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Variation in Cannibalistic Polyphenism between Populations in the Salamander *Hynobius retardatus*

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ABSTRACT—Organisms sometimes change their phenotype to maximize fitness according to local environments. If the frequency of the broad-headed "cannibal" morph in the larvae of the salamander *Hynobius retardatus* has been evolutionarily maintained at a certain level within a population as a result of local adaptation, variations in its frequency should be found among different populations with environmental variation. We investigated whether variations in the frequency of the broad-headed morph were present in 2 different populations, Nopporo (a low-density population) and Erimo (a high-density population), by raising larvae from the respective populations under the same experimental conditions. The occurrence rate of the broad-headed "cannibal" morph was significantly different between the 2 populations when examined with different experimental larval densities. These results suggest that the reaction norm with respect to the frequency of the broad-headed morph is different between the Nopporo and Erimo populations. Because the local populations are assumed to be selected for under different environments, the different reaction norm might have evolved in response to different selection pressures.

Key words: cannibal morph, phenotypic plasticity, polyphenism, salamander

INTRODUCTION

In general, phenotypic differences in life history and morphological traits observed among natural populations or among animals reared under experimental conditions may arise from genetic differentiation due to local adaptation and drift, or phenotypic plasticity (polyphenism) (Stemberger and Gilbert, 1987; Jackson and Semlitsch, 1993; Buskirk and Schmidt, 2000). Polyphenism is expressed as two or more discontinuous phenotypes (alternative phenotypes) from a single genotype across a range of environmental conditions (DeWitt *et al*., 1998; Gotthard and Nylin, 1995; Scheiner, 1993). Previous studies in amphibians have reported distinctive morphotypes and food habits, such as cannibal and typical morphs, in larvae of the salamander *Ambystoma tigrinum* (Collins and Cheek, 1983), broad-headed "cannibal" and typical morphs in *Hynobius retardatus* larvae (Wakahara, 1995; Nishihara, 1996) and in *H. nigrescens* larvae (Takahashi, 1992), and carnivores and omnivores in spadefoot toad tadpoles (Pfennig and Frankino, 1997; Pfennig and Murphy, 2000).

Cannibalistic polyphenism refers to phenotypic differences in behavior, morphology, and life history between cannibal and typical morphs (Polis, 1981; Crump, 1992). Larvae of the salamander *H. retardatus* show either a broad-headed "cannibal" morph that consumes conspecifics or a typical morph that mostly does not cannibalize (Wakahara, 1995, 1997; Michimae and Wakahara, 2001). A previous experimental study suggested that the "cannibal" morphs are induced by higher densities of conspecifics but are strongly suppressed in groups of siblings, even if larval densities are high (Michimae and Wakahara, 2001).

During the course of our field survey, we noticed that possible environmental factors, such as the densities of conspecifics, that affect the frequency of the broad-headed "cannibal" morph, vary considerably among natural ponds. If the broad-headed morphs and their frequency have been evolutionarily maintained within a population because of adaptive advantages and/or genetic drift, we should find some variation in their frequency among populations of different environments. In other words, we can detect different reaction norms among the remote populations. In this study, we examined whether variations in the frequency of the broad-headed morph are present among remote populations, that would be genetically isolated, even under the same experimental conditions.

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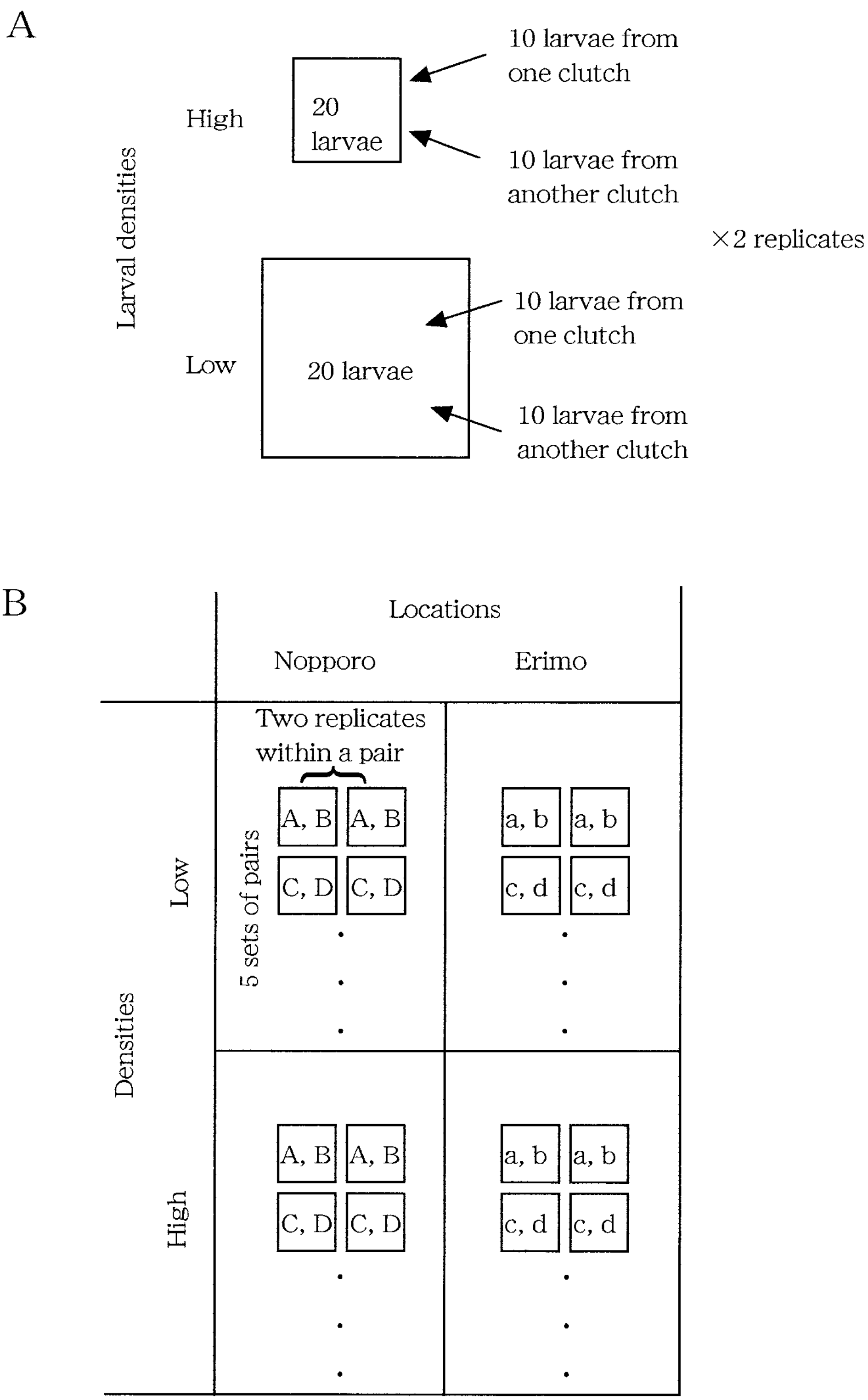

Fig. 1. Experimental design. **A**: Larvae hatched from 2 different egg clutches from each pond (Nopporo and Erimo) were mixed and reared in tanks of different sizes. Forty larvae hatched from a clutch were allotted to 4 tanks, 2 small (S, 17 cm×10 cm×11 cm) and 2 large (L, 30 cm×25 cm×17.5 cm) tanks, with 10 larvae placed in each tank. Forty larvae hatched from another clutch of the same pond were also allotted in the same way to the same tanks, so there were 20 mixed-sibling larvae per tank. The small tanks were filled with 0.9 l of dechlorinated tap water, and thus served as a high-larval-density treatment. The large tanks were filled with 3.7 l of dechlorinated tap water and served as a low-larval-density treatment. **B**: Five sets of experiments consisting of two replicates of the same, specific mixed-sibling pairs were performed by using 10 egg clutches from each pond.

MATERIALS AND METHODS

Animals

During the breeding season (from early April to late May) in 2001, fertilized egg clutches of *Hynobius retardatus* were collected from 2 different ponds at Nopporo (N43°02' E141°28'; surface area, approximately 10 m^2), located in the vicinity of Sapporo, and at Erimo (N42°7' E143°16'; surface area, approximately 150 m^2) located in Hidaka district, Hokkaido, Japan. Because the Nopporo pond is a temporary pond mainly made of the melting snow and thus very shallow (approximately 40 cm at the deepest point during the breeding season), the pond sometimes dries up by late autumn depending on the amount of rain of the year. Contrary to this, the Erimo pond is a deep (the maximum depth, 120 cm), permanent pond. Fishes were not observed at all in both ponds. At the Nopporo pond, spawned egg clutches of *H. retardatus* have been maintained in low density, and approximately 2 egg clutches/ m^2 are observed every breeding season. In contrast, high density has been maintained in the Erimo, and approximately 10 egg clutches/ m^2 are observed. The egg density in each pond has been confirmed at least 5 recent years. Ten egg clutches were collected from each pond (A–J clutches from Nopporo, a–j clutches from Erimo) and placed in the laboratory, separately into different medium tanks (22×15×12.5 cm) filled with 1.6 l of dechlorinated tap water at 4°C, until use. The hatching day of the embryos and the developmental stage of the larvae were controlled by placing the embryos or larvae either at 4°C or at room temperature (Arai and Wakahara, 1993).

Experimental procedures

In order to determine the occurrence rate of the broad-headed morph in each population, Nopporo and Erimo, larvae hatched from 2 different egg clutches from each pond were mixed and reared in tanks of different sizes (mixed-sibling experiment, see Michimae and Wakahara, 2001). Forty larvae hatched from a clutch were allotted to 4 tanks, 2 small (S, 17×10×11 cm) and 2 large (L, 30×25×17.5 cm) tanks, with 10 larvae placed in each tank. Forty larvae hatched from another clutch of the same pond were also allotted in the same way to the same tanks, making mixed-sibling groups of 20 larvae per tank (Fig. 1). The small tanks were filled with 0.9 l of dechlorinated tap water, and thus served as a high-larval-density treatment. The large tanks were filled with 3.7 l of dechlorinated tap water and served as a low-larval-density treatment.

The broad-headed morph in *H. retardatus* larvae has been described and defined previously (Michimae and Wakahara, 2001). To characterize the shape of the head quantitatively, we used the ratio of head width at the level of the eyes (HWE) to the largest head width (LHW). HWE/LHW≥0.9 defined a broad-headed cannibal morph, and HWE/LHW≤0.86 a typical morph (see Fig. 1 of Michimae and Wakahara, 2001). Numbers of broad-headed morphs were counted every morning. Because the broad-headed morph, once established, consumes conspecifics (Wakahara, 1995), the number of larvae in tanks with broad-headed morphs drastically decreases due to recurring cannibalism. Therefore, the broad-headed morphs were removed from the tanks as soon as they were detected. The larvae were fed with a live freshwater oligochaete (*Tubifex*) from 9:00 to 12:00 A.M. every 3 days. They were always given enough food to eat within 3 h, and any food remaining in their tanks was removed after the feeding period. The rearing water was exchanged every 3 days during the experiment. Our experiment in the laboratory was conducted at room temperature with a natural light/dark schedule.

Statistical analysis

Because it was difficult for all 10 clutches in each location used in this study to hatch out at the same time, we could not randomly allot the larvae hatched from a clutch to 2 treatments of low and high densities. For convenience of experiments, two replicates of the same, specific pairings with either low and high density were prepared (Fig. 1B). Larvae from 10 egg clutches at each pond were thus allotted to 5 sets of the specific pairings. In total, 800 larvae (400 larvae each from Nopporo and Erimo, respectively) were used in this experiment. When all the data were combined together irrespective of pairings in this type of experiment, biased conclusions might be drown. Thus, we used a test of homogeneity of odds ratios for replicated 2×2 tables (Sokal and Rohlf, 1995) to estimate 1) the effects of replicates within a pair in 4 cells of 2 locations (Nopporo and Erimo)×2 densities (low and high), and 2) the effects of replicated pairs in each location, respectively (Fig. 1B).

RESULTS

The first, the homogeneity of odds ratios of 5 separate tables (2 morphotypes×2 replicates within a pair) was examined. Because no significant differences were observed among 5 pairs of 4 cells (Nopporo-low density: χ^2=0.348, df=4; Nopporo-high density: χ^2=0.348, df=4; Erimo-low density: χ^2=5.367, df=4; Erimo-high density: χ^2=0.865, df=4), it was concluded that we could joint 5 odds ratios for each experimental group. The next step was to estimate the effects of replicates within a pair on overall average (joint) odds ratios for the 5 pairs. Because no significant differences were observed (Nopporo-low density: χ^2=0, df=1; Nopporo-high density: χ^2=0, df=1; Erimo-low density: χ^2=0, df=1; Erimo-high density: χ^2=0.135, df=1), the effects of replicates could be ignored and the data of the replicates were pooled.

The second, we estimated the effects of densities among 5 pairs in each location. If the homogeneity of their odds ratios was approved, we could pool the data, the numbers of broad-headed and typical morphs, among 5 pairs in each location. Because each odds ratio of 5 separate tables (2 morphotypes×2 densities) was approved to be homogeneous in the 2 locations (Nopporo: χ^2=0.93, df=4; Erimo: χ^2=6.027, df=4), it was statistically confident to pool the odds ratios of 5 separate tables. Finally, the effects of larval density were examined in each population based on the joint odds ratios for the 5 pairs.

The occurrence rate of the broad-headed morph was much higher in larvae derived from Erimo (representative of a high-density population; χ^2=14.02, df=1) than in those derived from Nopporo (representative of a low-density population; χ^2=0.144, df=1) under the same experimental conditions (Fig. 2). Larvae derived from the Nopporo population consistently showed a low occurrence rate of the broad-headed morph regardless of experimental larval density (Fig. 2). However, larvae from the Erimo population showed different occurrence rates of the broad-headed morph at different experimental larval densities: a lower occurrence rate at low larval densities but a higher occurrence at high larval densities. Larvae from different populations showed a different reaction norm (i.e., occurrence rate of the broad-headed morph) even in larvae reared experimentally at the same larval density (Fig. 2).

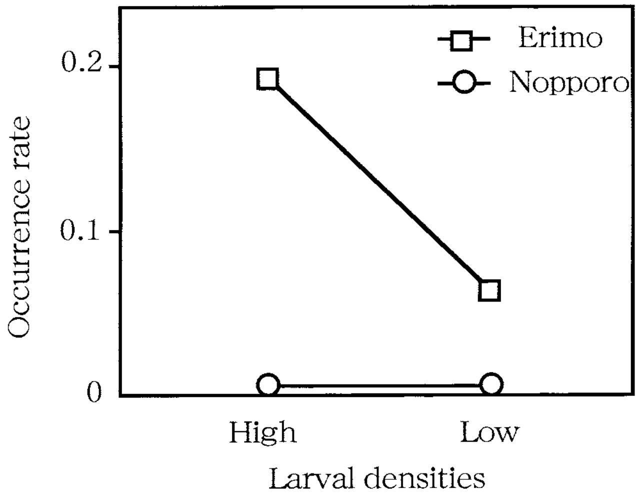

Fig. 2. The occurrence rate of the broad-headed "cannibal" morph (the number of the broad-headed "cannibal" morph/individual), analyzed for the effects of larval density (high or low density) and population (Erimo and Nopporo). Larvae derived from the Nopporo population showed the same, low occurrence rate of the broad-headed morph regardless of the experimental larval density. However, larvae from the Erimo population showed different occurrence rates of the broad-headed morph at different larval densities: a low occurrence rate at low larval density, but a high occurrence of the broad-headed morph at high larval density.

DISCUSSION

Genetic mechanisms and the evolutionary process of phenotypic plasticity and polyphenism are still controversial at present (Gotthard and Nylin, 1995; Via *et al.*, 1995; DeWitt *et al.*, 1998). We have found that variations of the frequency of the broad-headed "cannibal" morph in larvae of *Hynobius retardatus* are present between 2 different populations: the occurrence rate of the broad-headed morph was significantly different between the Nopporo (low density) and Erimo (high density) populations (Fig. 2). The broad-headed morphs were significantly affected by experimental larval density in the Erimo population, but not in the Nopporo population (Fig. 2). In other words, larvae from Nopporo population did not show a high occurrence rate of the broad-headed morph even though they were experimentally reared at a high larval density.

Previously, we showed that the broad-headed *H. retardatus* larvae might have evolved to overcome severe circumstances when the only available food items are conspecifics or heterospecific tadpoles (Michimae and Wakahara, 2002). The broad-headed morph is advantageous when many potential prey items (e.g., higher larval densities) but fewer relatives are present, because the indirect component of their inclusive fitness may be diminished if relatives are consumed (Hamilton, 1964; Waldman, 1988; Walls and Roudebush, 1991; Elger and Crespi, 1992). We have also demonstrated that the occurrence of the broad-headed morph, which continuously consumes conspecifics, is suppressed among siblings (Wakahara, 1997; Michimae and Wakahara, 2001).

We tentatively provide two possible reasons why the occurrence rate of the broad-headed morph was significantly different between the Nopporo (low density) and Erimo (high density) populations. Firstly, the variations in the frequency of the broad-headed morph between populations merely resulted from difference in actual kinship among egg clutches in two ponds. In the Nopporo population, kinship among egg clutches might be relatively high because of the smaller pond and fewer clutches. In contrast, that might be lower in the Erimo pond because of the larger pond and much more clutches. Therefore, it is possible that significant differences in the occurrence of the broad-headed morphs in the experimental conditions simply resulted from their different kinship between two populations. At present, however, it is unclear how the larvae discriminate kin and non-kin and how degree they estimate the kinship among conspecifics. Furthermore, actual kinship among egg clutches in each pond has not been examined yet.

Secondly, it is possible that the variations in the frequency of the broad-headed morph resulted from a local adaptation of phenotypic plasticity regulated by different genotypes. If an expression of plastic traits includes fitness costs that maintain the genetic and cellular machinery necessary to be plastic, every organism always must bear the costs regardless of the environment in which it develops (Scheiner, 1993). In other words, when the costs maintaining the plasticity (the costs of the genetic and cellular machinery necessary to be plastic) are larger relative to the benefits, the plasticity itself will be diminished by the selection. Assuming that the occurrence of the broad-headed morph is constrained by a specific but unknown genetic background, the results reported in this study can be explained comprehensively as follows. In the Nopporo population, the occurrence rate of the broad-headed morph is generally suppressed because many of the conspecifics encountered are relatives. Therefore, the occurrence of the broad-headed morph in the Nopporo pond (low density and high kinship among larvae) have been maintained at a relatively low level (or unnecessary) because the indirect component of their inclusive fitness may be diminished if relatives are consumed. In contrast, in the high density pond, it is expected that each larva frequently encounters conspecifics, and that many of the conspecifics encountered are nonrelatives. In the Erimo population, the occurrence of the broad-headed morph is thus maintained at a relatively high level. In the Nopporo pond, the broad-headed morph is not very adaptive so that the genetic traits to express the morph will weaken because the costs maintaining the plasticity exceed the benefits. Contrary, the genetic traits to express the broad-headed morph will intensify in the Erimo pond. Thus, the broad-headed morph and its frequency of occurrence might have been evolutionarily maintained within a local population as an adaptation. Because the abundance of prey items (conspecifics, tadpoles, *Tubifex* etc.) and kinship among larvae may vary depending on the local natural habitat, different occurrence rates of the broad-headed morph among populations might be attributable to adaptation to local environments. It seems thus absolutely necessary to elucidate these for further discussion in evolution of cannibalistic polyphenism.

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