SHORT COMMUNICATION

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Trophic specialisations in alternative heterochronic morphs

Abstract Polymorphisms are suspected of reducing competition among conspecifics in heterogeneous environments by allowing differential resource use. However the adaptive significance of alternative morphs has been poorly documented. The aim of this study is to determine food partitioning of two heterochronic morphs of the Alpine newt, *Triturus alpestris*, in mountain lakes. The morphs differ in the functional morphology of their feeding apparatus. Only paedomorphs are able to expel water during prey suction behind the mouth through gill slits. We observed a substantial trophic differentiation between morphs in all lakes. Paedomorphs preyed mainly on plankton, whereas metamorphs foraged on terrestrial invertebrates that fell upon the water surface. This resource partitioning may facilitate the coexistence of the alternative morphs in lakes devoid of vertebrate competitors. Food diversity may thus favour the evolutionary maintenance of facultative polymorphism in natural populations.

Introduction

Polymorphisms are components of functional biodiversity at the species level (Schlichting and Pigliucci 1998). They concern structures and behaviours involved in food processing (Smith and Skúlason 1996), dispersal (Roff

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1986) or protection (Roskam and Brakefield 1999). Their adaptiveness permits higher reproductive success in heterogeneous environments because of risk-spreading among different morphological or behavioural tactics, and a reduction in competition among conspecifics (Schmidt et al. 2000; Bolnick 2001). Resource-based polymorphisms are thus expected to occur in heterogeneous or variable habitats where the overall increase in parental fitness as a result of a variation in progeny composed of alternative morphs exceeds that of a parent whose progeny is monomorphic (Smith and Skúlason 1996). Alternatively, such polymorphisms may also be found in uniform habitats if resources are unpredictable over time (Schlichting and Pigliucci 1995).

Polyphenisms, or environmentally induced polymorphisms, often result from phenotypic plasticity, as their expression depends on environmental variation (Whiteman 1994). Developmental heterochronies are thus considered as mechanisms likely to produce polymorphism by modifying the relative timing of gene expression. In this respect, paedomorphosis results from the retention of juvenile characters in adult individuals. This may have considerable evolutionary implications, as small changes in the genetic control of ontogeny can produce large phenotypic novelties, which are then exposed to natural selection (McKinney and McNamara 1991). Although the theoretical framework of polymorphism is now well established, few empirical studies have been performed in order to evaluate the respective performance of alternative morphs (Whiteman 1994; Schlichting and Pigliucci 1998).

Facultative paedomorphosis in newts and salamanders is a suitable process for this purpose because comparisons of performance are made possible by the coexistence of alternative morphs within natural populations (Denoël et al. 2002). It then becomes possible to compare the ecological niches of the morphs and to evaluate their respective advantages. Low densities, permanent water, and earlier age at first reproduction are some of the factors favouring paedomorphosis (Semlitsch 1987; Denoël and Joly 2000). Because of the functional

specialisation of their feeding apparatus (Whiteman et al. 1996), alternative morphs would be expected to use different resources. As previous studies on the dietary habits of Alpine newt gave controversial results (Fasola and Canova 1992; Denoël and Joly 2001), we undertook further investigations comparing feeding specialisations of the two morphs in several lakes where they coexist.

Materials and methods

We studied three populations of *Triturus alpestris* (Amphibia, Caudata) inhabiting Alpine fishless lakes. One lake is located in southern France (La Cabane, 44°24′ N, 6°24′ E, elevation 1,950 m above sea level) and the other two are in Greece (Drakolimni in the Tymphi Mountains (Tymphi hereafter), 39°59′ N, 20°47′ E, 2,000 m elevation and Drakolimni in the Smolikas Mountains (Smolikas hereafter), 40°05′ N, 20°54′ E, 2,140 m elevation).

The three populations are composed of syntopic paedomorphic and metamorphic newts (Denoël et al. 2001a). In paedomorphic newts, the gill slits are open and support three pairs of external gills. In metamorphic newts the gill slits are closed and gills have resorbed. The two morphs are sexually mature and stay in the water during the active period, i.e. from the thawing of the lakes until the end of the summer (Denoël et al. 2001b).

We caught newts by dip-netting from the shore or from a boat, by snorkelling or by scuba-diving during the summers of 1997 in France (June–July) and 1999 in Greece (July–August). Samples consisted of 223 individuals at La Cabane (174 paedomorphs, 49 metamorphs), 184 at Tymphi (96 paedomorphs, 88 metamorphs) and 329 at Smolikas (169 paedomorphs, 160 metamorphs). Both paedomorphs and metamorphs were caught in all microhabitats of the lakes (water column, water surface, shore area, and substratum of deep parts). We collected prey items with the stomach-flushing technique (Joly 1987). This system consisted of injecting water with a sprayer into the stomach through the mouth and then collecting the prey items which exited the mouth. Newts were then housed in containers until the end of the sampling session (a couple of days) to avoid the recapture of already treated individuals. We classified prey according to three main categories: small crustaceans, insect aquatic larvae and terrestrial invertebrates. We also additionally used prey categories that were specific to each lake for

within-lake diet comparisons: pelagic Diptera, newt eggs and aquatic molluscs at Smolikas, large pelagic Chaoborids at La Cabane, and Hydracarina at Tymphi.

We analysed the effects of morph and lake on stomach contents with MANOVA by classifying each prey according to the three main prey types (crustaceans, aquatic and terrestrial insects) found in the three lakes. Data were square-root transformed after adding 0.5 to each value in order to avoid zero values in the statistical analyses. Pair-wise comparisons were performed by a post hoc protected LSD test. Subsequent MANOVAs were also separately performed for each lake to test for differences between morphs in prey types and microhabitat use.

Results

Stomach flushing resulted in 7,960 prey items at La Cabane (paedomorphs: 7,083, metamorphs: 877), 4,783 at Tymphi (paedomorphs: 3,863, metamorphs: 920) and 16,886 at Smolikas (paedomorphs: 9,646, metamorphs: 7,240). At all sites, both paedomorphic and metamorphic newts foraged on all available prey, from small planktonic crustaceans to aquatic insect larvae and terrestrial invertebrates. Locally, they also consumed eggs of their own species, molluscs and hydracarids.

Morph, lake, and their interaction had significant effects on the number of prey of the three main taxa per stomach (morph effect: $F_{3,728}=28.396$, $P<0.0001$; lake effect: $F_{6,1456}=26.144$, $P<0.0001$; interaction effect: $F_{6,1456}=10.957$, $P<0.0001$). At all lakes, the difference between morphs resulted in higher foraging by paedomorphs on small crustaceans (LSD test: La Cabane: $P<0.0001$, Tymphi: $P<0.0001$, Smolikas: $P<0.01$), and higher foraging by metamorphs on terrestrial invertebrates (La Cabane: $P<0.0001$, Tymphi: $P<0.05$, Smolikas: $P<0.01$; Fig. 1). Foraging on insect aquatic larvae differed among sites, as paedomorphs foraged more frequently on that prey than metamorphs at Smolikas ($P<0.0001$); to a

Fig. 1 Aquatic feeding of syntopic paedomorphs and metamorphs: number of small crustaceans and terrestrial invertebrates per newt stomach in three lakes (La Cabane, Tymphi and Smolikas). Presented values are transformed (square-root of values + 0.5). *Points* represent means, *boxes* represent SE, *whiskers* represent SD; *$P<0.05$, **$P<0.01$, ***$P<0.0001$ (ANOVA: LSD test)
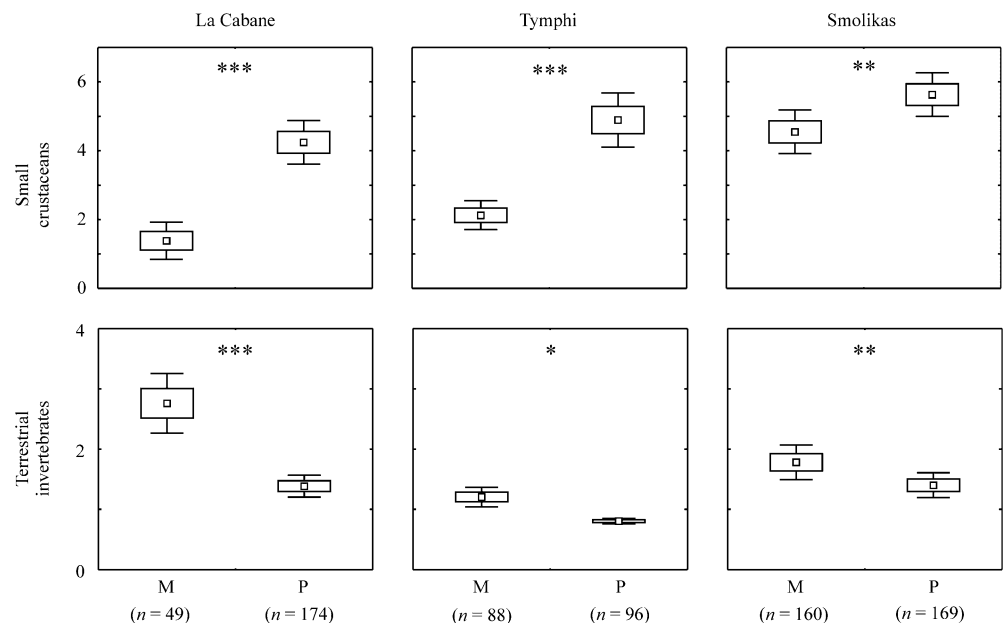
Table 1 Two-factor multivariate analyses for effects on the number of prey of the different taxa (La Cabane: small crustaceans, chirocephalids, aquatic insect larvae, terrestrial insects; Tymphi: small crustaceans, aquatic insect larvae, terrestrial insects, hydracarids; Smolikas: small crustaceans, pelagic diptera larvae, aquatic insect larvae, molluscs, eggs, terrestrial invertebrates) per stomach in paedomorphic and aquatic metamorphic Alpine newts from the four micro-habitats (water column, water surface, shore, deep substratum)

Lake	Source	F	df	P
La Cabane	Morph	2.628	4, 212	<0.05
	Habitat	14.156	12, 561	<0.0001
	Morph × Habitat	0.596	12, 561	0.85
Tymphi	Morph	5.215	4, 173	<0.001
	Habitat	5.582	12, 458	<0.0001
	Morph × Habitat	1.402	12, 458	0.16
Smolikas	Morph	14.352	6, 314	<0.0001
	Habitat	15.865	24, 1096	<0.0001
	Morph × Habitat	1.149	24, 1096	0.28

similar extent at Tymphi ($P=0.44$) and less frequently at La Cabane ($P<0.01$). Differences in diet were also detected for the lake-specific prey. Paedomorphs ate significantly more fairy shrimps at La Cabane ($P<0.0001$), Hydracarina at Tymphi ($P<0.05$), and *Chaoborus* and Bivalvia at Smolikas ($P<0.001$) than metamorphs did, but fewer newt eggs at Smolikas ($P<0.0001$).

Morph effect remained significant when microhabitat (water column, water surface, shore, deep substratum) was included in the multivariate model ($P<0.0001$; see Table 1). Microhabitat also had a significant effect in separate analyses of each site ($P<0.05$) with higher frequency of plankton prey in the water column and at the lake bottom, and higher frequency of terrestrial prey at the shore and water surface. The interaction between "Morph" and "Microhabitat" was never significant (Table 1).

Discussion

We observed similar differences between the two morphs in each lake, suggesting that trophic differentiation is a general trait of heterochronic Alpine newt populations. Whereas paedomorphs mainly foraged on plankton and pelagic prey, metamorphs concentrated on terrestrial invertebrates that fell upon the water surface. These results diverge from those of other studies, in which large food overlaps were found between morphs in Italian lowland ponds (Fasola and Canova 1992; Denoël and Andreone 2003). This divergence partially reflects differences in the scale of spatial heterogeneity between deep Alpine lakes and shallow ponds, the latter offering fewer possibilities for variation in foraging tactics (uniform habitat at the scale of newt foraging range).

Food partitioning can result from differences in both foraging behaviour and habitat use (Schoener 1974; Whiteman et al. 1996). Although the two morphs coexisted within the same lakes, they differed in their use of microhabitats. Whereas paedomorphs were usually more common in microhabitats where plankton was abundant (water column, deep benthic areas), metamorphs were often found at the water surface and along the shoreline (Denoël and Joly 2001; Denoël and Schabetsberger 2003). However, a difference between the diets of each morph does not only reflect variation in microhabitat use, because morph effect remains significant when microhabitats were included in the multivariate models (in this study).

Feeding specialisation may be brought about by differences in mouth morphology between morphs. In the paedomorphs, water is expelled backwards through the gill slits. In contrast, a reverse current through the mouth is the only way of expelling water in metamorphs (Lauder and Shaffer 1993). These differences may explain the variation in success rates between morphs according to prey type (Whiteman et al. 1996; M. Denoël, personal observation). Such variation may also contribute to the differentiation of trophic spectra between paedomorphs and metamorphs in natural habitats.

Competition theory predicts that resource use by coexisting species should differ in order to reduce the detrimental effects of resource depletion on reproductive success (Tokeshi 1999). The same issue has been addressed for sexual dimorphism (Shine 1989) and morphological polymorphism (Smith and Skúlason 1996). In this respect, polymorphism could reduce competition strength if the diets of the alternative morphs differ (Smith and Skúlason 1996). However, diet analyses of alternative morphs are not designed to reveal operating competition. Only the experimental removal of one morph would allow the investigation of potential competition between the two morphs.

The co-occurrence of distinct ecological niches is assumed to promote polymorphism by total or partial relaxation of intraspecific competition in heterogeneous habitats and/or higher resource availability (Smith and Skúlason 1996). In the three studied lakes, Alpine newts are the only top predators and they do not compete with other vertebrate predators. As a consequence, the only source of competition, if any, is intraspecific in such lakes. On the other hand, the morphology of these lakes leads to a great heterogeneity of resource availability. If there is emergence of a new successful morph in microhabitats that differ from those of the ancestral morph, the maintenance of polymorphism then becomes a possible evolutionary outcome, especially if the costs of its expression are low.

The sexual compatibility between alternative morphs probably results in genetic homogeneity at the population level (Denoël et al. 2001b). Paedomorphosis appears as a conditional strategy that may benefit each individual bearing a genotype capable of producing both morphs, each of them specialising on different prey types (Gross 1996; Denoël 2003; this study). Our study thus supports the idea that the maintenance of plasticity is driven by a more effective use of a structurally complex environment. Further studies are needed to test this hypothesis by

evaluating the respective performance and fitness of each morph under different conditions (Denoël et al. 2002). In particular, future research should use pond or cattle tank experiments to investigate the interplay between competition, habitat heterogeneity and polymorphisms.

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