

- L02379, and L02402); Australian onychophorans by D.M.R. (L02377, L02378, L02380, L02395, and L02414) and *Plicatoperipatus jamaicensis* by P. D. N. Herbert (Department of Zoology, University of Guelph, Canada) (L02410); chelicerate, *Liocheles waigiensis* by R. Moran (CSIRO) (L02397) and crustacean, *Cherax quadricarinatus* by R. Bedding (CSIRO) (L02396); hexapods, *Macropodexia* sp., *Scaptia* sp., *Ceromya cf. fergusonii*, and *Australofannia* sp. by D. H. Colless (CSIRO) (L02384, L02393, L02387, and L02401); *Lucilia cuprina*, *Musca domestica*, and *M. vetustissima* by P. Cranston (CSIRO) (L02391, L02409, and L02400); *Anopheles hillii* by P. Sweeney (Australian Army) (L02382); taxa within *Austrosimulium* by J.W.O.B. (L02383, L02385, L02386, L02390, L02398, and L02399); *Drosophila melanogaster* by W.A.O. (L02394); and *Ctenolepisma longicauda* by J. A. L. Watson (CSIRO) (L02381).
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 33. J. Trueman wrote the T-PTP randomizer and photograph for Fig. 1 was supplied by R. Oldfield. We acknowledge the technical help of R. Feldman, J. Oakeshott, D. L. Swofford, and E. Zurcher and constructive comments from K. J. Ballard and B. J. Richardson.

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Predator-Induced Phenotypical Change in Body Morphology in Crucian Carp

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In a field experiment where the presence or absence of piscivorous pike (*Esox lucius*) in ponds was manipulated, the morphology of crucian carp (*Carassius carassius*) diverged, such that individuals became deeper bodied in pond sections with pike. A laboratory experiment confirmed that the presence of this predator induced a change in body morphology in the carp. Estimation of prey vulnerability to predation by pike, a gape-limited predator, revealed that this increase in body depth resulted in crucian carp reaching a size that provided refuge from predation. However, this change in morphology incurs a cost through an increase in drag when the carp are swimming. Because crucian carp are limited by resources in the absence of piscivores and by the substantial cost of the defensive morph in their presence, phenotypic plasticity should be the optimal strategy for this species.

Various morphological structures in prey organisms function as efficient adaptations against predation (1), and these morphological defenses could be either constitutive or environmentally induced. The evolution

and maintenance of inducible defenses is favored when the defense incurs a fitness cost, when predation intensity varies temporally or spatially, and when prey have reliable cues for predator detection (2, 3). Predator-induced morphological defenses occur in a number of invertebrates, mainly aquatic taxa (2). Waterborne cues from predators or chemicals released by injured

conspecifics trigger the development of defenses that reduce predation rates (2, 4). However, induced defenses have been shown to incur a fitness cost through a reduction of growth or reproduction or both (2, 4, 5). Here, we report on a predator-induced change in body morphology in a vertebrate, the freshwater fish crucian carp (*Carassius carassius*).

Crucian carp are extremely vulnerable to predation (6, 7). In lakes with piscivores, especially pike *Esox lucius*, crucian carp populations consist of a small number of large individuals (6). However, without piscivores, crucian carp form dense populations of small individuals (6–8). The body morphologies of monospecific pond populations and multispecies lake populations differ, with lake individuals much deeper bodied. The two morphs originally were considered as separate species (*Cyprinus vulgaris* and *C. gibelio*); however, in the early 1800s it was shown by transplant experiments that these two species were one (9). The presence of two morphs has previously been considered a result of differences in resource levels; however, we show that increased body depth can also be an inducible morphological defense that reduces the risk of predation.

For part of a study evaluating the effects of trophic structure on freshwater communities, we divided into halves two small, eutrophic ponds (Severin's and Mats' ponds, 0.1 ha each) with monospecific crucian carp populations and introduced pike into one half (10). After 12 weeks, crucian carp had diverged in body shape; in pond sections with pike, carp tended to have a deeper body (Fig. 1). Given this result, we hypothesized that the change in body morphology could be a result of several things: (i) selective predation, (ii) an increase in resource availability, or (iii) a predator-induced phenotypic modification of body shape. The small variance in body depth and the absence of overlap between treatments (Fig. 1) suggested no polymorphism with regard to this trait in the original population; thus, selective predation on genetically determined morphs could not account for the increase in body depth.

High resource availability may be responsible for the shift in morphology, as suggested by a study in Finland where crucian carp increased in body depth when introduced at a low density of 187 fish per hectare to a fishless pond (8). In our ponds, the reduction of the crucian carp density by pike permitted an increase in the density of large, cladoceran zooplankton (11). This increase in food availability in the pike section could account for the differences in the carp body depth. However, in another experiment we transplanted crucian carp from a pond with a

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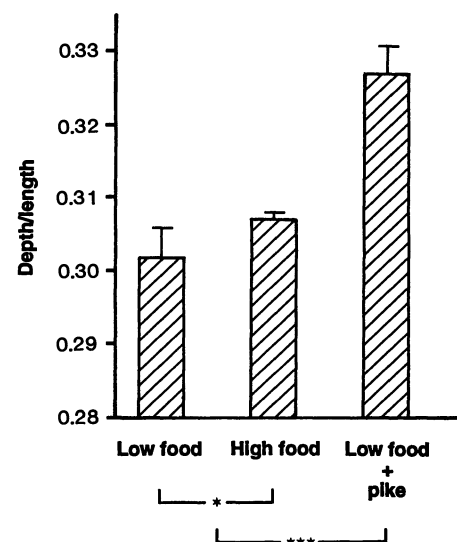
high-density, stunted population (John's Pond) to a fishless pond (Revinge Pond). Because the fishless pond had a much larger biomass of zooplankton and benthic invertebrates as compared to ponds with crucian carp (11), crucian carp grew faster there (0.319 g per day as compared to 0.096 and 0.097 g per day in the control sections of Severin's and Mats' ponds, respectively). Greater resource availability in Revinge Pond did not, however, increase body depth (the body depth-length ratio in Revinge Pond was 0.301 ± 0.011 , mean $\pm$ SD; in Severin's Pond (pike section), the ratio was 0.370 ± 0.015 ; and in Mats' Pond, it was 0.388 ± 0.013).

To investigate the mechanisms behind the shift in body morphology, we performed a laboratory experiment where we quantified body morphology of crucian carp as a function of food level (low or high) or presence of pike (12). High-food carp became marginally deeper bodied than low-food carp (Fig. 2). However, the presence of pike caused a large increase in body depth of crucian carp as compared to low- or high-food treatments without pike (Fig. 2). Thus, the presence of pike induced a phenotypical change in resource allocation, giving priority to growth in body depth.

Increasing body depth should benefit crucian carp by reducing predation. Piscivores, such as pike, are gape-limited pred-

ators (13); the body depth of prey relative to the mouth width of the pike constrains maximum prey size. Piscivores tend to feed on prey that are smaller than the maximum size possible (14, 15), but, as shown in laboratory experiments, prey that have not yet reached an absolute size refuge may still benefit from an increase in body depth because of longer piscivore handling times (15, 16) and a redirection of the strike caudally (17), which increases the probability of escape. Calculations of the relative vulnerabilities (18) for crucian carp of different body depths indicate that in sections of the ponds without pike, almost all crucian carp were vulnerable to predation (Fig. 3), whereas in sections with pike the increasing body depth provided an absolute size refuge for the remaining carp, with the exception of juveniles hatched during summer.

The plasticity of the morphological defense trait suggests that this trait incurs a fitness cost to the prey that could be avoided when predators are absent (2, 3). Minimizing costs should be especially important in situations where intraspecific competition is intense, such as in the dense crucian carp populations in systems without pike. A change in body morphology affects swimming performance. The theoretical total drag (19) for the body of a 140-mm crucian carp swimming at a speed of 10 cm/s is 32% greater for a deep-bodied carp from the pike section than for a shallow-bodied, fusiform carp from the control section. Thus, assuming that carp use the same foraging (swimming) mode in both sections, the cost of swimming increases markedly with the presence of pike. Gut content analysis of crucian carp indicated no difference in



diet between pike and pikeless sections (11), which suggests that carp use the same foraging mode in both sections. Once a prey has reached the absolute size refuge, we expect a loss of the induced, costly morphology (5), which for crucian carp means redirecting energy allocation toward growing longer. To date, no change in the body depth-length ratios of larger fish has occurred in our ponds. However, in three Finnish ponds with piscivores (6), the body depth-length ratio declined with increasing length for fish longer than 150 mm in total length (20).

When the cost of a defense is high, selection should favor the evolution of inducible defenses when the prey have reliable cues for detecting the predator and the predation pressure is variable (2, 3). Our experimental design could not discern the precise cue that triggered the body morphology modifications in crucian carp, but cyprinids do respond behaviorally to alarm substances released by conspecifics when attacked by piscivores (21). In habitats where severe winter conditions with anoxia eliminate other species, especially piscivores, crucian carp can survive by using alternative metabolic pathways (22). Stochastic environmental disturbances coupled

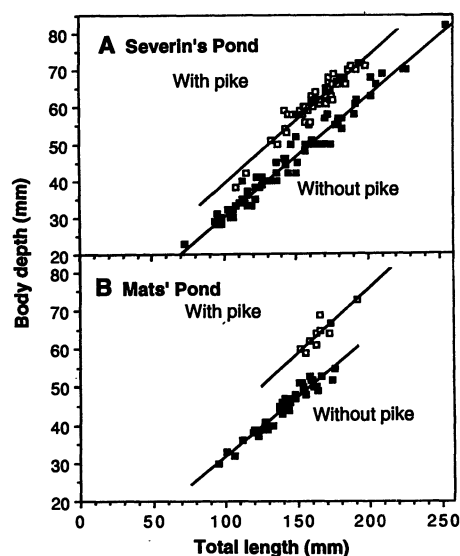
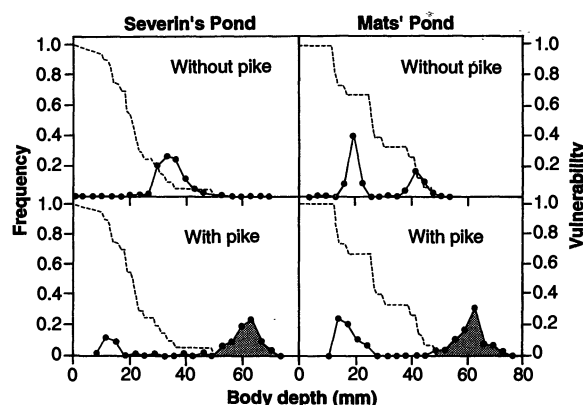


Fig. 1. The relation between total body length (L) and body depth (D) of crucian carp in the presence and absence of pike in two ponds in southern Sweden. Severin's control: $D = -2.728 + 0.328L$, $SE = 0.007$; Severin's pike: $D = 4.765 + 0.344L$, $SE = 0.014$; Mats' control: $D = 0.391 + 0.315L$, $SE = 0.012$; and Mats' pike: $D = 6.880 + 0.374L$, $SE = 0.066$. Regressions were significantly different between treatments in both ponds (analysis of covariance, $P < 0.001$), but slopes did not differ ($P > 0.05$) in either pond.

Fig. 3. Relative vulnerability to pike predation (dashed lines) and size distribution (circles) of crucian carp in two ponds as a function of crucian carp body depth. Shaded areas denote crucian carp that are invulnerable to predation because of their refuge in size.



with recolonization by piscivores could thus create the variability in predation pressure needed to promote the evolution of an inducible defense in crucian carp.

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12. Each of 15 165-liter aquaria was divided into two compartments of equal size by a plastic screen partition (mesh size: 20 mm). Three sides of the aquaria were covered externally with black plastic. Aquaria were filled with dechlorinated water that was continually filtered (one filter per aquarium), and their bottoms were covered with a thin layer (2 cm) of sand. One-third of the water was exchanged every week. Aquaria were aerated and kept at 20° ± 1°C with a regime of light to darkness of 10:14 hours. Crucian carp were collected on 10 to 11 October 1991 in Severin's Pond (section without pike) with trap nets. Length and body depth were measured. The experiment was started on 11 October when nine crucian carp were introduced into one of the compartments of each aquarium. Aquaria were randomly assigned to treatments: (i) low food, (ii) high food, or (iii) low food + pike; each treatment had five replicates. Initial body depth-length ratios were similar between treatments (0.295 ± 0.002, 0.295 ± 0.004, and 0.296 ± 0.002, respectively; mean ± SD). Pike were introduced to the second compartment of aquaria used for the treatment with pike. Crucian carp were fed daily with a mixture of trout pellets and frozen chironomids at two different rations, low or high. The low-food ration was initially set at 3% of crucian carp dry weight per day, whereas the high ration was 6% per day. Rations were increased to 6 and 12%, respectively, after 1 month, because low-food carp decreased in weight over this period. Pike were fed three times weekly with one crucian carp. The experiment ended after 58 days, when all fish were measured.
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18. The relative vulnerability (V) to ingestion for different prey body depths (d) was calculated as

$$V_d = 1 - \sum_{w=0}^d W$$
 where W is the frequency of pike mouth widths (w) in the population. Because we knew the exact size distribution of pike introduced into ponds, we could calculate mouth widths from $w = 0.087(TL) - 1.38$, where TL = total length. Equations are from K. D. Hambright, R. W. Drenner, S. R. McComas, N. G. Hairston, Jr., *Arch. Hydrobiol.* **121**, 389 (1991).
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Activation of a Plant Gene by T-DNA Tagging: Auxin-Independent Growth in Vitro

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A transferred DNA (T-DNA) tagging vector with the potential to produce dominant mutations was used with cocultured *Agrobacterium tumefaciens* and protoplasts to tag genes involved in the action of the plant growth substance auxin. Transgenic calli were selected for their ability to grow in the absence of auxin in the culture media. From one experiment, 12 calli that displayed this phenotype were recovered, of which 11 were able to regenerate into plants. In one plant studied in detail, protoplast division in the absence of auxin genetically cosegregated with a single T-DNA insert. A messenger RNA encoded by a 6.4-kilobase sequence of plant genomic DNA rescued from the mutant is overexpressed relative to untransformed plants. The genomic DNA, as well as a cognate complementary DNA, once transfected into protoplasts promote growth and cell division in vitro in the absence of exogenously added auxin.

In plants, auxins and cytokinins are required to induce cell division (1) and affect plant growth and development (2), although little is known of the molecular basis by which normal plant cells synthesize, perceive, or respond to plant growth substances (3). Several plant pathogens are able to induce growth and division of infected plant cells as a result of the synthesis of growth substances (4), and in the case of the tumor-inducing soil bacteria *Agrobacterium tumefaciens*, neoplastic growth results from the integration of a defined sequence

of bacterial DNA, the T-DNA, into the genome of the infected plant cell (5). T-DNA encodes proteins that interfere with the normal biosynthetic pathways of plant growth substances (6). T-DNA is used as a transformation vector (7) and gene tag (8). We describe here a tagging vector derived from T-DNA that produces dominant mutations and thus allows selection for specific mutations from the population of primary transformants.

The T-DNA tagging vector pPCVICE-n4HPT (Fig. 1A) contains multiple transcriptional enhancers derived from the cauliflower mosaic virus (CaMV) 35S RNA promoter located near the right border sequence. After insertion into the plant genome by *Agrobacterium*-mediated transformation, genes present in the plant DNA

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