ONTOGENETIC REACTION NORMS OF PREDATOR-INDUCED DEFENSIVE MORPHOLOGY IN DRAGONFLY LARVAE

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Abstract. The study of phenotypic plasticity, one of the most important mechanisms of phenotypic adaptation, is by tradition focused on differences in ontogenetically static phenotypic expression in different environments. Ontogenetic reaction norms, in contrast, describe how phenotypes unfold during growth in different environments. We studied the ontogenetic reaction norms of the morphological shape of a series of defensive abdominal spines in dragonfly larvae, both in the laboratory and in a number of natural populations. In a laboratory rearing experiment, we demonstrated that these spines grew more solid and elongated when waterborne environmental cues of fish predators were present: this is evidence of phenotypic plasticity in defensive spine morphology. The ontogenetic reaction norms of defensive spines were also found to differ in natural populations with and without fish. A detailed analysis of the growth trajectories showed that this differentiation was primarily due to ontogenetic acceleration in environments with fish, leading to relatively exaggerated spine shape in these environments. However, while the ontogenetic trajectories of shape in some spines diverged at the onset of ontogeny in the two environments, those of others remained parallel until a given phase of ontogeny. Hence, the timing of the developmental divergence of these phenotypically integrated traits differed, suggesting differences in the underlying regulatory mechanisms. Our results illustrate that a conceptual integration of environmental and ontogenetic approaches to the study of phenotypic differentiation can significantly promote our understanding of the ecology and evolution of adaptive phenotypic plasticity.

Key words: allometry; geometric morphometrics; induced defenses; Leucorrhinia dubia; Odonata; ontogenetic reaction norms; ontogeny; phenotypic plasticity.

INTRODUCTION

Understanding the ecology and evolution of phenotypic plasticity (i.e., environmentally based changes in the phenotype) and its role in adaptive morphological evolution has proven to be one of the major challenges in evolutionary ecology (Stearns 1989). While many important theoretical insights have recently been made, several key issues are still debated, and the paucity of empirical studies of plasticity in natural populations partly obstructs our understanding of its ecological relevance: it is impossible to deduce from laboratory studies alone how important plasticity is in the field (see Scheiner 1993, de Jong 1995, Schlichting and Pigliucci 1995, Via et al. 1995). Pigliucci and Schlichting (1995) recently introduced the concept of ontogenetic reaction norms, and demonstrated that combining the study of plasticity with that of ontogeny potentially broadens and deepens our understanding of phenotypic plasticity. Norms of reaction are typically studied statically at the end of ontogeny, and studies of the plasticity of ontogenetic trajectories are very rare (Pigliucci and Schlichting 1995). Considering the fact that much evolutionary change occurs through altera-

tions of the timing and rate of developmental events (Alberch et al. 1979, McKinney and McNamara 1991), this lack of theoretical and empirical integration of the concepts of ontogenetic allometry and phenotypic plasticity is unfortunate. Studies of the ontogenetic allometry of plastic traits in different environments can, in particular, reveal insights with regard to the plastic dynamics of growth patterns, and hence help in understanding the mechanisms by which adaptive reaction norms evolve (Werner 1982, Werner and Gilliam 1984, Pigliucci and Schlichting 1995).

One of the classical examples of adaptive phenotypic plasticity is predator-induced defenses in prey (Havel 1987, Harvell 1990). This has been used as a model system for adaptive phenotypic plasticity in both plants and animals, since predation is known to affect the phenotypic expression of a wide range of behavioral, life history, and morphological traits in a way that reduces the impact of predation (Lively 1986, Sih 1987, Dodson 1989, Stearns 1989, Adler and Harvell 1990, Crowl and Covich 1990, Spitze 1992). Studies of induced morphological defenses have, however, mainly been concerned with traits that exhibit switched plasticity (threshold characters) (Havel 1987, Harvell 1990), and have very rarely paid any attention to the ontogenetic growth trajectories of the defense structures (e.g., Lively 1986, Appleton and Palmer 1988,

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Brönmark and Miner 1992, Reimer and Tedengren 1996). An exception is defensive spine formation in *Daphnia,* where studies have indicated that the phenotypic expression of anti-predator morphology during ontogeny is maximized in the larval stages where vulnerability to predation is highest (Vourinen et al. 1989, Harvell 1990, Parejko and Dodson 1990, Hanazato and Ooi 1992, Tollrian 1993).

Odonates (Insecta; Odonata) are sexually reproducing organisms with a larval stage that is spent in aquatic habitats. Fish are major predators of dragonfly larvae, especially in the later larval instars, when invertebrate predators are no longer capable of handling the relatively large larvae (Benke and Benke 1975, Crowder and Cooper 1982, Martin et al. 1991, Diehl 1993), and different species of odonates differ considerably in their sensitivity to fish predation due to species-specific morphological and behavioral characteristics (e.g., Morin 1984, Henriksson 1988, Pierce 1988, McPeek 1990, 1995, McPeek et al. 1996). Since predation is a major factor in shaping life histories in this group of insects, we might expect that a suite of predator-induced defenses has evolved. Accordingly, studies of phenotypic plasticity of antipredatory behavior in odonates have shown that larvae alter their behavior adaptively in the presence of both fish (Heads 1985, Pierce 1988) and invertebrate (Heads 1986, Johansson 1993) predators. There are, however, no previous studies of induced morphological defenses in these or any other insects.

The larvae of several odonate species are provided with erect spines that protrude from the abdomen (Walker and Corbet 1975, Askew 1988, Johansson and Samuelsson 1994). These spines have been shown to offer protection from fish predation, much in the same way that defensive spines provide protection from fish predation in *Daphnia* (Tollrian 1994, Swaffar and O'Brien 1996) and in sticklebacks (Hoogland et al. 1957, Reist 1980*a*); fish predators have difficulty handling or swallowing larger *L. dubia* larvae with longer spines (Johansson and Samuelsson 1994). The defensive spines should, in contrast, offer little defense against most invertebrate predators (with piercing or chewing mouthparts; e.g., diving beetle larvae, backswimmers, other odonate larvae) (see also Reist 1980*b*). In accordance, Johansson and Samuelsson (1994) observed that the average length of abdominal spines in last-instar odonate larvae was higher in lakes with fish than in lakes without fish (Fig. 1), suggesting that spine growth may be induced during development as a defense to fish predation. Since odonate larvae typically grow out of the size range where they are sensitive to invertebrate predation and into the size range where they are relatively more exposed to fish predation (Diehl 1993), we would expect growth and differentiation of the spines to be synchronized with the potential defense benefits and thus to relate in a nonlinear way to size during ontogeny.
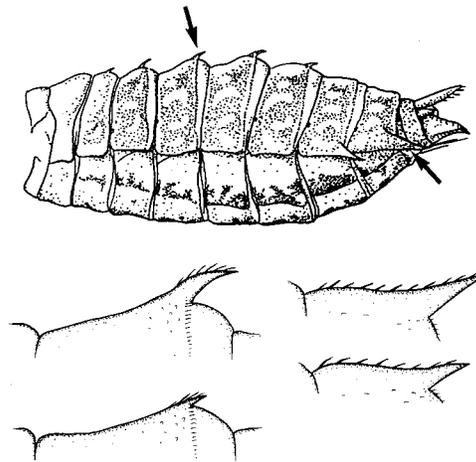

FIG. 1. Abdomen of a last-instar *L. dubia* larva in lateral view (top). Arrows indicate position of the dorsal V and lateral IX abdominal spines. To illustrate the range of natural shape variation in spine morphology, representative examples of these two spines are shown in magnification below. The bottom left part shows the dorsal V spine (lateral view), and the bottom right part the lateral IX spine (dorsal view), of last-instar larvae collected in lakes with (upper) and without (lower) fish. The abdominal spines are considerably broader and more extended in lakes with fish.

This paper examines plasticity in phenotypic expression of abdominal spines in dragonfly larvae, and our goals are three-fold. First, we assess experimentally whether abdominal spine growth is indeed induced by environmental cues. Second, we compare phenotypic expression in the laboratory with that in a number of natural populations, to relate induced phenotypic plasticity to naturally occurring environmental heterogeneity. Third, we determine the patterns of ontogenetic allometry of spine growth in natural environments, to gain insights into the developmental dynamics of these traits. We employ landmark-based geometric morphometrics (Bookstein 1991, 1996*a*, Rohlf and Marcus 1993), to allow analysis of the geometric shape of the defensive spines.

METHODS

The study was performed on the dragonfly *Leucorrhinia dubia* (Van der Linden) (Odonata; Libellulidae) (Fig. 1), and the morphometric data that form the basis for our analyses originate from two different sources: an extensive field sampling of larvae from a number of natural populations and a laboratory rearing experiment.

Field sampling

Larvae of *L. dubia* were collected at several different occasions during the fall of 1993 with a hand net from each of 15 different lakes and ponds, all situated within a restricted area in the vicinity of Umeå, northern Sweden. From each population, larvae were collected to represent a size range from the early to the very last

instar (range in total body length = 6.0–18.7 mm; average n = 24 per population; range of n = 15–30). *L. dubia* has 10–14 larval instars. Seven of the sampled populations inhabited lakes that also contained fish predators, and eight inhabited lakes without fish (see Johansson and Samuelsson [1994] for detailed data on the sampled lakes).

Laboratory rearing experiment

L. dubia eggs were collected in early August 1995 from four adult females captured at a fishless lake (Lilla Lomtjärn, 20 km southwest of Umeå). Females were induced to oviposit in the field into small glass jars as described by Boehms (1971). The eggs were brought to the laboratory for hatching. Twenty experimental aquaria (35 × 17 × 20 cm) were each partitioned into two compartments with 0.5-mm white Nitex net. Although nontransparent, the net allowed free exchange of water between the two compartments. The aquaria were maintained in the laboratory under a 14 h light : 10 h dark regime, at a water temperature of $17° ± 1°C$. Upon hatching, all first-instar larvae from the four females were pooled. Four randomly chosen larvae were then put into one of the compartments in each aquarium. We created a fish treatment in half of the aquaria (n = 10) by holding one perch (*Perca fluviatilis,* age 1+, body length ~3 cm) in the opposite compartment every second week. To avoid deterioration of the water quality in the aquaria, 1 L of water was changed in each aquarium once a week. During this procedure, the aquaria in the fish treatment received fresh water from a tank that held perch and the aquaria in the control treatment received fresh water that had not been in contact with fish.

The *L. dubia* larvae were fed according to a standardized protocol. Protozoans and brine shrimp from laboratory cultures were provided as food during the early phase of their development, and *Moina rectirostris* and brine shrimp during later ontogenetic stages (after larval instar 3–4). Equal amounts of food were supplied to each aquarium throughout the experiment, which was terminated in June 1996. By this time, almost all larvae had reached their ultimate or penultimate instar. Larvae were preserved in alcohol for subsequent morphometric analyses. In the four replicates where two individual larvae survived the experiment, only the largest individual (last instar) was used for statistical analyses, so as to avoid problems with pseudoreplication.

Morphometric measurements and analyses

We captured two-dimensional landmark data by viewing larvae in a dissecting microscope (Wild M5), and projecting the image through a camera lucida onto a digitizing tablet (Summasketch III). We recorded data for the two right lateral spines, located at the abdominal segments VIII and IX, and for three of the dorsal spines, located at the abdominal segments IV–VI (Fig. 1). Each spine was characterized by three landmarks, representing the three corners of a triangle describing the tip and bases of each spine (Fig. 2). We also collected landmarks of the head, for the purpose of providing an independent measure of the size of each larva. In total, 19 landmarks were collected for each individual by means of the computer program DS-DIGIT (Slice 1994), and all methods of data capture and analyses were identical for field-caught and laboratory-reared individuals. Landmarks for the head (four landmarks) and the right lateral spines (2 × 3 landmarks) were entered when viewing larvae from the dorsal side, and for the three dorsal spines (3 × 3 landmarks) when viewing larvae laterally (Figs. 1 and 2). To attain consistent lateral and dorsal projections, and in order to minimize measurement error (Arnqvist and Mårtensson 1998), the larvae were placed under the microscope on a flexible microscope cup stage, and the focal plane of the microscope was used to align each specimen.

Since head size is the most reliable and integrative measure of overall size in dragonfly larvae (Benke 1970), it was used as a measure of individual size. We calculated the centroid size of the head (the square root of the sum of squared distances of the four landmarks on the head from their centroid), and used this as a covariate in all subsequent analyses of the ontogenetic allometry of spine shape.

Abdominal spine ''length'' is difficult to define, and a simple consideration of static differences between pairs of landmarks does not satisfactorily describe and capture the morphometric variation and complexity in spine morphology during ontogeny. We thus used geometric morphometric analyses to assess morphological variation in the shape of lateral and dorsal spines. Rather than examining patterns of covariances among a set of linear measurements, as in traditional morphometrics, these recently developed techniques use point coordinate data to study the geometric shape of biological structures (see Rohlf and Marcus 1993, Bookstein 1991, 1996*a*, Marcus et al. 1996). These methods are generally more powerful since they take spatial relationships among all landmarks into account, unlike traditional morphometrics, which does not account for spatial relationships between distances. More importantly, the geometry of shape variation is retained throughout the analysis (in contrast, it is not possible to recover the shape of the original structure from a covariance matrix of distance measurements), and regions and modes of shape variation can thus be localized and visualized, which significantly improves the biological interpretability of the results. Geometric morphometrics is an especially valuable tool in studies of ontogeny, because it handles isometric size changes in an efficient way and because it permits full capture and visualization of the complex shape changes that often occur during growth (Bookstein 1991, Zelditch et al. 1992, 1993, Walker 1993, Monteiro et al. 1997).

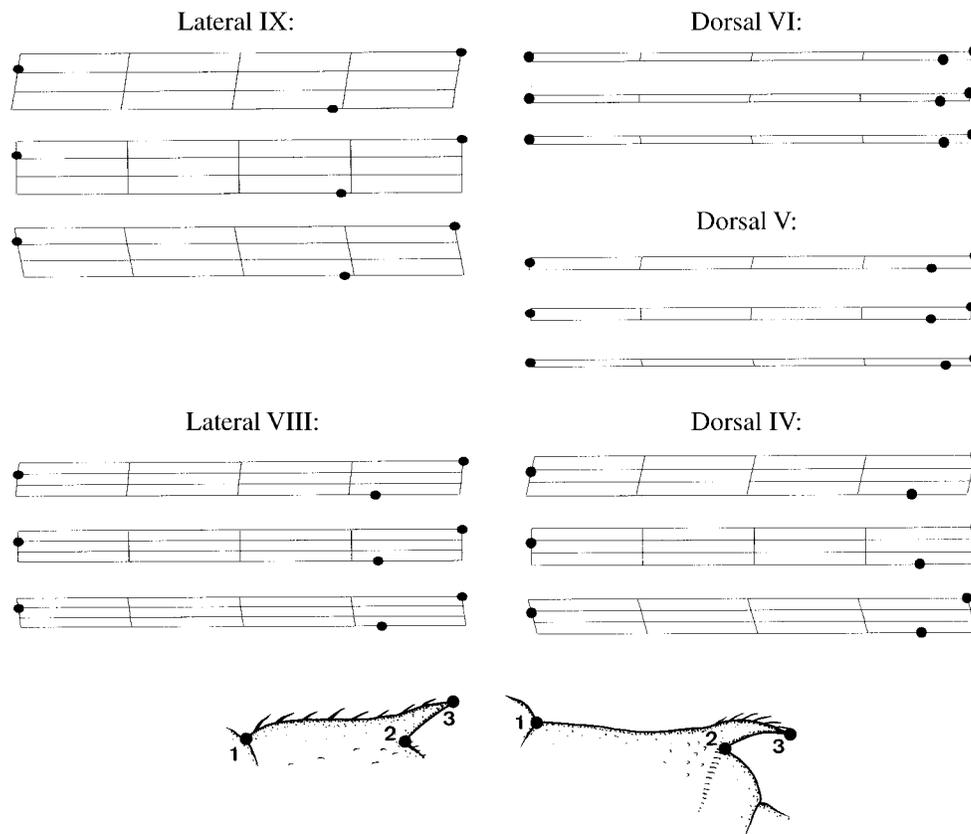Our geometric morphometric analyses of the abdom-

Fig. 2. Shape change in abdominal spine morphology during ontogeny, visualized as grid deformations. The two left panels represent lateral abdominal spines, and the three right panels represent dorsal, abdominal spines. The landmark locations are indicated at the bottom of the figure (cf. Fig. 1), as well as in the grid plots, with dots. Within each panel, the bottom grid shows the average configuration of spine landmarks for small-sized larvae, the middle grid that of mid-sized larvae, and the top grid that of large-sized larvae. The three grids within each panel can thus be thought of as showing the spine shape change that occurs as the larvae grow to larger sizes. The shape changes illustrated here represent the composite effects of the two uniform components (U1 and U2) and can also be understood as showing the shape change that results from simultaneously increasing the score of these two shape components. All five abdominal spines show a very similar pattern of shape change during ontogeny: the basis of the spine becomes broader (distances between horizontal gridlines increase) and the tip of the spine becomes extended (vertical gridlines rotate clockwise) as the larvae grow to larger body sizes.

inal spines were conducted on each of the five spines separately, but included data for all individuals collectively (field and laboratory larvae). We proceeded in two steps: (1) All spine landmark maps were first superimposed (scaled, translated and rotated, using the GLS option in the computer program GRF-ND (Slice 1993), to give the landmark maps the same size and location. This procedure effectively removes isometric differences in size, leaving a residual coordinate scatter that represents pure shape variation to be secondarily analyzed (e.g., two individuals [i.e., their landmark maps] that differ only by size and/or location will be identical after the superimposition). (2) Structures with only three landmarks represent the simplest of all geometric configurations, and show no non-uniform (inhomogeneous or localizable) shape variation. In such cases, all shape variation among specimens is spatially uniform, and the two ''uniform shape components'' (U1 and U2) are sufficient to collectively describe all

variation in relative displacement of landmark positions in a set of landmark maps (see Rohlf and Marcus 1993, Bookstein 1996a, b, c, for a discussion of the uniform and non-uniform shape spaces). Following the superimpositions of our spine landmark maps, shape variation in the shape of lateral and dorsal spines was characterized by estimating the two uniform components of shape variation for each spine (Bookstein 1996c) that form our measure of spine shape, using the computer program TPSRW (Rohlf 1993). In summary, our morphometric analysis generated an integrative measure of individual larval size (head size) and two shape components (U1 and U2) for each of the five spines, for all individuals included in the study.

Since we were interested in the patterns of ontogenetic allometry, the relationships between size and spine shape were linearized prior to being subjected to statistical analysis using linear models (e.g., ANOVA, MANOVA). This was achieved by basing all statistical

inferences on logarithmic transformations of raw data. The shape scores were all transformed as $X' = \log(c_1 + X)$, and the centroid size of the head was transformed as $X' = \log(c_2 + \sqrt{X})$, where c represents a constant.

RESULTS

Overall changes in spine shape during ontogeny

The pure shape change in spine morphology that occurs during ontogeny in the field, after removing isometric changes in the size of spines, is shown in Fig. 2. All five abdominal spines exhibit a very similar pattern of shape change during ontogeny. As *L. dubia* larvae grow to larger body sizes, the spine tips become relatively more extended and the bases of the spines become broader (see Fig. 2). In other words, the spines successively become more elongated and robust in later larval instars.

In order to assess the relative degree to which the two uniform components account for total variation in shape, we estimated the percentage of variance in procrustes distance (a measure of total shape variation) explained by the two uniform components (Bookstein 1996*a*, *c*). As expected, the first component captured the greatly dominating part of total variance in shape space (see Fig. 3). The first uniform component (U1) explained ≥71% of the variance in both lateral spines and ≥87% in all dorsal spines.

Ontogenetic norms of reaction in the wild

To characterize the variation in ontogenetic allometry of spine morphology in natural populations, the parameters describing the allometry between size and spine shape in each lake were tested in a series of multivariate analyses of variance (MANOVAs) using fish presence/absence as a categorical factor. Dependent variables were the slope and intercept of the allometric relationship, generated in univariate regressions between size and each of the two uniform shape components. Overall, the allometric relationship between size and spine shape differed between lakes with and without fish for all five abdominal spines (Table 1). Differences in the slope of the allometric relationships, especially for the first uniform shape component (Fig. 3), were consistently larger than differences in intercepts (Table 1). Thus, the most important conclusion that emerged from these analyses is that the abdominal spines of dragonfly larvae grow elongated and solid more rapidly in lakes with fish, compared to lakes without fish.

To further characterize differences in the allometric pattern in lakes with and without fish, we also performed nonparametric cubic spline regressions on data from the two types of lakes separately, and estimated bootstrap standard errors of all fitted functions (based on 1000 replications). This technique is particularly appealing for exploratory purposes, since it does not assume any specific form of allometry and thus pro-vides allometric functions with a local rather than a global fit (see Schluter 1988). The cubic spline analyses largely confirmed the MANOVAs: the growth of more elongated and broader spines among larvae in fish lakes was primarily due to accelerated development during ontogeny (Fig. 3). Interestingly enough, while the divergence in developmental rates occurred at the onset of development in the dorsal spines, this diverging pattern occurred during later ontogenetic stages in the lateral spines (see left panels in Fig. 3, and tests of the allometric intercept [the inception of divergence] in Table 1).

The magnitude of correlated growth across spines (phenotypic integration) was estimated by generating individual shape residuals of the first uniform components of all spines in models including lake, size, and their interaction. These residuals were then correlated with one another across all individuals. Shape of the defensive spines was correlated across spines within individuals (range of $r = 0.26–0.71$, $n = 358$, $P < 0.001$ in all cases).

Induction of spine shape in the laboratory

Given that dragonfly larvae in natural populations where predatory fish occur change spine shape more rapidly during ontogeny than do larvae from populations without fish, and hence attain more elongated and solid spines, we expect to see a similar pattern in our laboratory rearings if spine morphology is indeed phenotypically induced. We first performed a gross estimate of the effects of the fish treatment on spine shape in a series of multivariate analyses of covariance (MANCOVA). These analyses detected overall effects primarily for the lateral spines (see Table 2). More focused analyses of the effects of the fish treatment on the two uniform components separately, revealed strongest effects for the first, and statistically most important (Fig. 3), uniform component in three of the five spines (Table 3).

Since wild dragonfly larvae collected from lakes with fish had on average higher scores on all shape components of all spines than did larvae from fishless lakes (Fig. 3), this pattern of ordering should be mimicked in the laboratory as well. This overall prediction was assessed by comparing shape scores of larvae reared with and without fish in the following way. For all shape components, shape score was regressed on size using all individuals reared in the laboratory. The residuals from these allometric regressions represent deviation from expected spine shape at the observed size. Overall difference between the two laboratory groups in shape was then tested in a paired *t* test of the average residual value in the two groups, for all spines and shape components. As predicted, larvae reared with fish exhibited positive shape residuals (mean residual = 0.0065) and larvae reared without fish exhibited negative shape residuals (mean residual = −0.0072) across all 10 shape components ($t = 3.21$, df = 9, $P_{\alpha/2} <$
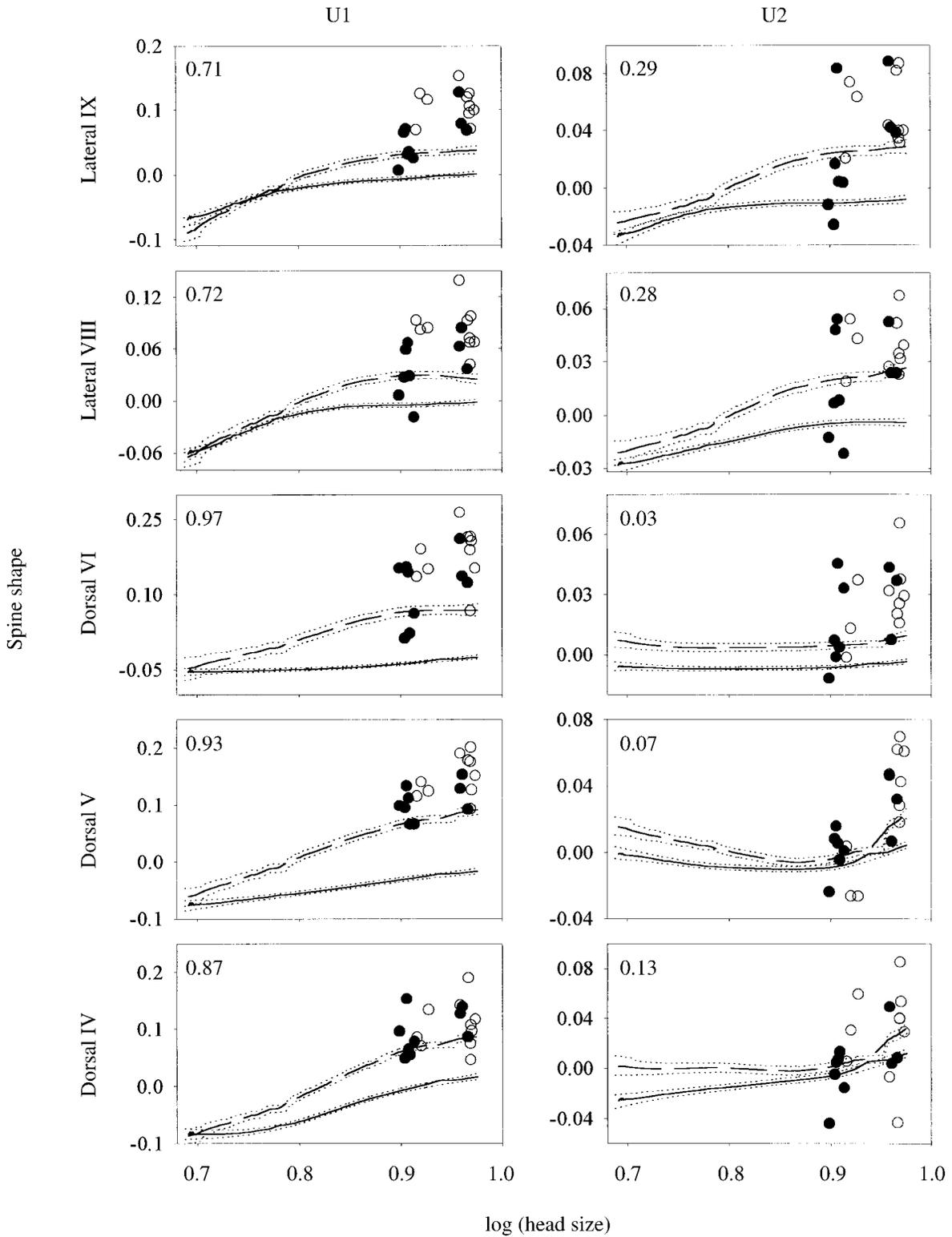
FIG. 3. Ontogenetic reaction norms in *L. dubia* larvae. The plots show average ontogenetic growth trajectories in two different environments: lakes with (dashed lines) or without (solid lines) fish predators, shown as the relationship between individual size and the shape (shape score) of the five defensive abdominal spines. Observed larval body lengths ranged between 6.0 and 18.7 mm. Fitted functions are cubic spline regression surfaces, with bootstrap SE (dotted lines) (Schluter 1988). Circles represent individual larvae reared in the laboratory with (open) or without (filled) fish present. The left column shows the first, and the right the second, uniform shape component, and the number in the upper left corner of each plot denotes the fraction of total shape variation explained by each of the two uniform shape components.

TABLE 1. Tests of difference between lakes with and without fish in the allometric relationship between larval size and abdominal spine shape. Analyses were performed with MANOVA.

Morphological component	Wilks' λ	F†	F‡	P
Lateral spine, segment IX	0.384	4.014		0.034
Slope	0.554		4.824	0.029
Intercept	0.598		4.037	0.046
Lateral spine, segment VIII	0.382	4.035		0.033
Slope	0.461		7.003	0.010
Intercept	0.525		5.432	0.021
Dorsal spine, segment VI	0.337	4.918		0.019
Slope	0.587		4.226	0.041
Intercept	0.618		3.701	0.056
Dorsal spine, segment V	0.266	6.904		0.006
Slope	0.491		6.211	0.014
Intercept	0.613		3.790	0.053
Dorsal spine, segment IV	0.243	7.805		0.004
Slope	0.565		4.615	0.033
Intercept	0.590		4.173	0.042

Notes: In a first series of tests, the slope and intercept of the regression of size versus spine shape for each lake and both uniform shape components (U1 and U2) were treated as dependent variables, and fish presence/absence as the independent variable. Hence, these tests represent tests of the null hypotheses that the overall allometry between size and spine shape is identical in lakes with and without fish (left F values). This effect was then partitioned into effects due to slope and intercept of the allometric relationship in a second series of analyses, where the slopes and intercepts of the two shape components were tested separately (right F values).

† Rao's F, df = 4, 10.

‡ Rao's F, df = 2, 12.

0.005). Hence, the relative ordination of larval spine shape in lakes with and without fish seen in the field was indeed mirrored in our laboratory rearings.

In conclusion, dragonfly larvae reared with fish developed more elongated and broader abdominal spines than did larvae reared without fish. Further, though the strength of this experimental effect varied across the five different abdominal spines, the overall pattern of spine shape differences seen between larvae reared with and without fish corresponds to the differences seen between wild larvae collected from lakes with and without fish.

Differences in spine shape allometry between laboratory-reared individuals and individuals caught in the field were assessed in two-way MANCOVAs including all individuals, where the spine shape scores (U1 and U2) were dependent variables, fish presence/absence and source (laboratory/field) dichotomous factors, and size the covariate (Table 4). For all five abdominal spines, spine shape differed between laboratory-reared larvae and those caught in the field. Fish presence/absence had a somewhat weaker, but equally general, effect on spine shape. In contrast, the relationship between abdominal spine shape and fish presence/absence did not differ in general between laboratory-reared individuals and individuals caught in the field (see interaction terms in Table 4). Hence, this analysis indi-

cated that while the multidimensional location of the ontogenetic reaction norms differed between field and laboratory conditions, the shape of the ontogenetic reaction norms did not.

DISCUSSION

The abdominal spines in *L. dubia* are induced by environmental predatory cues. Larvae reared in water carrying environmental cues from fish predators developed more elongated and more solid abdominal spines than did larvae reared in water that did not carry such cues. In the majority of documented cases, the inducible defense traits exhibit switched plasticity (threshold characters) (Harvell 1990). We show that the plastic induction of spines in *L. dubia* is graded, in the sense that spines are present to some extent in all environments, but grow more pronounced in environments containing predators. Considering that such graded plasticity is much less apparent, and hence potentially overlooked, graded inductions of defense morphologies may be much more widespread than has previously been appreciated. The findings of our study, together with those of other recent studies of induced defense morphologies in mollusks and fish (Appleton and Palmer 1988, Brönmark and Miner 1992, Brönmark and Pettersson 1994, Nilsson et al. 1995, Reimer and Tedengren 1996), suggest that this is the case.

Most empirical demonstrations of induced morphological defenses concern asexually reproducing microcrustaceans (Havel 1987, Harvell 1990), and our study represents one of the very few demonstrations of induced defense morphologies in obligate sexually reproducing animals (see also Lively 1986, Appleton and Palmer 1988, Brönmark and Miner 1992, Brönmark and Pettersson 1994, Nilsson et al. 1995, Reimer and Tedengren 1996). Harvell (1990) suggested that the apparent rarity of induced defenses in sexually reproducing organism is due to a high risk of mortality for genetic individuals in encounters with predators in such species, which would make selection on the individual level less cogent in driving the evolution of induced defenses compared to clonal species. However, whenever a given defense trait increases a prey individual's

TABLE 2. Tests of difference in abdominal spine shape between larvae (ultimate and penultimate larval stages only) reared with and without fish. Analyses were performed with multivariate analysis of covariance (MANCOVA) of uniform shape score (U1 and U2), with fish treatment as a fixed-effects factor and size as a covariate. The effects of the fish treatment on multivariate spine shape are given.

Morphological component	Wilks' λ	F†	P‡
Lateral spine, segment IX	0.741	2.617	0.053
Lateral spine, segment VIII	0.722	2.888	0.043
Dorsal spine, segment VI	0.880	1.024	0.191
Dorsal spine, segment V	0.776	2.169	0.074
Dorsal spine, segment IV	0.929	0.572	0.288

† Rao's F, df = 2, 15.

‡ One-tailed P values.

TABLE 3. Tests of difference in spine shape, partitioned into the uniform components 1 and 2, between larvae (ultimate and penultimate larval stages only) reared with and without fish. Analyses were performed with ANCOVA of shape score, with fish treatment as a fixed-effects factor and size as a covariate. The effects of the fish treatment on spine shape are given.

Morphological component	F†	P‡
Lateral spine, segment IX		
U1	5.537	0.016
U2	0.530	0.238
Lateral spine, segment VIII		
U1	6.139	0.012
U2	1.193	0.145
Dorsal spine, segment VII		
U1	1.978	0.089
U2	0.015	0.452
Dorsal spine, segment V		
U1	3.257	0.045
U2	0.324	0.288
Dorsal spine, segment IV		
U1	0.001	0.497
U2	1.060	0.159

† df = 1, 16.
‡ One-tailed P values.

probability of surviving encounters with predators, classic individual selection will favor the expression of such traits. Thus, there are reasons to believe that the predominance of asexual organisms in the literature on induced defenses is due in part to taxonomic bias and in part to more dramatic inductions in asexual organisms.

The ontogenetic allometry of induced defenses

Assuming that there is at least some cost of expressing morphological defense traits (Harvell 1990), we would expect such traits to be most pronounced during the ontogenetic stages where the net benefits are largest. Our study provides some support for this prediction, as do studies of *Daphnia* where the phenotypic expression of defense traits varies during ontogeny and is most pronounced during the stages where individuals are believed to be most exposed to predation (Vourinen et al. 1989, Harvell 1990, Parejko and Dodson 1990, Hanazato and Ooi 1992, Tollrian 1993). In *L. dubia,* the abdominal spines grew proportionally more elongated and solid during ontogeny. This most likely represents an adaptive growth pattern, since significant defense benefits of spines in this species should not occur until the larvae reach a certain critical size. This is not only because dragonfly larvae grow out of the size range when they are sensitive to invertebrate predators (see *Introduction*), but also because the fish predators of *L. dubia* are to some extent gape limited, and such limitations will not influence the outcome of predator–prey encounters during ontogeny until larvae approach a size corresponding to the gape limit of the smallest size class of potential predators (cf. Hambright

et al. 1991, Damsgård 1995). Such size-related defense benefits of anti-predatory spines, due to predator gape limitation, have been demonstrated in *Daphnia* (Swaffar and O'Brien 1996) and in sticklebacks (Reist 1980*a, b*). The main fish predator of *L. dubia,* the perch *Perca fluviatilis,* goes through ontogenetic niche shifts and switches from planktivory to benthivory at a certain size (Persson and Greenberg 1990). The critical prey size for the smallest benthivorous perch seems to be ~10–12 mm (Diehl 1993)—a critical size that corresponds well with the pattern of accelerated defensive spine growth during prey ontogeny seen in *L. dubia* (a body length of 10–12 mm corresponds to a log head size of 0.85–0.9; see Fig. 3). Balancing costs of spine growth in *L. dubia* may result from direct metabolic/material expenses, indirect costs in terms of interference during molts (Arnqvist 1994), or trade-offs between defensive morphology and anti-predator behavior (cf. Andraso and Barron 1995, Andraso 1997).

Ontogenetic norms of reaction

The study of adaptive phenotypic plasticity in morphology traditionally focuses on the adult stages only, and dynamics are examined in the trait–environment space under ontogenetic stasis. Pigliucci and Schlichting (1995) recently suggested that our definition of reaction norms should be broadened to include phenotypic changes during ontogeny, so as to define ontogenetic norms of reaction as three-dimensional sur-

TABLE 4. Tests of the effects of fish presence and larval environment (natural or laboratory) on the allometry of abdominal spine shape. Analyses were performed with two-way MANCOVA of uniform shape (U1 and U2), where the dichotomous variables fish presence (presence/absence) and source (natural population/laboratory) were factors and larval size was the covariate.

Morphological component/effect	Wilks' λ	F†	P
Lateral spine, segment IX			
Fish presence	0.946	10.536	<0.001
Source	0.861	29.875	<0.001
Fish presence × Source	0.994	1.016	0.363
Lateral spine, segment VIII			
Fish presence	0.948	10.095	<0.001
Source	0.896	21.530	<0.001
Fish presence × Source	0.992	1.499	0.225
Dorsal spine, segment VI			
Fish presence	0.927	14.528	<0.001
Source	0.774	54.001	<0.001
Fish presence × Source	0.998	0.346	0.707
Dorsal spine, segment V			
Fish presence	0.922	15.682	<0.001
Source	0.815	42.035	<0.001
Fish presence × Source	0.985	3.062	0.053
Dorsal spine, segment IV			
Fish presence	0.964	6.926	<0.01
Source	0.916	16.980	<0.001
Fish presence × Source	0.975	4.660	<0.05

† Rao's F, df = 2, 372.

TABLE 5. Mean lengths (mm) and standard deviations of the tips of the abdominal spines of last instar *L. dubia* larvae, from environments with and without fish, for comparative purposes only. Lengths represent distances between landmarks 2 and 3 (see Fig. 2) for each spine and are given separately for larvae caught in the field and those reared in the laboratory.

Abdominal spine	Field samples		Laboratory-reared	
	Fish ($n = 39$)	Fishless ($n = 55$)	Fish ($n = 7$)	Fishless ($n = 3$)
Lateral spine (segment IX)	0.81 (0.13)	0.66 (0.09)	1.05 (0.10)	0.99 (0.14)
Lateral spine (segment VIII)	0.49 (0.09)	0.39 (0.07)	0.65 (0.08)	0.59 (0.05)
Dorsal spine (segment VI)	0.25 (0.17)	0.06 (0.10)	0.49 (0.14)	0.43 (0.11)
Dorsal spine (segment V)	0.32 (0.12)	0.13 (0.09)	0.47 (0.10)	0.39 (0.04)
Dorsal spine (segment IV)	0.28 (0.07)	0.19 (0.06)	0.33 (0.05)	0.33 (0.04)

faces in a trait–environment–size/time space. Such a conceptual fusion of the developmental and environmental processes of phenotypic diversification can yield a number of new insights into the mechanisms, and hence the evolution, of phenotypic plasticity. Comparisons of ontogenetic trajectories in different environments can, for example, be informative of the causal variation in the timing and rate of developmental processes that generate plasticity, and can show where alternative ontogenetic trajectories in different environments lead to similar phenotypic end products (Pigliucci and Schlichting 1995).

In general, the ontogenetic norms of reaction of defensive spine shape in *L. dubia* seen in the field showed that phenotypic modulation arises primarily as a result of accelerated development (allometric acceleration) in lakes with fish. However, spine morphology unfolded in a different manner in lateral and dorsal spines: while the ontogenetic trajectories of shape in dorsal spines diverged at the onset of ontogeny, those in lateral spines remained parallel until a given phase of ontogeny. Hence the timing, and thus the regulatory mechanism, of the development of these phenotypically integrated traits apparently differed. Very little is known about the genetic basis of adaptive phenotypic plasticity, and which types of genes and gene actions cause plasticity (Via et al. 1995). It has been suggested that allelic sensitivity is responsible for graded plasticity, whereas switched (or discrete) plasticity is thought to be caused by regulatory loci that turn structural genes on or off in different environments (de Jong 1995, Schlichting and Pigliucci 1995). Via et al. (1995) suggested that these two mechanisms need not be mutually exclusive: regulatory genes may affect graded plasticity as well, by turning subsets of the structural genes on or off. By incorporating ontogeny into the phenotype–environment space, we suggest another viable alternative to this possibility: regulatory genes may affect graded plasticity by turning loci on or off during *different phases* of ontogeny in different environments. Though direct genetic information is not available, our results suggest that this may be the case for the lateral abdominal spines in *L. dubia*. The difference in phenotypic expression of lateral spine shape in different environments seems to be at least in part due to a temporal difference in the action/effect of regulatory genes: the ontogenetic trajectories remain parallel until a certain point, after which they diverge (e.g., Fig. 3). This illustrates the importance of combining the study of plasticity with that of ontogeny, not only to deepen the understanding of the action of selection (Pigliucci and Schlichting 1995), but also to potentially gain insights into the epigenetics of phenotypic plasticity.

Allometric growth (ontogenetic allometry) is often thought of as constraining adaptive evolution (Maynard-Smith et al. 1985). It seems, however, that allometric growth commonly forms the basis for graded phenotypic plasticity (Pigliucci and Schlichting 1995); if the parameters of growth trajectories vary in different environments, phenotypic plasticity will result. To the extent that genetic variation in the environmental sensitivity of the parameters of growth trajectories is present, patterns of ontogenetic reaction norms, as well as those of allometry itself, may evolve in complex adaptive ways. Hence, a novel arena for phenotypic adaptations emerges by integrating environmental and ontogenetic aspects of development, where ontogenetic allometry can be viewed as a mediator of adaptation as much as a constraining factor.

Environmental heterogeneity in the field

Much of the work on phenotypic plasticity has been performed by experimental manipulations of single environmental factors in the laboratory, and the link between theory and empiricism is thus biased: our knowledge of actual patterns of environmental heterogeneity and phenotypic expression in unmanipulated natural populations, and hence the role of phenotypic plasticity in natural selection, is limited (see Via et al. 1995). The current contribution is one of the few studies that combine measurements of phenotypic plasticity in natural environments with controlled inductions in the laboratory. While it is clear that the experimentally induced response in defensive morphology in *L. dubia* corresponds very well qualitatively with that occurring in natural populations with and without fish in terms of induction of spine growth, it is also clear that overall defensive morphology differed quantitatively in the laboratory and in the field (Fig. 3; Tables 4 and 5). This may, of course, be due to genetic differences in

ontogenetic reaction norms between laboratory and field individuals. However, considering the limited geographic sampling area of this study (Johansson and Samuelsson 1994) and the documented high migration rates of adult *L. dubia* during the prereproductive period (Pajunen 1962), a major effect of genetic differentiation is very unlikely. Instead, we suggest that the phenotypic expression of spine shape during growth is affected by additional environmental variables (e.g., additional predatory cues, food availability and quality, water temperature and chemistry), which differed between natural populations and the laboratory setting and which exert an influence on the expression of spines above and beyond that of fish presence. Hence, our results collectively form a very strong case for adaptive phenotypic plasticity observed in nature, but they also illustrate a common problem with experimental studies of phenotypic plasticity: while adaptive patterns can be deduced from qualitative aspects of the results of controlled laboratory experiments, these results may only very rarely correspond quantitatively with the phenotypes expressed in analogous environments in natural populations. In nature, organisms face variations in a multitude of environmental factors, all of which may interactively affect phenotypic expression of morphological traits, leading to a dilution of the phenotypic effects of any single factor.

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