Predator induction of spine length in larval *Leucorrhinia intacta* (Odonata)

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ABSTRACT

Questions: Do larvae of a dragonfly with a broad habitat distribution have longer abdominal spines when they co-exist with fish, and are these differences the result of phenotypic plasticity?

Hypothesis: Phenotypic plasticity will result in larvae having longer spines when they are exposed to cues from predatory fish.

Organism: Larvae of *Leucorrhinia intacta* (Odonata: Libellulidae).

Research site: Natural ponds and cattle tanks on the E.S. George Reserve in southeast Michigan.

Methods: We compared the morphology of larvae collected from two natural ponds before and after a drought resulted in the extirpation of fish from one pond. We also compared spine morphology of larvae reared in an experiment where they were either exposed to caged fish or empty cages. Finally, we use a phylogeny for this genus to begin reconstructing the evolutionary history of plasticity and spine morphology within *Leucorrhinia*.

Results: Larvae collected from ponds with fish present had longer spines than larvae collected from ponds without fish. In the experiment, exposure to fish resulted in longer spines for some but not all of the spines measured. These results indicate that at least some of the variation in spine length is the result of plasticity. *Leucorrhinia intacta* is not a sister species to a European *Leucorrhinia* in which similar plasticity has been found. Mapping plasticity on to the phylogeny of this genus indicates that either plasticity is ancestral to the two major clades of this genus or that it has arisen independently twice.

Keywords: habitat distribution, *Leucorrhinia*, phenotypic plasticity, predator–prey interactions

INTRODUCTION

Anti-predator morphological defences are common in prey species and can be either fixed or phenotypically plastic in response to varying predator conditions. Induced morphological defences have been documented in numerous animal taxa in response to the presence of predators (see reviews in Havel, 1987; Tollrian and Harvell, 1999; Lass and Spaak, 2003; Benard, 2004). Spatial

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and temporal variation in the presence of predators or in predator type may favour inducible defences over fixed defences (Levins, 1963; Lively, 1986). In this context, prey that arrive at sites without predators can avoid the costs associated with producing defensive structures (DeWitt, 1998; Tollrian and Dodson, 1999; Van Buskirk, 2000; Relyea and Auld, 2004), while prey in sites with alternative predator types may exhibit corresponding predator-specific defences (DeWitt *et al.*, 2000; Relyea, 2004; Benard, 2006).

Morphological plasticity has been demonstrated in larvae of a European dragonfly, *Leucorrhinia dubia* (VanderLinden), in response to cues from predatory fish: fish induce the development of longer spines in both laboratory studies (Arnqvist and Johansson, 1998; Johansson, 2002) and in the field (Johansson and Wahlström, 2002). Longer spines in *L. dubia* may decrease mortality by increasing larval handling times for fish (Johansson and Samuelson, 1994). Studies of other European *Leucorrhinia* species also indicate that spine length affects fish handling behaviour and that individuals or species with long spines are more likely to be rejected by fish (Mikolajewski and Johansson, 2004; Mikolajewski and Rolff, 2004). Although the advantages of longer spines for survival in the presence of fish can be inferred from these studies, the costs of longer spines include increased larval vulnerabilities to invertebrate predators (Mikolajewski *et al.*, 2006). This cost may be a factor favouring the evolution of morphological plasticity as a means of minimizing the mortality risk associated with a generalist distribution that encompasses habitats with fish and invertebrate top predators.

Leucorrhinia is a holarctic genus that includes seven Palearctic species and seven Nearctic species. The phylogeny of *Leucorrhinia* includes three major clades: a group restricted to North America that has six species, a group restricted to Europe with five species, and a clade that split from these two groups earlier that includes one North American and two European species (Hovmöller and Johansson, 2004) (Fig. 1). All species have lateral spines on the margins of abdominal segments VIII and IX (Fig. 2), and these spines vary in length between species. Some species also have prominent spines along the dorsal portion of abdominal segments. Within the North American clade, two species have prominent dorsal spines and four have either no dorsal spines or vestigial dorsal spines. In the European clade, only one species lacks dorsal spines. Plasticity in these spines has only been described in one species of *Leucorrhinia* to date, so whether these spines are fixed or plastic in other species is currently unknown.

In this study, we wished to determine whether the length of dorsal and lateral larval spines in one species within the North American clade, *Leucorrhinia intacta* (Hagen), were fixed or whether they were plastic in response to the presence of fish. This species was chosen because it is the most common *Leucorrhinia* in the region where we worked, and it has a highly generalist distribution, occurring frequently in lakes and ponds with alternative types of top predators (fish and invertebrates) (S.J. McCauley *et al.*, unpublished data). It also possesses prominent dorsal and lateral spines. We assessed correlations between morphological variation in spine length and the presence of fish predators under field conditions and then conducted a mesocosm experiment to identify the mechanism generating observed variation. Finally, we use these data in conjunction with results from previous work in *L. dubia* (Arnqvist and Johansson, 1998; Johansson, 2002; Johansson and Wahlström, 2002) and the phylogeny of Hovmöller and Johansson (2004) to begin examining patterns of morphological plasticity across this genus, and discuss insights this system may have for our understanding of the evolution of phenotypic plasticity.
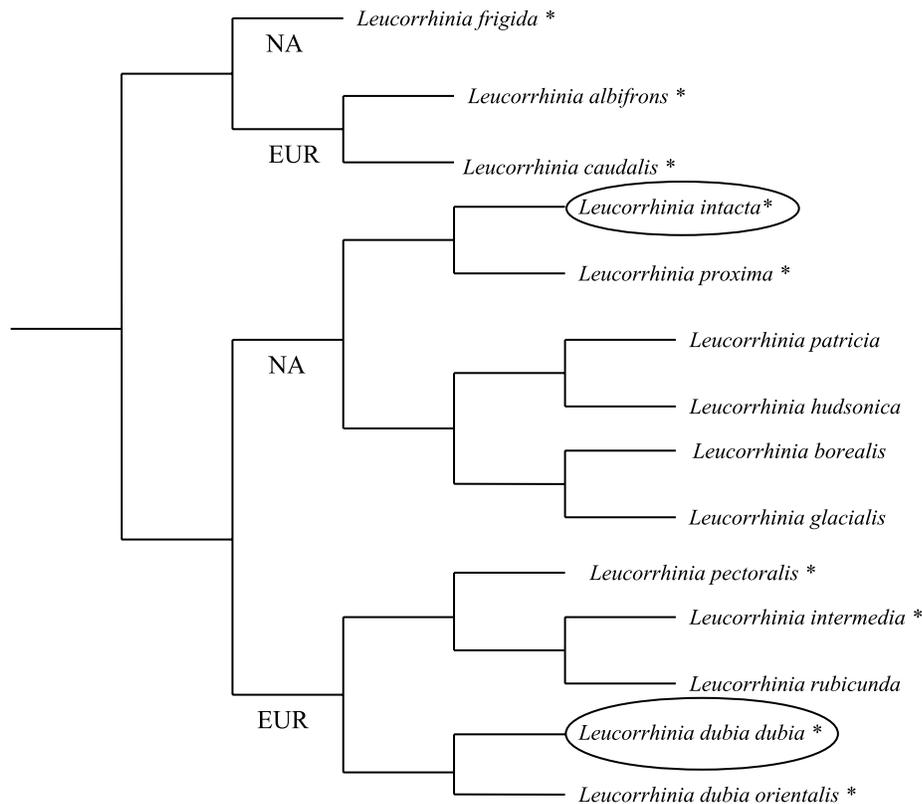
Fig. 1. Phylogeny of *Leucorrhinia* [redrawn from Hovmöller and Johansson (2004) with permission from Elsevier]. The biogeographic distributions of species are indicated as NA = North America, EUR = Europe. Asterisks indicate species with prominent dorsal spines. Species in which plasticity has been found are circled.

METHODS

Study system

Leucorrhinia intacta (Odonata: Anisoptera: Libellulidae) is a widely distributed uni-voltine dragonfly found across the northern, sub-arctic, portion of North America. Dipnet sampling of 22 ponds was conducted in 2000 and 2001 (McCauley, 2005) and used to assess larval densities of this species across top predator environments. Ponds were sampled four times per year, once monthly May through August. Sampling was conducted using D-frame dipnets to collect invertebrates from multiple micro-habitats within the pond. The time spent sampling in each site was adjusted based on pond size. Catch per unit effort of *L. intacta* was calculated for each water-body as the number of individuals collected per person-hour of sampling in each pond. Catch per unit effort was used as an estimate of larval densities. An analysis of variance (ANOVA) was used to compare larval density estimates in water-bodies with alternative top predators, including large-bodied fish (e.g. Centrarchidae), small-bodied fish (e.g. Umbridae, Cyprinidae), and invertebrates (e.g. Aeshnidae).
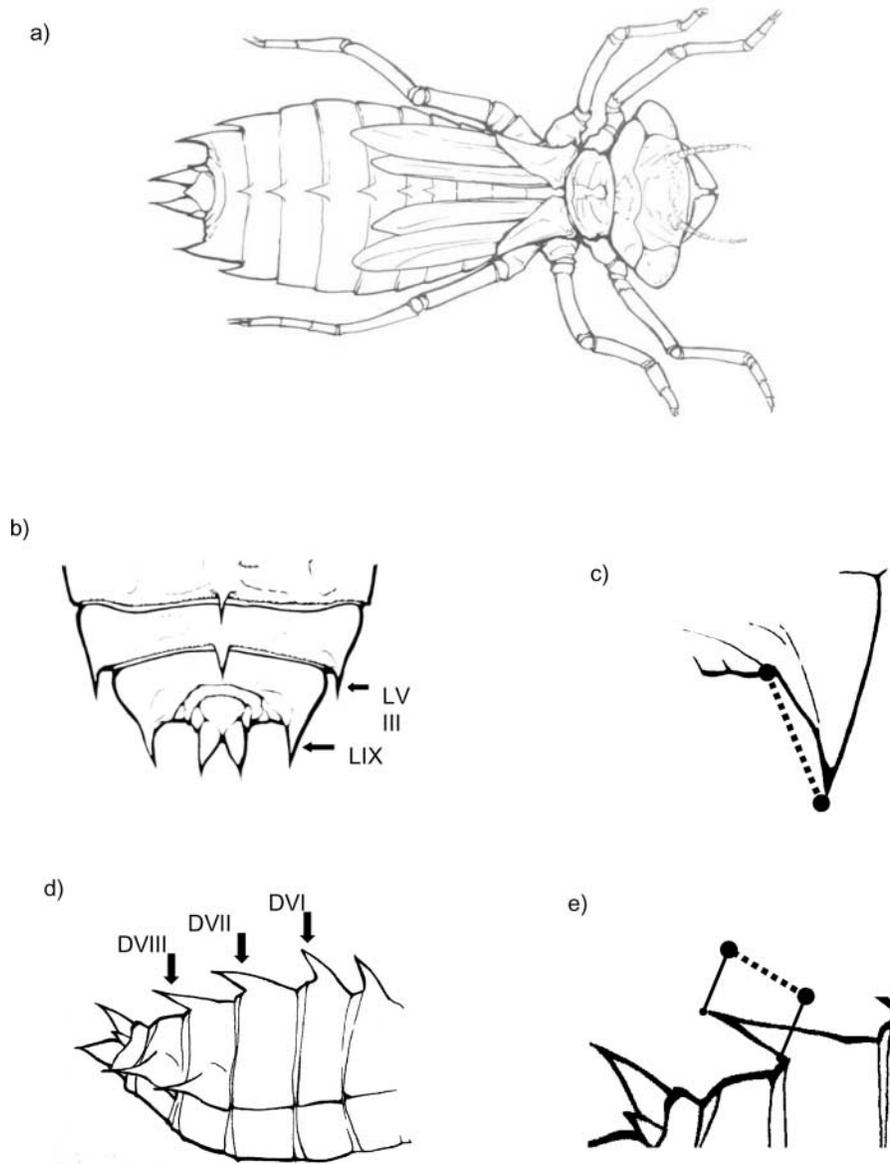
Fig. 2. Full body (a) and abdominal (b–e) views of a larva that co-existed with predatory fish. Arrows indicate spines that were measured in this study. Lateral spines are labelled with an L followed by the segment number (b). Measurements of lateral spines were made from the base of the segment to the tip of the spine along the interior margin of the spine. Dots represent landmark points and the dashed line is the linear measurement of spine length made on lateral spines (c). Dorsal spines that were measured are labelled with a D followed by the segment number (d). Measurements of dorsal spines were made from the base of the segment to the tip of the spine along the interior margin of the spine. The straight lines point to the landmark points at the base and tip of the spine and the dashed line represents the linear measurements of length made on dorsal spines, displaced above the spine for ease of viewing (e).

Natural experiment

Larval *L. intacta* were collected from ponds on the University of Michigan's E.S. George Reserve (hereafter ESGR) as a part of a survey of amphibians and their predators (separate from the survey described above) that began in 1996 and continues to the present. Surveys were conducted in the third week of May and July with supplemental collecting in October 2000. Larvae were preserved in 70% EtOH and then identified and measured in the laboratory. Before a period of drought in 1998–1999 the ESGR had five ponds with fish. After this drought, fish had been extirpated by drying in three of these ponds. The proximity and different drying histories of two ESGR ponds, Crane Pond and Fishhook Marsh, provided an appropriate context in which to assess the relationship between the presence of fish and larval morphology in *L. intacta* from natural populations. These two ponds are separated by approximately 10 m at their closest point. In 1996 and 1997, both of these ponds had fish (dominated by eastern mudminnows, *Umbra limi*, Kirtland). During the drought in 1998–1999, Fishhook dried, eliminating both fish and larval *L. intacta* from this site. Crane Pond did not dry in this period and fish as well as *L. intacta* larvae persisted there. Fishhook Marsh was rapidly re-colonized by *L. intacta* and a larval population was well established by 2000, but fish remained absent from this site during this period. Crane Pond was the closest and largest potential source for the *L. intacta* colonists in Fishhook Marsh. However, the site of origin for colonists in Fishhook following the drought is unknown and individuals may be drawn from multiple sites including those with and without fish present.

To assess the relationship between larval morphology and the presence of fish under field conditions, we made a series of contrasts between larvae collected from these two ponds in 1996–1997 (hereafter 1996, the year when most specimens were collected) and in 2000. This natural experiment is not replicated, as we have only the two ponds at two periods, and it cannot determine the mechanism generating variation across predator conditions (i.e. plasticity or differential mortality resulting from predation). However, these contrasts provide insight into the association between spine morphology and predator community under natural conditions. We compared larvae collected from these two ponds in 1996–1997 and in 2000. Our comparisons were of spine length relative to head width, a standard measure of larval odonate body size (Benke, 1970). We made four contrasts: (1) Crane 1996 vs. Crane 2000, (2) Fishhook 1996 vs. Fishhook 2000, (3) Fishhook 2000 vs. Crane 2000, and (4) Fishhook vs. Crane in 1996. Because each set of data was used in two contrasts, we set *P*-values equal to or less than 0.025.

Larvae were photographed (Fuji Finepix S1Pro) through a camera tube attached to a Wild M8 Dissecting Scope. Three photos were made of each larva to capture the head, dorsal spines, and lateral spines. Each photo had a metric ruler present for scale. We measured five spines from photos, the lateral spines on abdominal segments VIII and IX, and the dorsal spines on abdominal segments VI, VII, and VIII. These are the posterior-most set of spines on the abdomen, and evidence from studies in *L. dubia* (Mikolajewski and Rolff, 2004) and preliminary trials in *L. intacta* (S.J. McCauley, unpublished data) indicate that spines act as a defence when a larva is attacked from the back. Since these spines are the first to be encountered by attacking fish, they were expected to be the most important as defences. Spine lengths and head widths were measured by obtaining linear measurements from landmark points in TPSDIG (Rohlf, 2006) (Fig. 2). Differences in spine length were analysed using a separate multivariate analysis of variance (MANOVA) for each contrast, with pond or year as the fixed factor and head width as a covariate. Analyses were conducted in SPSS 11.5.

Induction experiment

We conducted an experiment to determine whether exposure to fish predator cues affected the expression of larval spine length. The experimental units were cattle watering tanks (1.9 m diameter, ~0.5 m deep) filled with 1300 litres of water from an underground well and covered with lids of green shade cloth. Mated pairs of *L. intacta* were captured from Crane Pond on the ESGR between 3 and 5 July 2005. Eggs were collected from females by trailing the tip of the abdomen through well water in a Petri dish. This stopped the eggs from being exposed to predator cues before being placed in the experiment. Eggs were held overnight to determine whether they were fertile (Corbet, 1999). Fertilized clutches were then randomly assigned to a tank. Although the division of clutches across tanks might have been preferable, the eggs rapidly adhere to the surface of the dish and attempts to move them can result in high egg mortality (S.J. McCauley, personal observation). Consequently, whole clutches were placed into tanks still in the Petri dish where they were laid. Eight clutches were obtained in this way (clutch size: 137 ± 19, mean ± 1 standard error). The eight tanks, each with a single clutch, were then randomly assigned as either fish or fishless units. Fish tanks contained a single bluegill (*Lepomis macrochirus*, Rafinesque; standard length: 10.1 ± 1.6 cm, mean ± 1 standard error) housed in a window-screening cage that extended from the bottom of the tank to past the top of the water line (~25 cm diameter, 80 cm long). Tanks without fish had an empty cage. Bluegill were used, rather than mudminnows, because *L. intacta* are common in habitats with bluegill and because bluegill have much better survivorships in mesocosm settings than do *U. limi* (E.E. Werner, personal observation). Each fish was fed a cube of frozen mosquito or chironomid larvae (all tanks were fed the same food type on a given day) and one libellulid larva three times per week. Initially (approximately first 2½ weeks), the libellulid larvae were not *L. intacta* (most commonly *Pachydiplax longipennis*, Burmeister but also *Tramea lacerata*, Hagen). This was done because the phenology of *L. intacta* meant that during the initial period of this experiment, *L. intacta* in natural ponds were either still in the egg stage or too small to collect and identify. Once they had matured enough to be collected from the field, fish were fed *L. intacta* larvae and the frozen food. Two fish died on the first day they were placed into the experiment and were replaced; no fish mortality occurred after the first day.

To simulate a more natural pond community and provide food resources for larval *L. intacta*, each tank received 25 copepods and 5 *Daphnia* two to three days after eggs were introduced. Each tank received 50 additional *Daphnia* on 28 July and 100 *Daphnia* on 17 August, which helped to maintain the abundant zooplankton cultures that developed in tanks. Larvae were collected on 5 October using separate equipment for each treatment to prevent the accidental transfer of larvae between treatments. Larvae collected from each tank were preserved in 70% EtOH. No larvae were collected from one tank in the no-fish treatment, which was consequently dropped from analysis. The clutch in this tank was notably smaller than the others used (40 eggs vs. 100 for the next smallest clutch) and it is possible that this clutch was not viable.

The 20 largest larvae from each tank were each photographed twice using a Fuji Finepix S1Pro camera and a Nikon 60-mm Af Micro-Nikkor lens. Larger larvae were chosen to increase measurement accuracy. Larger individuals are easier to photograph and measure and so may be subject to less error in measurement, and the error inherent to taking any measurements will be a smaller fraction of the overall measurement. One photo was taken to capture the head and dorsal view of the larva for measuring the head width and lateral

spines. Another photo was taken with the larva positioned on its side and the dorsal spines visible. A metric ruler was present in each photo for scale. The same measurements were made as in the natural experiment – head width, lateral spines on abdominal segments VIII and IX, and dorsal spines on abdominal segments VI, VII, and VIII. Measurements were made using ImageJ software (Rasband, 2006).

Spine lengths were compared using separate mixed-model procedures for each spine measured. Spine lengths were used as dependent variables and head width as a covariate. Tank was nested within treatment and this was entered as a random factor. Treatment was a fixed factor. The treatment effect was analysed with a type III sum of squares appropriate for the unbalanced design that resulted from different sample sizes in each treatment.

RESULTS

Based on catch per unit effort (CPUE) measures, densities of *L. intacta* were similar across sites with alternative top predators ($F_{2,19} = 0.219$, $P = 0.81$; CPUE counts in habitats with invertebrate, small-bodied fish, and large-bodied fish top predators: 7.3 ± 4.1, 10.2 ± 2.9, and 8.7 ± 1.8, respectively; mean ± 1 standard error).

Natural experiment

There were no significant differences in spine lengths for larvae collected from Crane Pond (which retained fish) in 1996 and 2000 (Wilks' λ, $F_{5,25} = 0.34$, $P = 0.89$, Table 1). However, larvae collected from Fishhook Marsh (which lost fish) in 1996 and 2000 differed significantly in spine lengths (Wilks' λ, $F_{5,33} = 29.11$, $P < 0.001$). Larvae collected in 1996 when fish were present had longer spines than larvae collected in 2000 after fish had been lost in this site (Table 1). Furthermore, comparing larvae collected from the two ponds in 2000 indicated that all lateral and dorsal spines in Crane were longer (relative to head width) than in Fishhook (Wilks' λ, $F_{5,26} = 18.29$, $P < 0.001$, Table 1). In 1996, when both ponds had

Table 1. Natural experiment: Pair-wise comparison *P*-values of spine length relative to head width for larvae collected in Fishhook Marsh and Crane Pond

Spine	Crane 1996 vs. 2000	Fishhook 1996 vs. 2000	Fishhook vs. Crane 2000	Fishhook vs. Crane 1996
Lateral VII	0.82	**<0.001**	**<0.001**	0.56
Lateral IX	0.59	**<0.001**	**<0.001**	0.51
Dorsal VI	0.47	**<0.001**	**<0.001**	0.42
Dorsal VII	0.65	**<0.001**	**<0.001**	**0.01**
Dorsal VIII	0.74	**<0.001**	**<0.001**	**<0.001**

Note: Fishhook Marsh had fish in 1996 but not in 2000 (indicated by the X through the fish symbol), while Crane Pond had fish throughout this period. In the first three columns for all significant differences indicated, we found longer spines where larvae co-exist with fish. In the last column, the significant differences indicate longer spines in Crane Pond than in Fishhook Marsh.

Table 2. Means and standard errors of head widths and spine lengths (mm) in Crane Pond and Fishhook Marsh in the two periods sampled

Measurement	Crane 1996	Fishhook 1996	Crane 2000	Fishhook 2000
Head width	4.66 ± 0.07	3.79 ± 0.14	3.91 ± 0.13	4.32 ± 0.14
Lateral VIII	0.63 ± 0.02	0.49 ± 0.02	0.52 ± 0.03	0.38 ± 0.01
Lateral IX	0.97 ± 0.03	0.77 ± 0.03	0.78 ± 0.04	0.53 ± 0.03
Dorsal VI	0.66 ± 0.03	0.44 ± 0.02	0.56 ± 0.03	0.33 ± 0.01
Dorsal VII	0.71 ± 0.03	0.53 ± 0.02	0.59 ± 0.03	0.39 ± 0.02
Dorsal VIII	0.65 ± 0.03	0.52 ± 0.02	0.50 ± 0.03	0.35 ± 0.02

Note: The absence of fish is indicated by the X through the fish symbol.

fish, larvae collected from the two ponds differed in the length of only two spines (Wilks' λ, $F_{5,32} = 5.86$, $P = 0.001$, Table 1), which were longer on larvae from Crane. Spine length measurements from both sites and years are presented in Table 2.

Induction experiment

Longer spines, relative to head width, were observed in the fish treatment than in the no-fish controls for the two most posterior abdominal spines (lateral spine IX: $F_{1,5} = 7.44$, $P = 0.041$, Fig. 3a; dorsal spine VIII: $F_{1,5} = 18.61$, $P = 0.008$; Fig. 3b). A non-significant trend towards longer spines in the presence of fish was found in two other spines (lateral spine VIII: $F_{1,5} = 4.40$, $P = 0.09$, Fig. 3c; dorsal spine VII: $F_{1,5} = 4.98$, $P = 0.076$, Fig. 3d). No treatment effect was observed for dorsal spine VI ($F_{1,5} = 0.34$, $P = 0.58$, Fig. 3e). Body size, measured as head width, did not differ between treatments ($F_{1,5} = 2.32$, $P = 0.188$).

DISCUSSION

Leucorrhinia intacta has a broadly generalist habitat distribution, occurring in equal densities across the three alternative top predator habitat types in the region. Consequently, this species experiences significant spatial variation in the form of predation pressure it encounters. We found that this species also displays significant morphological variation in spine length under natural conditions of when fish are present or absent. Comparing larvae from a single pond, Fishhook Marsh, before and after a drought that resulted in fish being extirpated from this site, we found significantly longer spines on larvae collected while fish were still present in this site. Larvae from an adjacent pond, Crane Pond, retained fish and did not exhibit any significant changes in spine length over this same period. Comparing larvae from these two ponds in 2000 when they differed in whether fish were present or absent also found significantly longer spines on larvae collected from the site with fish. Although this field study is constrained to a contrast of two ponds in two time periods, a limit imposed by the natural experiment in fish removal provided by the drought, these results indicate a strong positive relationship between the presence of fish and larval spine length in natural habitats. Results from this comparative study provided the impetus to experimentally test for plasticity in spine morphology.
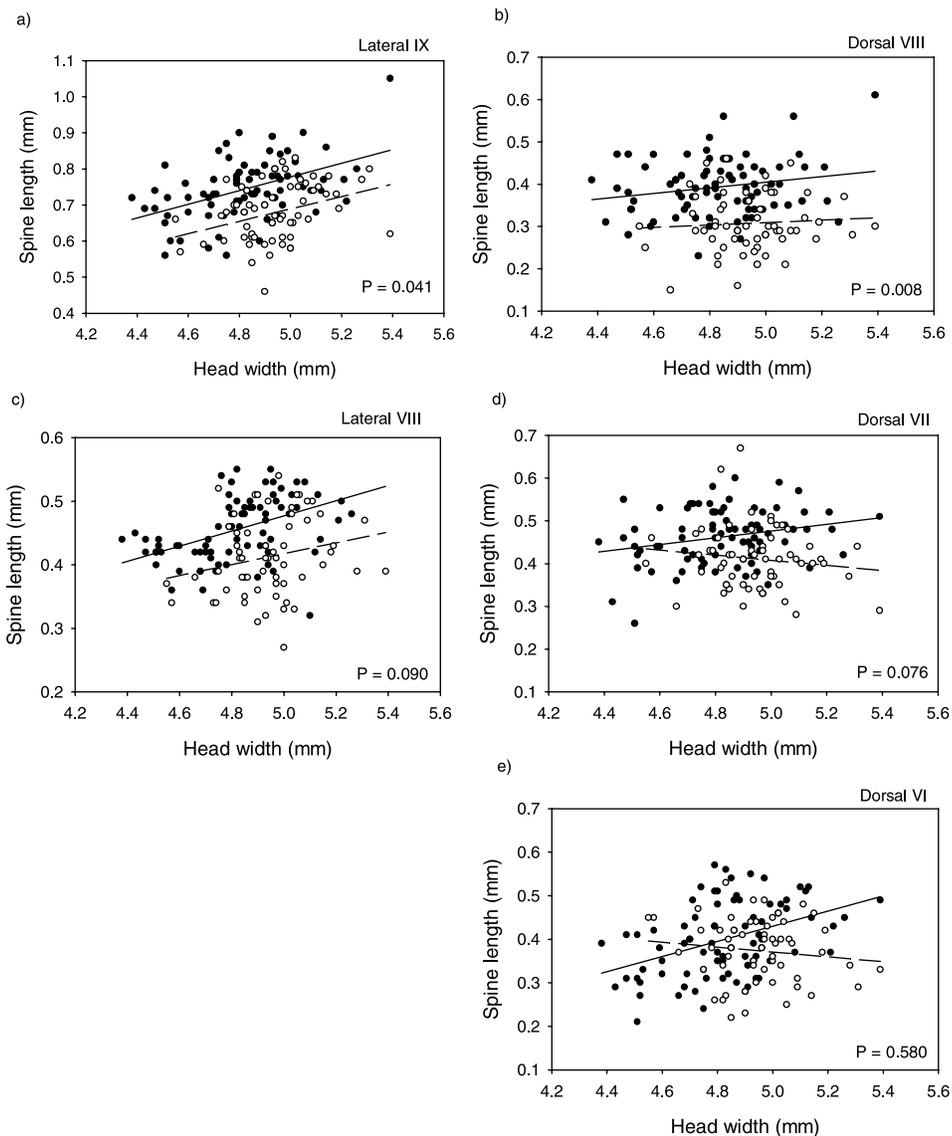
Fig. 3. Relationship between head width and spine length for lateral spines on abdominal spines VIII (a) and IX (b) and for dorsal spines on abdominal spines VI (c), VII (d), and VIII (e). Closed symbols (●) are individuals from the fish treatment, while open symbols (○) are individuals from the no-fish treatment. Each point represents a single individual. The solid line represents the relationship between head width and spine length for the fish treatment, while the dashed line represents the relationship between head width and spine length for the no-fish treatment. Note that scales differ between plots.

Our induction experiment confirmed that plasticity plays a role in *L. intacta* spine length. Several defensively important spines were longer in mesocosms containing caged fish. These results were significant for dorsal and lateral spines on the posterior-most abdominal segments, and there was a strong trend towards longer spines on abdominal segments VII

and VIII. The combination of the two studies indicates that *L. intacta* exhibits morpho-logical plasticity in spine length in response to the presence of fish, and that this pattern is found in the field as well as under laboratory conditions. This plasticity is likely to be an adaptive response because long spines provide protection from fish predators by increasing handling time (Johansson and Samuelson, 1994; Mikolajewski and Johansson, 2004; Mikolajewski and Rolff, 2004). However, long spines increase the risk from invertebrate predators by providing places to hold larvae during capture (Mikolajewski *et al.*, 2006). Plasticity in spine morphology can provide a mechanism for minimizing the risk of predation associated with both predator types to which this habitat generalist is exposed, minimizing the costs of the trade-off and acting as an important selective force in the evolution of plasticity (Mikolajewski *et al.*, 2006). Spatial variation in the top predator community and temporal changes in this community within sites, such as those observed during the drought which removed fish from Fishhook, may both act as important factors favouring the evolution of plasticity.

The results of these two studies are largely congruent. In both studies, the presence of fish was associated with the development of longer larval spines, and these effects were observed in both lateral and dorsal spines. However, we found exposure to fish was associated with longer spines on more segments in the field study than in the experiment. Three factors were considered potential mechanisms causing the differences observed between these two studies. First, in the induction experiment, eggs were collected from adults that were all captured at Crane Pond, a site with fish. Local adaptation of reaction norms could constrain the expression of plasticity in the induction experiment. However, this is unlikely to explain the difference between the induction experiment and the natural experiment because the drought also eliminated *L. intacta* larvae from Fishhook and larvae collected from this site in 2000 had to be derived from recent colonists. Although the origin of these colonists is unknown, the drought that eliminated fish and *L. intacta* larvae in Fishhook also resulted in the drying of many other fishless ponds in the region, which are typically smaller and shallower than ponds with fish. This makes it unlikely that the colonist population in Fishhook had a large input of individuals derived from fishless sites. Given that Crane Pond was the largest and closest source of *L. intacta* for Fishhook, and that much of the regional population that could re-colonize previously dried sites was derived from habitats with fish, it is unlikely that there are distinct, locally evolved reaction norms in Crane and Fishhook by 2000.

The two other factors that may explain the differences between the field and laboratory studies are: (1) reinforcement of the pattern by predation and (2) a dosage-response to the intensity of predation cues. In natural habitats, larvae are exposed to both non-lethal cues and predation. If individuals vary in the extent to which they express longer spines in the presence of fish, differential mortality based on spine length could increase the observed difference in larval spine length between fish and fishless habitats. This would result in larger differences between fish and fishless conditions in the natural habitats where both plasticity and predation are acting than in the experimental conditions where predators were non-lethal. The length and intensity of exposure to predator cues may also explain differences between the two studies. In *L. dubia*, spine length increases over ontogeny with later instars showing more elongated spines than earlier instars (Arnqvist and Johansson, 1998). Larvae in natural ponds had a longer period of exposure to fish cues than larvae in the induction experiment, potentially explaining why we found a greater difference in larval morphology in the field. Additionally, the intensity of predator cues has been shown to affect the degree of expression of both behavioural and morphological plasticity in tadpoles (Van Buskirk and

Arioli, 2002) and dosage-responses to predator risk may be common in plastic defence responses. In natural habitats, *L. intacta* larvae are exposed to a greater range of cues, including mechanical and visual cues, than in the induction experiment where cues were principally chemical. This latter difference, the intensity of fish cues, may also explain the differences observed in larvae collected from Fishhook and Crane in 1996. Larvae from Crane had significantly longer dorsal spines on two segments in 1996 than larvae from Fishhook. In 1996–1997, the density of fish biomass (wet weight, including both *U. limi* and *Phoxinus eos*) was approximately twice as high in Crane as in Fishhook [in May 1996, estimates of *U. limi* biomass were 2334 mg$\cdot$m^{-2} and 1003 mg$\cdot$m^{-2} in Crane and Fishhook, respectively (E.E. Werner *et al.*, unpublished data)]. Differences in fish biomass between these two ponds are likely to result in a greater intensity of fish cues in Crane Pond, which may explain why we found longer spines on larvae collected from this pond during this period, although further research on dosage-responses in this genus are warranted.

The morphological responses of *L. intacta* are similar to those observed in the European *L. dubia*. Both species responded to cues indicating the presence of fish by increasing spine length relative to body size, and these responses were observed in dorsal and lateral spines under laboratory and field conditions (Arnqvist and Johansson, 1998; Johansson and Wahlström, 2002). These two species are not sister to each other and the phylogeny of Hovmöller and Johansson (2004) places them in separate North American and European clades (Fig. 1). Both of these clades also have species that do not possess prominent dorsal spines, which has been hypothesized to be two independent losses of these defensive spines (Hovmöller and Johansson, 2004). The observation that phenotypic plasticity in the length of these spines is also found in both clades raises a number of questions about the evolution of plasticity and the context in which defensive spines have been independently lost twice in this genus. Either plasticity evolved before the split between the North American and European clades or very similar forms of plasticity have arisen independently twice in these two lineages. Testing these two alternatives will require determining the distribution of plasticity in spine morphology across the entire genus of *Leucorrhinia*. Either outcome will provide insight into the forces selecting for the evolution and maintenance of plasticity. If this form of morphological plasticity is ancestral in *Leucorrhinia*, potentially the most parsimonious interpretation, then dorsal spines have been repeatedly lost despite a capacity for plasticity in this trait. This is particularly striking in that Johansson (2002) found that the production costs of longer spines are minimal in *L. dubia*, and there does not appear to be a clear habitat shift associated with the loss of dorsal spines in species for which there are published data. Within both the European (*L. rubicunda*) and N. American (*L. glacialis*) clades, two species that lack prominent dorsal spines are frequently found in lakes both with and without fish [*L. glacialis* (Bendell and McNicol, 1995; Strong and Robinson, 2004) and *L. rubicunda* (Johansson and Brodin, 2003)]. In contrast, if plasticity in spine morphology is not ancestral within the *Leucorrhinia*, then we have a striking example of convergent evolution occurring in species occupying similar habitats on two continents. In either scenario, these results, in conjunction with work done by Johansson and colleagues, establish *Leucorrhinia* as a useful model system in which to explore the evolution of phenotypic plasticity.

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