Ecology, 80(3), 1999, pp. 1076–1080

PROXIMATE CAUSES OF CANNIBALISTIC POLYPHENISM IN LARVAL TIGER SALAMANDERS

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Abstract. Larval tiger salamanders, *Ambystoma tigrinum*, exist as small-headed ''typical'' morphs that feed mainly on aquatic invertebrates and as large-headed ''cannibal'' morphs that often prey on conspecifics. Previous experiments have demonstrated that cannibals are induced facultatively when crowded with conspecific larvae. We asked: (1) What sensory cues trigger expression of cannibals? (2) Does larval age influence expression of cannibals? (3) Are cannibals also induced by crowding with heterospecifics? (4) Do cannibals suppress others from developing into cannibals? We found that tactile cues from other salamander larvae were necessary to elicit cannibals. Cannibals were produced when tiger salamander larvae were crowded with congeneric larvae, and these heterospecific-induced cannibals were expressed at an earlier age than those induced by conspecifics. Furthermore, younger larvae were more likely to develop into cannibals. Finally, visual and chemical cues from cannibals did not suppress other larvae from developing into cannibals. We infer the adaptive significance of polyphenism in this and other species.

Key words: Ambystoma tigrinum; *cannibalistic polyphenism; phenotypic plasticity; salamander, eastern tiger; trophic polyphenism.*

Introduction

Polyphenism is the occurrence in a population of alternative phenotypes that are produced from a single genotype in response to different environmental stimuli (West-Eberhard 1989). Organisms that exhibit polyphenism provide an excellent arena for examining the mechanistic and functional bases of phenotypic variation because the variation is discrete and easily manipulated. Also, polyphenism often results in expression of the phenotype most advantageous under current environmental conditions, thereby facilitating an examination of the fitness consequences of different phenotypes (West-Eberhard 1992).

Some of the best systems for studying proximate and evolutionary causes of polyphenism are species or populations where individuals occur as alternative ''cannibalistic'' or ''noncannibalistic'' phenotypes (Polis 1981). Cannibalistic polyphenisms are ideal for such studies because predictions regarding when individuals should assume the cannibalistic phenotype are generally clear (Crump 1992). In particular, cannibalistic polyphenisms are thought to evolve in response to limited alternative prey availability and to situations where rapid development and large size induced by cannibalism are advantageous (Crump 1992). However, the environmental cues that trigger cannibalistic and noncannibalistic phenotypes are largely unknown. Previous research has revealed that distinct cannibal morphs are triggered by starvation (in some ciliates and cellular slime molds: Waddell 1992), diet (in rotifers and spadefoot toad tadpoles: Gilbert 1973, Pfennig 1990), and crowding with conspecifics (in larval tiger and *Hynobius retardatus* salamanders: Collins and Cheek 1983, Nishihara 1996).

In this paper, we examine the proximate basis of cannibalistic polyphenism in larval eastern tiger salamanders, *Ambystoma tigrinum tigrinum.* Larval tiger salamanders often occur in nature as a ''typical'' morph that feeds mostly on invertebrates and as a larger, faster-developing ''cannibal'' morph that often preys on conspecifics (Collins and Holomuzki 1984). Cannibals are induced facultatively (Collins and Cheek 1983, Pfennig et al. 1991), and morph determination is reversible (up to a certain age) if conditions change (Reilly et al. 1992).

Although previous studies have suggested that crowding with conspecifics induces cannibal morphs, the precise proximate mechanisms that mediate this response are unclear. Moreover, factors other than con-

Manuscript received 9 January 1998; revised 5 May 1998; accepted 31 May 1998; final version received 26 June 1998.

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specific density may trigger cannibal morphs. For instance, when reared in mixed sibship groups, *A. tigrinum* salamander larvae are more likely to transform into cannibals than when reared with siblings only (Pfennig and Collins 1993), suggesting that an animal's kinship environment influences production of cannibals. Increased variation in larval size distribution also appears to stimulate development of cannibal morphs (Maret and Collins 1994), presumably because greater disparities in size facilitate cannibalism (Polis 1981). Additionally, density of heterospecific prey may be an important stimulus. In some populations, cannibal density is positively correlated with density of *Pseudacris* tadpoles, which are preyed upon by cannibals (Loeb et al. 1994).

We sought to clarify the proximate basis of cannibalistic polyphenism in tiger salamanders by addressing four questions: (1) What sensory cues trigger expression of cannibals? (2) Does larval age influence expression of cannibals? (3) Are cannibals produced when crowded with heterospecific salamander larvae? (4) Do cannibal morph larvae suppress other larvae from developing into cannibals?

METHODS AND RESULTS

General experimental procedures

In all experiments, salamander larvae were reared in the laboratory in 38-L aquaria containing aged tap water. Larvae were fed live brine shrimp nauplii (*Artemia* sp.; San Francisco Bay, San Francisco, California USA) daily ad libitum (the hatch from 0.015 g of eggs/larva). Larvae were monitored daily and scored as being either typical or cannibal morphotypes using criteria given by Powers (1907) and Pedersen (1991). Specifically, as illustrated in Lannoo and Bachmann (1984), larvae were scored as cannibals based on their enlarged vomerine ridge and elongate teeth (see also Pfennig et al. 1991, Pfennig and Collins 1993). All statistical analyses were done using the SAS JMP computer statistics package version 3.1.5 (SAS 1994).

Experiment 1.—To investigate what sensory cues trigger expression of cannibals, *A. t. tigrinum* eggs were collected on 8 April 1995 from a single pond on the Max McGraw Wildlife Foundation, Kane County, Illinois, USA. Egg masses consisting of 10–50 eggs each were collected throughout the pond (to ensure that multiple families were represented) and immediately transferred to two 38-L aquaria filled with 22 L of aged tap water. Upon hatching, larvae were assigned to one of five different treatment groups that modified the ability of focal larvae to sense the presence of conspecifics in their surroundings (Fig. 1). We used 16 replicates of each of five treatments testing for effects of sensory cues on production of cannibals. We simultaneously tested effects of (a) visual cues only; (b) visual cues and chemical cues only; (c) chemical cues only; (d) chemical cues, visual cues, and tactile cues; and (e) no cues (isolated focal animals).

Similarly sized focal animals were placed individually in clear plastic 2-L bottles and allowed to have varying degrees of sensory access to stimulus animals. Focal animals were initially larger than stimulus animals (mean snout–vent length [SVL] for focal animals = 14.99 mm; mean SVL for stimulus animals = 10.17 mm). By using slightly larger focal animals, we increased the likelihood that these animals would develop into cannibals, since cannibals originate from the upper end of the larval size distribution (Maret and Collins 1994). Bottles containing focal animals were either wrapped in aluminum foil (no visual cues treatment) or not (visual cues treatment) or perforated (chemical cues treatment) or not (no chemical cues treatment). To test for the effect of tactile cues, focal animals were either placed alone inside a bottle (no tactile cues treatment) or were allowed to swim among (and thereby contact) the stimulus animals (tactile cues treatment). Each treatment group (except isolates) was then placed in the center of a 38-L aquarium containing 21 L of water and 14 stimulus animals. Perforations were made such that each bottle contained ~11 rows of 8 holes (diameter = 5 mm). A ball of crushed aluminum foil was placed in visual treatments to control for the aluminum foil in the no visual treatments. The bottles holding isolates were placed on a shelf separated from the aquaria containing the other treatment groups by a cardboard partition. Thus, all animals experienced similar temperatures and photoperiods. The experiment ended after 24 d, which was sufficient time for cannibals to be produced. Focal animals were killed by immersion in a 1:2000 solution of 3-aminobenzoic acid ethyl ester (MS-222) and preserved in 90% ethanol. Focal animal morphotype was assessed by visual examination of vomer ridge.

The cannibal phenotype was produced only when focal animals had access to combined chemical, visual, and tactile cues associated with conspecifics (Fig. 1). All 16 of the focal animals in this treatment group developed into cannibals. In contrast, no cannibals were produced in any of the other four treatment groups ($N = 64$). Thus, tactile cues are necessary to induce the cannibal morphology; chemical cues alone, visual cues alone, or a combination of visual and chemical cues are not sufficient.

Experiment 2.—To determine if larval age influences rate of cannibal production, eggs of *A. t. tigrinum* were collected on 15 March 1995, from the Sam Parr Biological Station, Marion County, Illinois, USA. Eggs were immediately partitioned into one of two treatments. In the ‘‘young’’ treatment, larvae were placed

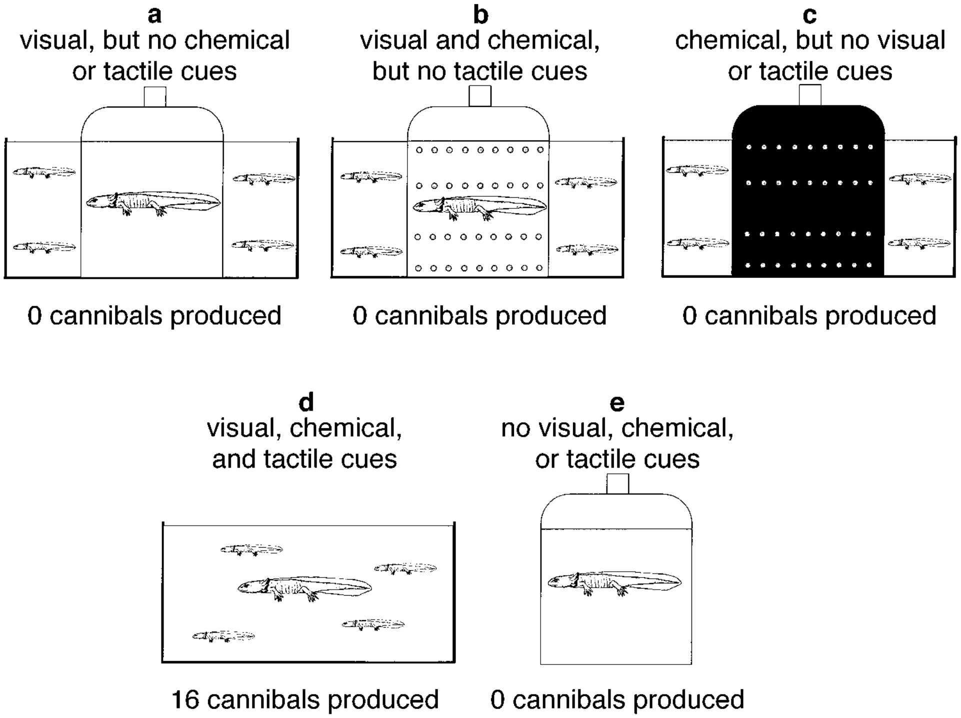

FIG. 1. Diagrammatic representation of treatment groups used to alter a focal larva's perception of cues associated with 14 smaller, conspecific stimulus larvae. The basic experimental setup was to place the focal larvae in a 38-L aquarium containing stimulus larvae. To vary the focal animal's tactile access to stimulus animals, focal larvae were either allowed to swim freely among stimulus larvae (d) or they were placed inside of a 2-L plastic bottle, which was then positioned inside the aquarium (a–c, e). To vary the focal animal's chemical access to stimulus animals, focal larvae were either placed in a perforated bottle (b, c) or an intact bottle (a, e). To vary the focal animal's visual access to stimulus animals, focal larvae were either placed in a clear bottle (a, b) or in a bottle wrapped with aluminum foil (c), or their bottle was placed on a shelf (e). There were 16 replicates of each of the five treatment groups, and the numbers below each group refer to the number of cannibals that were produced in each treatment category.

in groups of 15 into one of 30 tanks filled with 21 L of aged tap water. In the ''old'' treatment, 500 eggs were isolated into 250-mL cups filled with aged tap water. These larvae were isolated for 37 d and then placed in groups of 15 larvae into 11 aquaria filled with 21 L of aged tap water. The experiment was ended 40 d after each treatment was initiated and morphotype was assessed as before by visual examination of vomer ridge.

Twenty-one of 30 aquaria (70%) containing young larvae produced cannibals. By contrast, only 3 of 11 aquaria (27%) containing old larvae produced cannibals. Thus, younger larvae were significantly more likely to develop into cannibals (Fisher's exact test, $P = 0.029$).

Experiment 3.—We sought to determine if cues from heterospecific larvae are sufficient to induce the cannibal morphology in *A. tigrinum,* or if cues specific to conspecific larvae are required to induce the cannibal morphology. To test this, we collected eggs of *A. t. tigrinum* and small-mouthed salamanders, *A. texanum,* on 15 March 1995, from several ponds on the Sam Parr Biological Station, Illinois. Eggs were separated according to species and transported to the laboratory. Upon hatching, the larvae were divided into two treatments. One treatment consisted of 30 replicates of aquaria containing 15 *A. tigrinum* larvae reared in 22 L of aged tap water. The other treatment consisted of 26 replicates of aquaria containing one *A. tigrinum* larva reared with 14 slightly smaller *A. texanum* larvae in 22 L of aged tap water. The size difference increased the likelihood of a cannibal being produced (Maret and Collins 1994), yet did not alter the difference between treatments because equal-sized animals were used in both treatments. The experiment ended on day 40, whereupon focal animals were killed by immersion in a 1:2000 solution of 3-aminobenzoic acid ethyl ester and preserved in 90% ethanol.

To determine if cannibals induced by conspecifics differed from those induced by heterospecifics, vomer ridge width and snout–vent length were measured on all cannibals with digital calipers. Larvae were individually tagged so that the person conducting measurements was unaware of each animal's treatment group.

We found that cannibals were induced when individual *A. tigrinum* larvae were crowded with heterospecific larvae (*A. texanum*) and at a frequency equal to that when *A. tigrinum* were crowded with conspecifics. Sixteen of 26 (62%) larvae reared with *A. texanum* expressed the cannibal morphology, whereas 21 of 30 (70%) larvae reared with conspecifics expressed the cannibal morphology (Fisher's exact test, $P > 0.50$). Expression of the cannibal morphology, specifically log of vomer ridge width, was no more pronounced when larvae were reared with heterospecifics (*A. texanum*) than when larvae were reared with conspecifics (ANCOVA with log of snout–vent length as the covariate, $F_{1,29} = 0.051$, $P > 0.50$). However, cannibals were produced at a significantly earlier age when reared with heterospecifics than where reared with conspecifics (mean ± 1 SE, age = 26.1 ± 2.3 d when reared with heterospecifics and 35.3 ± 2.7 d when reared with conspecifics; chi-square = 7.37; df = 1; $P = 0.007$; Wilcoxon test).

Experiment 4.—We investigated whether cannibals suppress other larvae from developing into cannibals. Generally, no more than one cannibal is produced per aquarium, regardless of the number of larvae occupying the tank (Collins and Cheek 1983, Pfennig and Collins 1993; E. A. Hoffman, *personal observation*). Thus, in aquaria containing relatively few larvae, cannibals may inhibit other larvae from developing into cannibal morphs through chemical or visual cues or by diminishing the food supply, thereby making it less profitable for other larvae to develop into cannibals (Pfennig and Collins 1993). Alternatively, no inhibition may occur, but the first cannibal to develop may consume other larvae before they have a chance to develop into cannibals.

The experiment consisted of two treatments with *A. t. tigrinum* larvae experiencing varying access to cannibals. We collected eggs on 8 April 1995 from a single pond on the Max McGraw Wildlife Foundation, Kane County, Illinois, USA. Egg masses consisting of 10–50 eggs each were collected throughout the pond (to ensure that multiple families were represented) and immediately transferred to two 38-L aquaria filled with 22 L of aged tap water. Upon hatching, larvae were distributed into each aquarium at a density of 15 larvae per 21 L of aged tap water. For one treatment ($N = 11$ replicates), a cannibal was placed in a perforated clear plastic 2-L bottle, which was positioned in the center of the tank (identical to the bottles used in Experiment 1) and fed one conspecific two times each week. In the other treatment ($N = 11$ replicates), no cannibals were placed in the aquaria. The experiment was ended after 40 d and morphotype was assessed by visual examination of vomer ridge.

Of treatments with stimulus cannibals, 9 of 10 aquaria (90%) produced a second cannibal. Of the treatments without a stimulus cannibal, only 3 of 11 aquaria (27%) produced an additional cannibal. Therefore, contrary to expectation, the presence of a cannibal in a tank significantly increased the likelihood that another larva would develop into a cannibal (Fisher's exact test, $P = 0.008$).

DISCUSSION

This study clarifies some of the proximate mechanisms underlying cannibalistic polyphenism in larval tiger salamanders. Previous studies had determined that cannibals are produced only when larvae are crowded with conspecifics (Collins and Cheek 1983, Pfennig et al. 1991) and that the expression of cannibalistic polyphenism is mediated by various ecological factors, such as kinship environment (Pfennig and Collins 1993), larval size distribution (Maret and Collins 1994), and presence of alternative prey (Loeb et al. 1994). We found that tactile cues from other salamander larvae are necessary to elicit the cannibal phenotype. We also found that cannibal morphs can be induced when larvae are crowded with heterospecifics, suggesting that cues that induce the cannibal morphology are not limited to conspecifics. Additionally, older larvae are less likely to develop into cannibals than are younger larvae, implying that a critical window of time exists during which salamander larvae must experience the environmental stimuli that trigger cannibal morphs.

We also found that cannibal morph larvae were more likely to be produced in the presence of a cannibal in a bottle than when no cannibal or bottle was present in the focal animals' tank. However, it is not clear whether cannibals actually induced other larvae to develop into cannibals, or if the greater production of cannibals in the presence of a cannibal in a bottle was simply a perceived volume effect. In particular, because there was no bottle in the control tanks, cannibals may have been more likely to develop in the presence of the cannibal in a bottle because the larvae were more crowded. Nevertheless, our data do suggest that combinations of visual and chemical cues from a cannibal do not impede other larvae from developing into cannibal morphs.

There are at least two possible mechanisms by which tactile cues may trigger cannibal morphs in tiger salamander larvae. First, the cannibal phenotype may be elicited when a focal animal eats another salamander. However, some of the salamanders in Experiments 1 and 3 expressed the cannibal phenotype before any other larvae were eaten, indicating that predation is not necessary to induce the cannibal phenotype. Second, cannibal production may be triggered by increased tactile stimuli or stress associated with crowding. The physiological mechanisms by which such cues could stimulate cannibal morph production are unclear, however.

Regardless of the precise proximate mechanism by which cannibals are produced, there are at least two factors in addition to crowding with conspecifics that are important in mediating induction of cannibals in tiger salamanders. First, younger larvae appear to be more sensitive than older larvae to cues that induce cannibals. Second, crowding with heterospecific larvae also induces cannibals. It is likely that the cues necessary to stimulate cannibal production are produced only by phylogenetically similar organisms because tadpoles fail to induce expression of cannibals (Loeb et al. 1994; but see Walls et al. 1993).

What is the adaptive significance of the mechanisms of morph determination in tiger salamander larvae? In particular, why does crowding with both conspecific and heterospecific salamander larvae (but not tadpoles) induce cannibals? It may be more advantageous for a larva to develop into the large-headed cannibal morph, which is an adaptation for consuming large prey (Reilly et al. 1992), in the presence of large potential prey, especially if smaller prey are absent. Moreover, other salamander larvae are close competitors for food, further favoring the switch to an alternative cannibal morph in the presence of other salamanders if cannibals experience reduced competition for food. Indeed, cannibal morphs occur commonly in extremely ephemeral ponds inhabited only by conspecifics (see Rose and Armentrout 1976, Pfennig et al. 1991). In such settings, the enhanced growth rate of cannibals may be favored. Moreover, the proximate signal that stimulates cannibal morph production, tactile cues, would appear to predict the above condition that benefits cannibals: increased contacts with other salamanders would indicate that the focal animal is in a pond that is drying rapidly, and/or one that has many conspecifics and perhaps few alternative prey. Thus, the use of tactile cues to induce cannibals may enable these larvae to respond to their environment adaptively.

ACKNOWLEDGMENTS

We thank S. Collins, D. Enstrom, S. Juliano, M. Loeb, K. Pfennig, and two anonymous referees for commenting on the manuscript, and S. Ho, S. Naseesuddin, M. Pulins, and M. Reetz for laboratory assistance. This research was supported by the University of Illinois Graduate College Dissertation Travel Grant to E. Hoffman and by the Arnold O. Beckman Research Fund and NSF grant IBN 9512110 to D. Pfennig.

LITERATURE CITED

Collins, J. P., and J. E. Cheek. 1983. Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum.* American Zoologist **23**:77–84.

Collins, J. P., and J. R. Holomuzki. 1984. Intraspecific variation in diet within and between trophic morphs in larval tiger salamanders (*Ambystoma tigrinum nebulosum*). Canadian Journal of Zoology **62**:168–174.

Crump, M. L. 1992. Cannibalism in amphibians. Pages 256–276 *in* M. A. Elgar and B. J. Crespi, editors. Cannibalism: ecology and evolution in diverse taxa. Oxford University Press, Oxford, UK.

Gilbert, J. J. 1973. Induction and ecological significance of gigantism in the rotifer *Asplanchna sieboldi.* Science **181**: 63–66.

Lannoo, M. J., and M. D. Bachmann. 1984. Aspects of cannibalistic morphs in a population of *Ambystoma t. tigrinum* larvae. American Midland Naturalist **112**:103–109.

Loeb, M. L. G., J. P. Collins, and T. J. Maret. 1994. The role of prey in controlling expression of a trophic polymorphism in *Ambystoma tigrinum nebulosum.* Functional Ecology **8**: 151–158.

Maret, T. J., and J. P. Collins. 1994. Individual responses to population size structure: the role of size variation in controlling expression of a trophic polyphenism. Oecologia **100**:279–285.

Nishihara, A. 1996. Effects of density on growth of head size in larvae of the salamander *Hynobius retardatus.* Copeia 1996:478–483.

Pedersen, S. C. 1991. Dental morphology of the cannibal morph in the tiger salamander, *Ambystoma tigrinum.* Amphibia-Reptilia **12**:1–14.

Pfennig, D. W. 1990. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. Oecologia **85**:101–107.

Pfennig, D. W., and J. P. Collins. 1993. Kinship affects morphogenesis in cannibalistic salamanders. Nature **362**:836–838.

Pfennig, D. W., M. L. G. Loeb, and J. P. Collins. 1991. Pathogens as a factor limiting the spread of cannibalism in tiger salamanders. Oecologia **88**:161–166.

Polis, G. A. 1981. The evolution and dynamics of intraspecific predation. Annual Review of Ecology and Systematics **12**:225–251.

Powers, J. H. 1907. Morphological variation and its causes in *Ambystoma tigrinum.* Studies of the University of Nebraska **7**:197–274.

Reilly, S. M., G. V. Lauder, and J. P. Collins. 1992. Performance consequences of a trophic polymorphism: feeding behavior in typical and cannibal phenotypes of *Ambystoma tigrinum.* Copeia 1992:672–679.

Rose, F. L., and D. Armentrout. 1976. Adaptive strategies of *Ambystoma tigrinum* Green inhabiting the Llano Estacado of West Texas. Journal of Animal Ecology **45**:713–739.

SAS. 1994. Statistics made visual. SAS Institute, Cary, North Carolina, USA.

Waddell, D. R. 1992. Cannibalism in lower eukaryotes. Pages 85–101 *in* M. A. Elgar and B. J. Crespi, editors. Cannibalism: ecology and evolution in diverse taxa. Oxford University Press, Oxford, UK.

Walls, S. C., S. S. Belanger, and A. R. Blaustein. 1993. Morphological variation in a larval salamander: dietary induction of plasticity in head shape. Oecologia **96**:162–168.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics **20**:249–278.

———. 1992. Behavior and evolution. Pages 57–75 *in* P. R. Grant and H. S. Horn, editors. Molds, molecules, and metazoa. Princeton University Press, Princeton, New Jersey, USA