

Research Paper

Expression of sexual ornaments in a polymorphic species: phenotypic variation in response to environmental risk

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Abstract. Secondary sexual traits may evolve under the antagonistic context of sexual and natural selection. In some polymorphic species, these traits are only expressed during the breeding period and are differently expressed in alternative phenotypes. However, it is unknown whether such phenotypes exhibit phenotypic plasticity of seasonal ornamentations in response to environmental pressures such as in the presence of fish (predation risk). This is an important question to understand the evolution of polyphenisms. We used facultative paedomorphosis in newts as a model system because it involves the coexistence of paedomorphs that retain gills in the adult stage with metamorphs that have undergone metamorphosis, but also because newts exhibit seasonal sexual traits. Our aim was therefore to determine the influence of fish on the development of seasonal ornamentation in the two phenotypes of the palmate newt (*Lissotriton helveticus*). During the entire newt breeding period, we assessed the importance of phenotype and fish presence with an information-theoretic approach. Our results showed that paedomorphs presented much less developed ornamentation than metamorphs and those ornamentations varied over time. Fish inhibited the development of sexual traits but differently between phenotypes: in contrast to metamorphs, paedomorphs lack the phenotypic plasticity of sexual traits to environmental risk. This study points out that internal and external parameters act in complex ways in the expression of seasonal sexual ornamentations and that similar environmental pressure can induce a contrasted evolution in alternative phenotypes.

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Keywords: natural selection; newt; paedomorphosis; secondary sexual traits; sexual selection

Introduction

In 1871, Darwin was the first to suggest that sexual selection is a substantial determinant of morphological diversity among species (Darwin, 1871). Indeed, in many species, males express conspicuous ornaments, whereas females usually lack such traits. The evolution and maintenance of these traits are explained by their major role in intersexual communication (Candolin, 2003). In particular, they can evolve as a result of female selection, as these traits may reflect male genetic quality and long-term viability (Kokko et al., 1999; Scheuber et al., 2004). However, male ornaments may also evolve as a trade-off between sexual and natural selection (Fowler-Finn & Hebets, 2011a; Hernandez-Jimenez & Rios-Cardenas, 2012). Indeed, because conspicuous ornamentation is often associated with a higher risk of predation (Endler, 1983; Stuart-Fox et al., 2003; Fowler-Finn & Hebets, 2011b), natural selection can favour more cryptic traits (Zuk & Kolluru, 1998). Predation is thus an important selective force in the evolution of morphological adaptation (Sih et al., 1985); cryptic traits allow animals to invest

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more time and energy on other activities without the risk of being detected by predators (Lima, 1998).

Many species are polymorphic, with alternative phenotypes differing in their morphological traits (West-Eberhard, 1989; Moran, 1992). Sexual differences between alternative phenotypes have been found in most cases, such as horn dimorphism in beetles (Emlen et al., 2007), leg specializations in acarid mites (Unrug et al., 2004), dichromatism in many birds (Badyaev & Hill, 2003) and frogs (Bell & Zamudio, 2012), and facultative paedomorphosis in newts and salamanders (Denoël et al., 2001). The latter process is a polyphenism in which one phenotype undergoes metamorphosis (the metamorph) and an alternative phenotype retains larval traits such as gills at the adult stage (the paedomorph) (Gould, 1977; Denoël et al., 2005b). Monomorphic species are known to display plastic traits over time, such as during the breeding season (Svensson et al., 2009) or according to predation risk in fish (Endler, 1991; Candolin, 1998). For polyphenic species, secondary sexual traits are often considered to be fixed at the adult stage and consequently, when produced, they cannot be adjusted to local risks. A well-studied case is that of horned beetles, where natural selection in individuals at the larval stage explains body size and phenotype specializations at the adult stage (Emlen, 1994). Alternatively, some polyphenic species can exhibit seasonal ornaments, that is sexual traits that are developed only during the period of reproduction (Cornuau et al., 2012). This is typical of both paedomorphic and metamorphic phenotypes of newts and salamanders (Denoël et al., 2001). For instance, in metamorphosed adult palmate newts (*Lissotriton helveticus*), males exhibit hind foot webs, a long tail filament and a large cloaca, whereas females are devoid of the former and develop a much smaller filament and cloaca (Griffiths & Mylotte, 1988). Sexual selection experiments have shown that these traits are effectively selected by females (Haerty et al., 2007; Cornuau et al., 2012). It is therefore likely that these traits could show variation over time and according to environmental context differently across phenotypes, but this has not yet been explored.

Studying the effect of natural selection on ornamentation is particularly important to determine whether alternative phenotypes can both express phenotypic plasticity of sexual attributes and whether their response could give similar or different signalling expression. Although both phenotypes have been reported to have a similar courtship success, the ornamentation of paedomorphs is usually less developed than that of metamorphs (Denoël et al., 2001; Denoël, 2002). It can therefore be expected that paedomorphs may have less potential response than metamorphs and that any reduction of sexual traits may be detrimental in terms of mate choice. This could therefore have profound consequences on the outcome of the process because paedomorphosis is dependent on a genetic underpinning and could rapidly be selected, as shown by artificial crossing (Semlitsch & Wilbur, 1989; Voss et al., 2012). It is indeed thought that environmental pressures can lead to maintenance of the polyphenism or fixation of only one of the two phenotypes (Denoël et al., 2005b; Bonett et al., 2014). Heterogeneous habitats favour diversity in maintaining the polyphenism (Whiteman et al., 1996; Denoël et al., 2005b), whereas unfavourable environments, such as drying, are thought to favour metamorphosis over paedomorphosis (Semlitsch, 1987; Denoël & Ficetola, 2014).

Goldfish (*Carassius auratus*) is the most frequently introduced ornamental fish in the world (Savini et al., 2010), particularly in ponds used by amphibians such as newts (Denoël et al., 2013). Goldfish are predators of newts eggs and larvae, but not usually of adult stage newts (Monello & Wright, 2001); it causes avoidance behaviour and reduced sexual behaviour in adult newts in laboratory conditions (Winandy & Denoël, 2013a,b) suggesting that newts perceive it as a threat. In the field, goldfish has been shown to affect newt populations by

limiting their occurrence and decreasing their abundance (Denoël & Ficetola, 2014). This is particularly the case for the paedomorphs that disappear after goldfish introductions (Denoël et al., 2005a). However, the reasons for the stronger effects of fish on populations of paedomorphs are not well known (Denoël et al., 2005a). However, paedomorphs are thought to be more vulnerable because they cannot escape water and often forage in the same microhabitats as fish (Denoël et al., 2005a). On the other hand, it was shown in mesocosm experiments that larvae develop less as paedomorphs in the presence of fish (Jackson & Semlitsch, 1993). Studying the effects of goldfish on sexual ornamentation in alternative phenotypes could therefore help to disentangle the complex ways in which paedomorphs are disadvantaged under predatory risk.

In this context, our aim was to show whether phenotypic plasticity explains the development of a seasonal secondary trait differently in alternative morphs produced by facultative paedomorphosis. More specifically, we tested the hypotheses that the development of sexual ornamentation is inhibited by the risk of predation, leading to a less marked development of sexual traits in both phenotypes and to a different response of phenotypes in the presence than in the absence of predation risk. To test our hypothesis, we carried out a replicated controlled experiment on paedomorphic and metamorphic palmate newts over their entire reproductive period in the absence or presence of goldfish.

Materials and methods

Study organism

We caught 96 palmate newts (48 metamorphs and 48 paedomorphs with 24 individuals of each sex) by dipnetting in a pond (Le Coulet North-East, Larzac Plateau, France) on 29 March 2013. Capture took place at the start of the reproduction period. At this time, the population contained more than 300 palmate newts (48.9% of metamorphs). After capture, newts were stored for 1 day in four large tanks (80 × 40 cm, 20 cm water depth) and then brought to the laboratory in several tanks, keeping morphs, and sex-separated in six 3-L tanks placed in a refrigerated box (230 L). At the end of the experiment, all newts were transported in the same way and released into their capture pond (7 July 2013) in accordance with capture permits.

Laboratory maintenance

On 31 March 2013, we distributed the newts into 24 identical and independent tanks (60 × 60 cm, 40 cm water level; 135 L) with four individuals per tank: one male and one female of each phenotype. We chose a balanced sex ratio because our aim was to test the main effect of predation risk rather than sexual competition on male ornamentation. An oxygen diffuser was placed in each tank. The bottoms of the tanks were covered with pieces of slate, and one large shelter (20 × 60 cm) was provided in each tank. The ambient air temperature was regulated to maintain water temperature at an average of 14.69 °C (SE = 0.05 °C). We established a photoperiod (with one Lumilux de lux 2350-lm daylight tube, L36W/12-950; and one Sylvania Professional tube, 36W DECOR183) that reflected the natural cycle at the capture location (12 h light/12 h dark). Subjects were fed with 50 mg of defrosted *Chironomus* larvae per newt every day in the afternoon. This prey is a typical newt prey, and the quantity given was ad libitum, corresponding to the needs of the newt. Food was provided in a shelter behind a grid to avoid consumption by fish.

The goldfish came from a fish retailer (Blue Coral, Herstal, provider of the University of Liège Aquarium). They were stored in a large tank (180 × 80 cm, 60 cm water depth) at an average temperature of 18 °C and a photoperiod of 11 h light/13 h dark. Goldfish had a mean (\pm SE) total length of 11.8 ± 0.2 cm ($n = 12$), which is a typical size found in the wild. At the start of the experiment, we placed one goldfish in each of 12 of the 24 experimental newt tanks as described above, so the conditions applied to fish were similar to those applied to newts. The newts and goldfish were placed in tanks at the same time. Goldfish were in direct contact with newts but could not go behind the grid to the shelter. From the day of their arrival to the end of the experiment, they were fed with 200 mg of defrosted *Chironomus* larvae per fish every day. Food was provided to the goldfish at the surface of the water, so newts did not eat it (goldfish consumed *Chironomus* larvae quickly). After the experiment, goldfish were stored in a large tank (180 × 80 cm, 60 cm water depth) for further studies.

Morphological measurement

The morphological measurements were taken every month for 3 months, on 27 April (after the first month of the experiment), 26 May and 23 June 2013, to cover the whole breeding season. This protocol provided three periods of repeated measurements to assess the effect of time on the expression of sexual traits.

We analysed secondary sexual traits that are developed during the breeding season only: filament length, hind foot web and cloaca size (Fig. 1). The hind foot web area (not considering the toes themselves) was measured on photos taken with a Nikon D90 with a 60-mm lens and using Able Image Analyser software for calibrated measurements. The cloaca was measured using an electronic calliper and the tail filament with a ruler. We did not use the crest as it is very small in palmate newts (Green, 1991; Cornuau et al., 2012). Finally, the snout–vent length (SVL) was also measured as a factor that may influence the size of sexual traits. Indeed, a previous study showed that larger males expressed larger cloaca and hind foot web (Haerty et al., 2007). Paedomorphs had a smaller SVL than metamorphs in the study population (mean \pm SE: 33.67 ± 1.97 mm and 40.4 ± 1.91 mm, respectively, $t_{46} = 12.03$, $P < 0.001$). In the beginning of the study, newts were distributed in control and fish tanks so that there were no significant differences between the treatments for body size, that is snout–vent length ($t_{46} = -0.11$, $P = 0.91$) and each sexual trait (Filament: $t_{46} = -0.00$, $P > 0.99$; Cloaca: $t_{46} = -0.17$, $P = 0.87$; hind foot web: $t_{46} = -0.60$, $P = 0.55$).

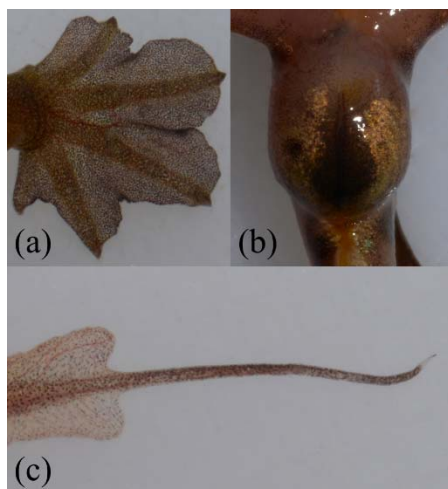


Figure 1. Secondary sexual traits in male palmate newts: (a) hind foot web, (b) cloaca and (c) tail filament.

Statistical analyses

As the three variables representing sexual traits are strongly correlated ($r \geq 0.8$, $P < 0.001$ for all pairwise comparisons), we used a principal component analysis (PCA) to summarize variables in a lower number of uncorrelated components. The first factor of extracted component explained 87.3% of the total variance. The positive correlation between the original variables and the extracted component was 0.94 for filament length, 0.93 for hind foot web and 0.93 for cloaca size. On the extracted component, renamed secondary sexual traits, we used an information-theoretic approach based on the Akaike information criterion (AIC) to evaluate the support of hypotheses explaining variation of sexual traits. The AIC analysis gives a numerical value used to rank competing models. Parsimonious models explaining more variation have the lowest AIC values and are considered to be the best models (Burnham & Anderson, 2002). We used a generalized linear mixed model (GLMM) to test the effect of phenotype (fixed factor), fish (fixed factor) and time (ordinal variable, included as both fixed and random effect) on the development of secondary sexual traits. To avoid the concomitant effect of body size, we included the snout–vent length (continuous variable) as a covariable in the model (García-Berthou, 2001). Individuals and aquariums were also included as random factors. We built GLMMs representing combinations of these hypotheses. We then calculated the AIC corrected for small sample size (AICc) for each model and ranked models on the basis of AIC (Burnham & Anderson, 2002). For each candidate model, we calculated the Akaike weight w_i , which represents the probability that a given model is the best approximating model, given the data and the set of candidate models. We also calculated the importance of parameters (sum of the weight of all models including the given parameter), which can be interpreted as the probability that a predictor is part of the best model and the model average parameters (Burnham & Anderson, 2002).

Finally, from the AIC analyses, we calculated the significance of parameters included in the best models to improve the interpretation of the role played by each variable. We used the model averaging function from AIC on models with a delta AIC ≤ 2 (suggesting substantial evidence for the models). This model averaging test consists of making inferences based on the top set of candidate models, instead of basing the conclusions only on the single best model (Mazerolle, 2006). For significant interactions between phenotype and fish, we used a Tukey contrast test to assess significant differences between groups.

For all analyses, we chose an *a priori* level of significance of 0.05. All analyses were performed in R using factominr, lme4, mumin and multcomp packages (R Foundation for Statistical Computing, Vienna, Austria).

Results

The best AIC model (Table 1) showed that the expression of secondary sexual traits was significantly influenced by three of the four parameters considered representing our *a priori* hypotheses: fish, phenotype and SVL. Time was included in the second best model ($\Delta\text{AIC} = 2$). With respect to the interactions between factors, the presence of fish affected the expression of ornamentation differently in the two phenotypes. The interaction between SVL and phenotype was not included in the best models ($\Delta\text{AIC} > 4$), showing that SVL influenced the expression of ornamentation in the same way in both phenotypes.

Table 1. Comparison of models explaining the expression of secondary sexual traits in palmate newts

Rank	Fish	Phenotype	SVL	Time	Fish x Phenotype	SVL x Phenotype	K	AIC _c	Weight
1	-1.04	-1.51	0.22		0.98		4	340	0.50
2	-1.04	-1.48	0.22	-0.25	0.99		5	342	0.18
3	-0.56	-1.05	0.21				3	343.1	0.10
4	-0.06	-5.30	0.16		1.04	0.10	5	344.1	0.07
5	-0.55	-1.01	0.22	-0.24			4	345.1	0.04
6		-0.94	0.23				2	345.7	0.03
7	-1.06	-5.21	0.16	-0.24	1.05	0.10	6	346.1	0.02
Parameter importance	0.90	0.90	>0.99	0.27	0.77	0.11			

The models are ranked from best to worst ($\Delta AIC < 7$), according to AIC_c scores (small-sample-corrected Akaike information criterion) and Akaike weights. Coefficients of included parameters with the sign of the relationship (+ or -) are reported. The importance of all parameters is indicated in the table, and *K* represents the number of parameters in each model. The tested parameters were as follows: Fish (control treatment vs. fish treatment), Phenotype (paedomorph vs. metamorph), SVL (snout-vent length) and Time (three periods).

The test of significance on the model averaging ($\Delta AIC \leq 2$) showed that in the presence of fish, newts presented less developed sexual traits compared to the control treatment (Table 2; Fig. 2a). Phenotype also had a strong main effect: paedomorphs had less developed sexual traits compared to metamorphs (Table 2; Fig. 2b). There was also an interaction between phenotype and fish (Table 2; Fig. 2c). The Tukey contrast tests brought out a significant difference between metamorphs with fish and control metamorphs ($Z = -5.25$, $P < 0.001$). There was no significant difference between paedomorphs with fish and control paedomorphs ($Z = -1.30$, $P = 0.57$). The reduction in sexual trait size of fish group in comparison with control group has a factor of 2.7 for metamorphs and 1.3 for paedomorphs. There was no significant variation in sexual traits over time (Table 2). Finally, in both phenotypes, there was a strong effect of SVL on ornamentation (Table 2). Indeed, secondary sexual traits were strongly positively correlated with SVL ($r = 0.85$, $R^2 = 0.71$, $F_{1,46} = 114.82$, $P < 0.001$): the larger newts had more developed ornamentation (Fig. 3). However, the correlation between sexual traits and SVL in paedomorphs is much stronger than in metamorphs ($r = 0.74$, $R^2 = 0.55$, $F_{1,22} = 26.82$, $P < 0.001$ and $r = 0.40$, $R^2 = 0.16$, $F_{1,22} = 4.13$, $P = 0.05$, respectively).

Table 2. Effect of parameters on the expression of secondary sexual traits in palmate newts.

Variables	Parameters	Averaged coefficients	SE	Z	P
Secondary sexual traits	Fish	-1.04	0.30	3.53	<0.001
	Phenotype	-1.50	0.41	3.63	<0.001
	SVL	0.22	0.04	4.952	<0.001
	Fish x Phenotype	0.99	0.42	2.351	0.02
	Time	-0.25	0.15	1.619	0.1

Significance of model averaged parameters estimates (based on best models with $\Delta AIC \leq 2$). The tested parameters were: Fish (control treatment versus fish treatment), Phenotype (paedomorph versus metamorph), SVL (snout-vent length) and Time (three periods). Significant values are highlighted in bold.

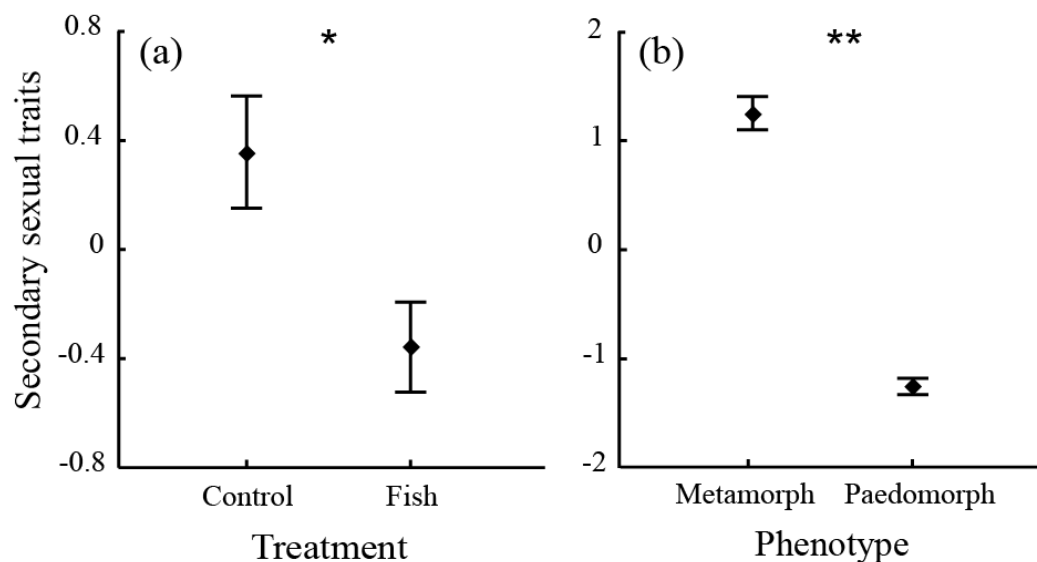


Figure 2. Significant effects of fish (a), phenotype (b) and the interaction between fish and phenotype (c) on the expression of secondary sexual traits (extracted component of a principal component analysis) in male palmate newts (mean \pm SE). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. See results for statistical analyses.

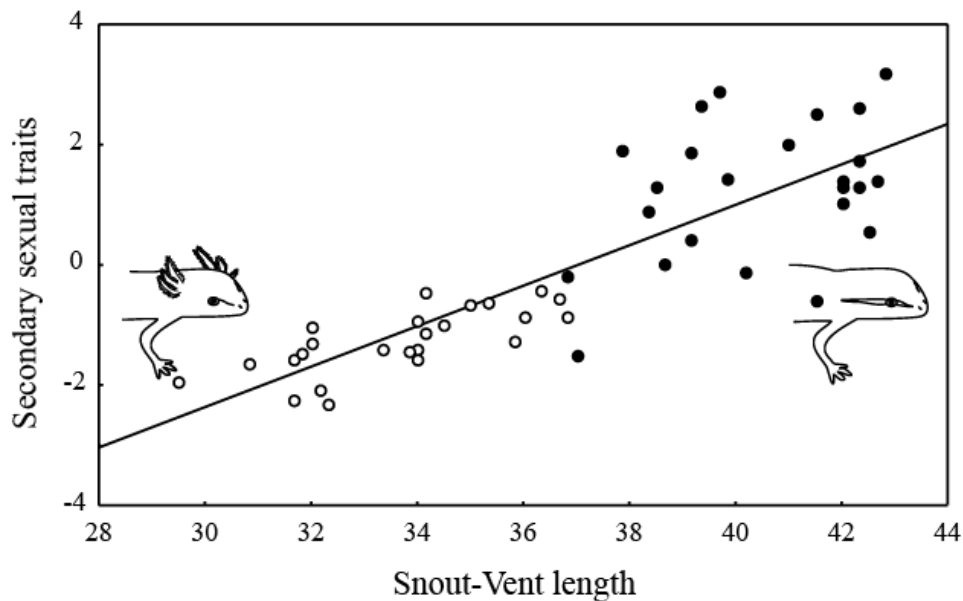


Figure 3. Relation between snout–vent length (mm) and secondary sexual traits (extracted components of a principal component analysis) in male palmate newts. Open circles: pedomorphs; full circles: metamorphs. See results for statistical analyses.

Discussion

We found that newts exhibited phenotypic plasticity of sexual ornamentation in response to an environmental pressure caused by the presence of fish. The two alternative phenotypes – the pedomorphs and the metamorphs – differed in the expression of these seasonal sexual traits and responded differently to predation risk. To the best of our knowledge, this is the first study to highlight the effect of predatory threat on the development of seasonal secondary sexual traits in a polyphenic species. The differences in responses of the two phenotypes may differently affect their fitness and therefore have profound implications on the evolution of facultative pedomorphosis. As pointed out by Whiteman (1997) on the importance of integrating sex when studying pay-offs of alternative phenotypes in salamanders, the present results show that plastic secondary sexual traits can be understood not only by studying mate choice but also by considering natural selection.

Sexual ornaments increase conspicuousness and thus the risk of predation (Zuk & Kolluru, 1998; Stuart-Fox et al., 2003). The pressure of predation can therefore interact with sexual selection to shape the expression of these traits. However, the degree to which selected sexual traits are plastic to the perception of predation risk is poorly understood (Cothran et al., 2012). The plasticity of the expression of these traits may allow fitting the level of perceived risk. In this study, newts responded to the presence of goldfish by reducing the expression of their ornamentations. The mechanisms by which sexual traits are reduced are not known. This could be a deleterious by-product of a response to a stressful environment or an adaptive strategy to appear less conspicuous (Ruell et al., 2013). In guppies, it was suggested adaptive as it reduces the risk of predation (Ruell et al., 2013). Similarly, among the sexual traits of the palmate newt, the filament is particularly conspicuous during the courtship display with the

male using it as a lure to attract the female (Halliday, 1975). A shorter filament is less attractive to females (Cornuau et al., 2012), but might decrease the risk of detection by the fish during courtship.

As expected, the paedomorphs showed less developed sexual traits than the metamorphs. This is typical of paedomorphs, which retain a mixture of larval and adult traits, as shown in other species (Denoël et al., 2001), but the traits were particularly underdeveloped in the paedomorphic palmate newts. Newts with conspicuous sexual secondary traits are more successful in attracting females (Hedlund, 1990; Green, 1991; Gabor & Halliday, 1997), suggesting that paedomorphic males may have less success than metamorphic males. However, female palmate newts consider both morphological and behavioural traits when choosing a mate: facing a male with reduced traits, females may value the information content of display activity over ornament size (Cornuau et al., 2012). Moreover, in several salamandrid species, the two morphs can interbreed successfully and display similar behavioural patterns (Whiteman et al., 1999; Denoël et al., 2001; Denoël, 2002). Such sexual compatibility likely contributes to the maintenance of phenotypic plasticity instead of fixation of paedomorphosis.

Sexual ornamentations were strongly correlated with body size (SVL) in palmate newts. This is in line with results from other polyphenisms, such as in horned beetles, where the smallest males either do not exhibit a horn at all or produce only a reduced horn in comparison with larger males (Emlen, 1994). In metamorphic newts, larger males are known to be more ornamented, that is to express larger cloaca and hind-foot web (Haerty et al., 2007). In our study, all of the best models included both body size and phenotypes, showing that both parameters directly affected the development of sexual traits. There was no interaction between body size and phenotype, suggesting that in both morphs, larger individuals develop more conspicuous ornaments. However, a much stronger correlation was found between sexual traits and body size in paedomorphs than in metamorphs. This is probably caused by the smaller size of paedomorphs than metamorphs, which has been shown to be associated with an earlier maturity in paedomorphs (Denoël & Joly, 2000). Indeed, paedomorphs need to invest more in somatic growth than the metamorphs (Denoël & Joly, 2000), and thus, ornamentations could thus be less costly to produce in the largest individuals that invest less in growth.

The presence of fish did not affect the two phenotypes in the same way. Whereas metamorphs showed a strong decrease of sexual trait expression, paedomorphs did not exhibit a significant reduction of these traits. In comparison with metamorphs, the lack of impact on paedomorphs' ornamentation does not mean that they are not affected by the presence of fish. Indeed a long-term field study showed a strong effect of goldfish presence on the occurrence of paedomorphosis in palmate newt populations: whereas metamorphs can persist in the presence of goldfish, paedomorphs almost always disappeared (Denoël et al., 2005a). This suggests that paedomorphs could be more sensitive to fish than metamorphs. However, the cost of reducing sexual traits in paedomorphs can be expected to be higher than in metamorphs as paedomorphs had very small ornamentation, even in the control group. Moreover, the metamorphs that develop reduced ornamentation in contact with fish had larger ornaments than paedomorphs from the control group. Any reduction of their small ornaments may therefore excessively affect their reproductive success, resulting in counterselection, and thus explaining their lack of response to fish presence in contrast to the metamorphic phenotype. This is in agreement with the hypotheses of a trade-off between mating benefits and the cost of predation risk, suggesting a greater response to the risk of predation for the most ornamented males, that is those that are the most at risk and those with the largest potential for trait reduction without impairing too much fitness by sexual selection (Fowler-Finn & Hebets, 2011a; Hernandez-

Jimenez & Rios-Cardenas, 2012). Through a lack of phenotypic plasticity of their sexual traits to fish presence, paedomorphs could then maintain breeding activities which make the persistence of polyphenism possible, which has been shown to be adaptive in previous studies (see review in Denoël et al., 2005b).

Conclusions

Through an experimental approach, combining several internal and external parameters, we highlighted how predatory pressure could differently affect phenotypes and how complex the determinants of seasonal secondary sexual traits can be. This shows that polyphenisms such as facultative paedomorphosis are excellent models for understanding the development and evolution of sexual ornamentation. The coexistence of alternative phenotypes with a common evolutionary history makes it possible to test the effect of environmental pressures and to disentangle the roles of natural and sexual selection.

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