less difficulty swallowing a larva than small fish (Table 1, Fig. 3).

Table 1. Results of generalized linear models on (1) the probability of being rejected depending on the direction of fish attack (front/back), and on (2) the probability of being rejected when possessing dorsal and lateral, only dorsal or only lateral spines (spine type), by perch on larval *Leucorrhinia caudalis*

(1) Source	Deviance	LLR	d.f.	*P*
Fish attack direction	60.63	–	43	–
front/back	–	20.56	1	<0.001
fish size	–	11.46	1	<0.001
front/back × fish size	–	0.08	1	>0.100
(2) Source	**Deviance**	**LLR**	**d.f.**	***P***
Spine cooperation	106.28	–	77	–
spine type	–	18.42	2	<0.001
fish size	–	4.26	1	<0.050
spine type × fish size	–	0.90	2	>0.100

Note: Fish size was used as covariate. LLR is the value of the log-likelihood ratio test.

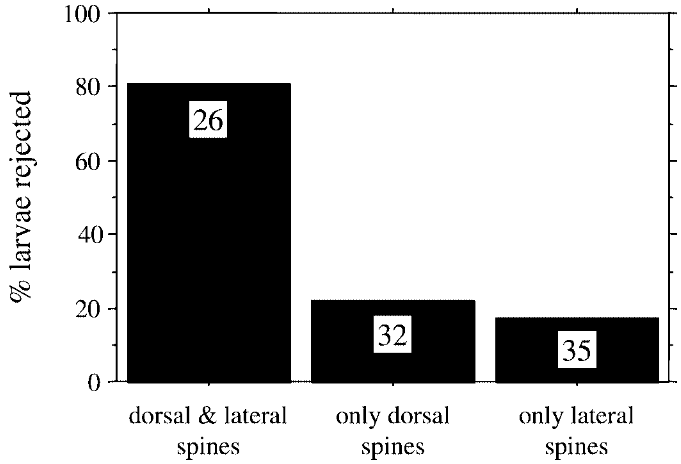

Fig. 2. Percentage of larvae being rejected after fish attacks from behind. X-variable describes which spines were present in larval dragonflies. Numbers in bars are *n*-values.

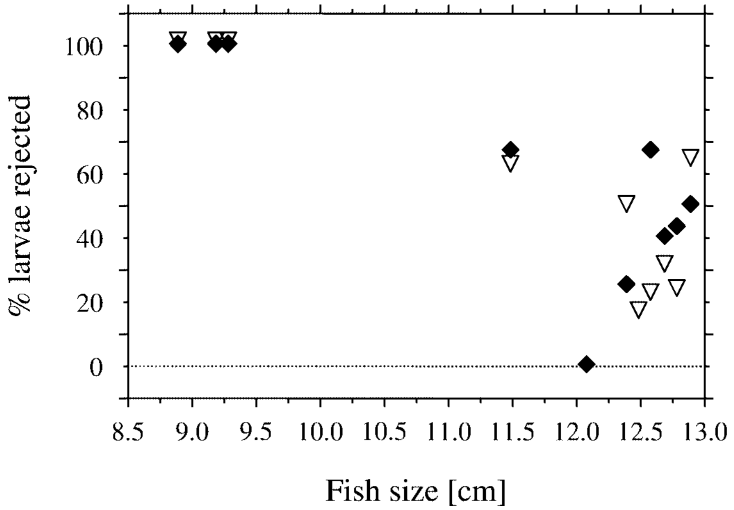

Fig. 3. Percentage of larvae being rejected after fish attacks versus fish size (cm). For both experimental parts, (1) fish attack direction (◆) and (2) spine cooperation (▽), all fishes of one length and treatment were pooled.

DISCUSSION

Our results indicate that even small changes in morphology can alter prey vulnerability. Elongated abdominal spine length increases survival after an encounter with predatory fish, as shown here by direct manipulation of the trait. Larval spines in dragonflies represent a post-contact defence, offering protection when already detected by a predator. When attacked by a fish, larval dragonflies swim away from the predator (D.J. Mikolajewski, personal observation) and are therefore most likely to be attacked from behind. They use jet propulsion to escape over a short distance and hide beneath substrate (Henrikson, 1988).

Fish swim faster than larval dragonflies (Henrikson, 1988; McPeek, 1990), so backwards pointing spines offer a great survival benefit. Here, we used a novel experimental approach, which excluded other traits such as behaviour, to demonstrate that spines offer protection against attacks by fish. This is somewhat different from comparing individuals with induced defence structures such as spines (Johansson and Samuelsson, 1994). In most experiments, traits such as body shape, which might be important for gape-limited predators, are not controlled for. This problem is overcome by the direct manipulation of the defence structure applied here.

Additionally, our response variable – rejection by perch – demonstrated directly that survival probability increased. Rejecting the larvae by spitting them out offers prey organisms a chance to escape by hiding between plants or substrate, since under natural circumstances larval environments are very complex. Additionally, in all cases we observed, larvae rejected by a fish were not attacked again by the same fish in the following few minutes. Fish that rejected larvae did so immediately and inspected larvae again by touching them with their mouth but did not try to predate on them.

Abdominal spines in larval dragonflies are presumed to have several functions, including supporting movement among vegetation and serving as a ratchet to ease the abdomen out of the exuvia (see Corbet, 1999). Johansson and Samuelsson (1994) demonstrated that the handling-time of predatory fishes increases with elongated abdominal spines. Mikolajewski and Johansson (in press) showed that dragonfly species with longer spines are better protected against fish predation than species with short spines. However, our results not only support the assumption that longer spines are an effective protection against fish predators, but also that lateral and dorsal spines interact in offering protection against fish attacks. We did not cut dorsal and lateral spines simultaneously, since no further effects are likely to be detected. Survival probability was decreased by 60% in larvae missing one spine type, hence any further decrease in survival was likely to be relatively limited.

In aquatic insects, only two examples of inducible morphological defence are known (Johansson and Samuelsson, 1994; Dahl and Peckarsky, 2002). During development, larval dragonflies grow out of the prey size range of invertebrate predators against which spines are suggested to offer no protection (Reist, 1980), and reach the selective size range of fish (Diehl, 1993). *Leucorrhinia dubia* acquires more elongated and solid spines in the later instars (Arnquist and Johansson, 1998). This expression of morphological defence during ontogenetic development overlaps with the critical prey size for the smallest perch (Arnquist and Johansson, 1998). Our findings are interesting for understanding inducible defences in other *Leucorrhinia* species. Mikolajewski and Johansson (in press) showed that in larval *Leucorrhinia* species, including *L. dubia*, survival probability after an attack by fish depends on spine length and not on larval size. Additionally, *L. caudalis* do not differ in size from *L. albifrons* (unpublished data), which was the largest species used in their investigation. However, the difference in spine length between larval *L. dubia* of lakes with and without fish (see Johansson and Samuelson, 1994) is relatively smaller than the length of spines we cut in our experiment. Hence, the protection efficiency of the abdominal spines might be overestimated in our experiment. However, the difference in survivorship between larvae with and without spines was four-fold in our experiment. Even small changes in protective morphology should not be underestimated. Neckteeth in second instar *Daphnia*, for example, offer the same protection against a size-limited predator as body size in fifth instar individuals without neckteeth that are twice as big (Tollrian, 1995). The current expression or increase in morphological defence only needs to be protective against the most frequently appearing predator in the particular shared microhabitat and their benefits have to be evaluated with the counterbalancing costs.

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