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Correspondence

The Alpine "axolotl": a remarkable example of phenotypic plasticity in *Ichthyosaura alpestris* (Amphibia: Salamandridae)

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The phenomenon of paedomorphism describes the retention of juvenile characters in the reproductive adult (Gould 1977) and defines a heterochronic or alternative developmental process (Denoël & Poncin 2001). The commonly known textbook example of paedomorphism is certainly the axolotl (*Ambystoma mexicanum*), which provided a suitable early model to describe and study this pattern in salamanders (Gould 1977). In general, paedomorphism can be based on (i) an accelerated development of the gonads, which become functional significantly earlier than normal in a still larva-like body, a process referred to as "progenesis"; (ii) on a delayed somatic development with gonads maturing at a regular rate, referred to as "neoteny" (Gould, 1977, Denoël & Joly 2000, Denoël, et al. 2005). Both progenesis and neoteny lead to a sexually mature animal that will have retained larval somatic features, such as external gills and open gill slits. Paedomorphism is widespread in salamanders and found both as an obligate and a facultative ontogenetic process in nine out of ten recognized salamander families (Denoël & Poncin 2001, Denoël et al. 2005, Wiens et al. 2005). In species with obligate paedomorphism, adults never undergo metamorphosis. In species with facultative paedomorphism, both paedomorphic and metamorphosed adults can be found (Denoël & Poncin 2001). Facultative paedomorphism is also well known from the Alpine Newt (*Ichthyosaura alpestris*) where facultative paedomorphism is currently known from 87 out of thousands of known European populations (Zuiderwijk 1997, Denoël et al. 2001).

The cause of facultative paedomorphism in *I. alpestris*, its appearance in certain populations, and its lack in others, are not fully understood yet. It was suggested that both genetic and environmental factors have to be considered (Denoël et al. 2001a). In fact, it seems that environments such as permanently cool and oxygen-rich waters with abundant food and a surrounding hostile terrestrial habitat might favour skipping metamorphosis to a terrestrial stage (Wilbur & Collins 1973). On the other hand, Denoël et al. (2001a) reported paedomorphs from semi-temporary waters located in the proximity of suitable terrestrial environments. Furthermore, despite the wide distribution of *I. alpestris*, populations with facultative paedomorphism are almost exclusively found in southern Europe (Italy, Balkans, France) although habitats that would appear to favour paedomorphism also exist in Northern Europe (Denoël et al. 2001a). It appears that although paedomorphism is related to external conditions, it is not strictly correlated with these and a genetic predisposition to paedomorphism seems to be a prerequisite for this alternative developmental process (Denoël et al. 2001a).

Interestingly, paedomorphic Alpine Newts can still undergo metamorphosis under certain environmental conditions such as (i) increased population density (Harris 1987, but see Denoël 2003a); (ii) decreased aquatic prey availability (Denoël & Poncin 2001, Denoël 2003a); (iii) decreased water levels (Denoël 2003a). Nevertheless, individuals react differently to such changes of environmental conditions as a result of age or genetic differences between individuals (Denoël 2003a, Voss & Smith 2005).

Similar to paedomorphism, the phenomenon of leucism can be considered a rare form of genetically determined intraspecific polyphenism. Leucism (sometimes also referred to as "xanthism" or "partial albinism") is defined as an alteration in the function of dermal pigment via recessive allele expression and characterized by a partially or predominantly yellowish to white skin (Dunham & Childers 1980). Leucism is typically found in single individuals in populations that otherwise consist of normally pigmented individuals, and this phenomenon is occasionally encountered in a variety of vertebrates, including salamanders and newts (Brandon & Rutherford 1967, Lenders 1989, Nöllert & Nöllert 1992, Thiesmeier & Schulte 2010).

Paedomorphism or leucism in *I. alpestris* can be found in very few out of thousands of known populations and are considered extraordinary but still poorly understood instances of polyphenism that lead to unique forms of intraspecific diversity (e.g., Veith 1986, Lenders 1989, Nöllert & Nöllert 1992, Denoël et al. 2005, Thiesmeier & Schulte 2010). Intraspecific diversity is of special interest from an evolutionary point of view, because such phenotypic diversity is supposed to play an important role in the development of evolutionary radiations by triggering a cascade of diverse structural changes (Galis 1996).

Here I report on a unique and very rare form of paedomorphism combined with leucism in a population of "regular" *I. alpestris* in an irrigation reservoir in a region where paedomorphism used to be unknown before. This should contribute to our knowledge of paedomorphism, leucism, and the combination of both as special forms of intraspecific polyphenism and stimulate further studies on these patterns and their possible role in speciation.

Animals were observed and collected from an artificial irrigation reservoir in September of 2012. The pond was constructed in the Province of Bolzano (South Tyrol) in the Alps of northern Italy at an altitude of 1,245 m in 1998 (46°34'22.2" N, 11°16'13.0" E). The irrigation reservoir covers a surface of 5,700 m^2, with a maximum depth of 7 m, and contains an average water volume of 40,000 m^3. Water is continuously supplied by a small stream with an average water flow rate of 130 l/s^{-1}. The water level in the pond is subject to seasonal variation and fluctuates especially during the summer period due to increased tapping for agricultural irrigation. The bottom and bank insulation of the pond consists of white plastic pond liner, and only a small section of the bank is covered with gravel. The submerse vegetation of the pond consists only of algae and no vascular plants were observed. The pond itself is surrounded by pastures that are encircled by dense forest mainly composed by gymnosperms (European spruce, *Picea abies*). The reservoir and the supply stream are devoid of any fish. During springtime, the pond is densely populated by breeding Alpine Newts (*Ichthyosaura alpestris*), common toads (*Bufo bufo*), frogs (*Rana dalmatina* and *Rana arvalis*), and fire salamanders (*Salamandra salamandra*) can occasionally be found at some distance to the pond. While all toads and many frogs will leave the pond after breeding, single *I. alpestris* can be seen in it throughout the year.

For the current study, newts were observed from the banks of the pond. Pictures in the natural environment were taken with a waterproof digital camera Olympus Tough (Olympus Imaging Corp., Tokyo, Japan). In order to document animals from other than dorsal views, they were caught and photographed in a small glass aquarium with a Canon Eos 1 digital SRL camera (Canon, Tokyo). Animals were sampled by dip-netting under collection permit No. 63.01.05/120963, granted by the local government of the Province of Bolzano. Nine normally pigmented metamorphs (5 males, 4 females) and four leucistic neotenic newts were brought to the laboratory (Laboratory for Functional Morphology at the University of Antwerp, Belgium) for taking measurements and making further observations. The animals were anaesthetized by immersing them in an aqueous 0.05% solution of dissolved MS222 (protocol after Cecala et al. 2007) and total length and snout–vent length (SVL) were measured. All animals recovered well from anaesthesia within 15 minutes.

The leucistic paedomorphs were housed in a 60-l aquarium provided with aquatic plants and hiding facilities. Water was oxygenated by an air pump and constantly filtered with an internal aquarium filter. The animals were kept at a 12/12 h photoperiod and fed twice a week with live red mosquito larvae (chironomids), daphnia, *Tubifex* sp., and enchytraeids.

For further analysis, especially to identify their sex, two of the four paedomorphs in captivity were euthanised. Euthanasia was necessary because these two animals suffered from a fungal infection. The animals were first anaesthetized by immersing them into an aqueous solution of dissolved MS222 and then killed by intraperitoneal injection of sodium pentobarbital, after which they were fixed in an aqueous 4% formaldehyde solution. Their dissection was performed under a stereomicroscope (Olympus SZX16, Olympus, Tokyo) and documented using a digital camera (Olympus SC30, Olympus, Tokyo) mounted on the microscope. The vouchers are kept in the State Museum of Natural History Stuttgart (SMNS 16344 and 16345).

Animal maintenance and treatment was in accordance with national and international laws as approved by the Ethical Commission for Animal Experiments of the University of Antwerp (code: 2010-36).

Besides hundreds of "normally patterned" metamorphosed *I. alpestris*, 21 yellowish white paedomorphs were counted as living in the irrigation reservoir described above. The collected paedomorphic newts showed typical larval features. Compared to metamorphs, their head was narrower and somehow triangular in shape (Fig. 1). Posteriorly, the head bore well visible external gills on both sides and open gill slits were present. The tail was laterally compressed with ventral and dorsal fin folds (Fig. 1). The dorsal fin fold extended well anteriorly to the level of the shoulder girdle (Fig. 1). The SVL of 44 ± 3.6 mm (mean ± SD) in the four paedomorphic individuals did not differ significantly from the nine metamorphic adults (SVL 44.3 ± 3.5 mm) caught from the same population. The dissection of two paedomorphic individuals revealed large ovaries with well-visible oocytes (Fig. 2). The remaining two individuals showed no signs of metamorphosis even after 29 months in captivity. Based on their large size, the well-developed gonads, and the fact that they did not undergo metamorphosis in captivity, it can be assumed that they are not regular larvae or larvae with retarded metamorphosis, but represent true paedomorphs.

Progenetic paedomorphic *I. alpestris* are known to be significantly smaller than metamorphs from the same population (Denoël & Joly 2000). Furthermore, progenetic paedomorphs are mainly found in temporary ponds. By contrast, neotenic paedomorphs are similar in size to metamorphs from the same population and typically found in permanent waters (Denoël & Joly 2000). At the study site, paedomorph sizes were comparable to those of metamorphs. The pond varied in its water level throughout the year, but would not usually dry out. Accordingly, it can be concluded that the instance of paedomorphism reported here represents true neoteny.

All four paedomorphs studied herein showed a similar "pigmentation disorder" referred to as "leucism". In sharp contrast to the typical colour pattern found in "normally pigmented" *I. alpestris*, head, trunk, limbs, and tail where yellowish white with black spots dorsally and laterally (Figs 1C, D). The ventral body exhibited a homogenous orange colouration without black spots and the gills were pinkish red. The animals reported in this study are exceptional with regard of their exhibiting a combination of neoteny and leucism. Alpine newts showing such a combination are extremely rare, and only single individuals with these characteristics have been reported in the past (Lenders 1989, Nöllert & Nöllert 1992). However, to the best of my knowledge, no larger group or a subpopulation of such animals has ever been reported before.

There does not seem to be a clear ecological pattern favouring leucism or paedomorphism in wild *I. alpestris*. Leucistic individuals seem to be very rare and appear randomly in normally coloured populations. However, the irrigation reservoir reported herein represents an artificial habitat with a white bottom so that lighter coloured individuals might benefit from improved camouflage and, accordingly, favour the selection of the leucistic form. Paedomorphism on the other hand occurs far more often in the wild, but is almost exclusive to populations in Southern Europe, even though *I. alpestris* are also widely distributed in Northern Europe (Denoël et al. 2001a, see Fig. 1A). My record of paedomorphic Alpine Newts from the northern

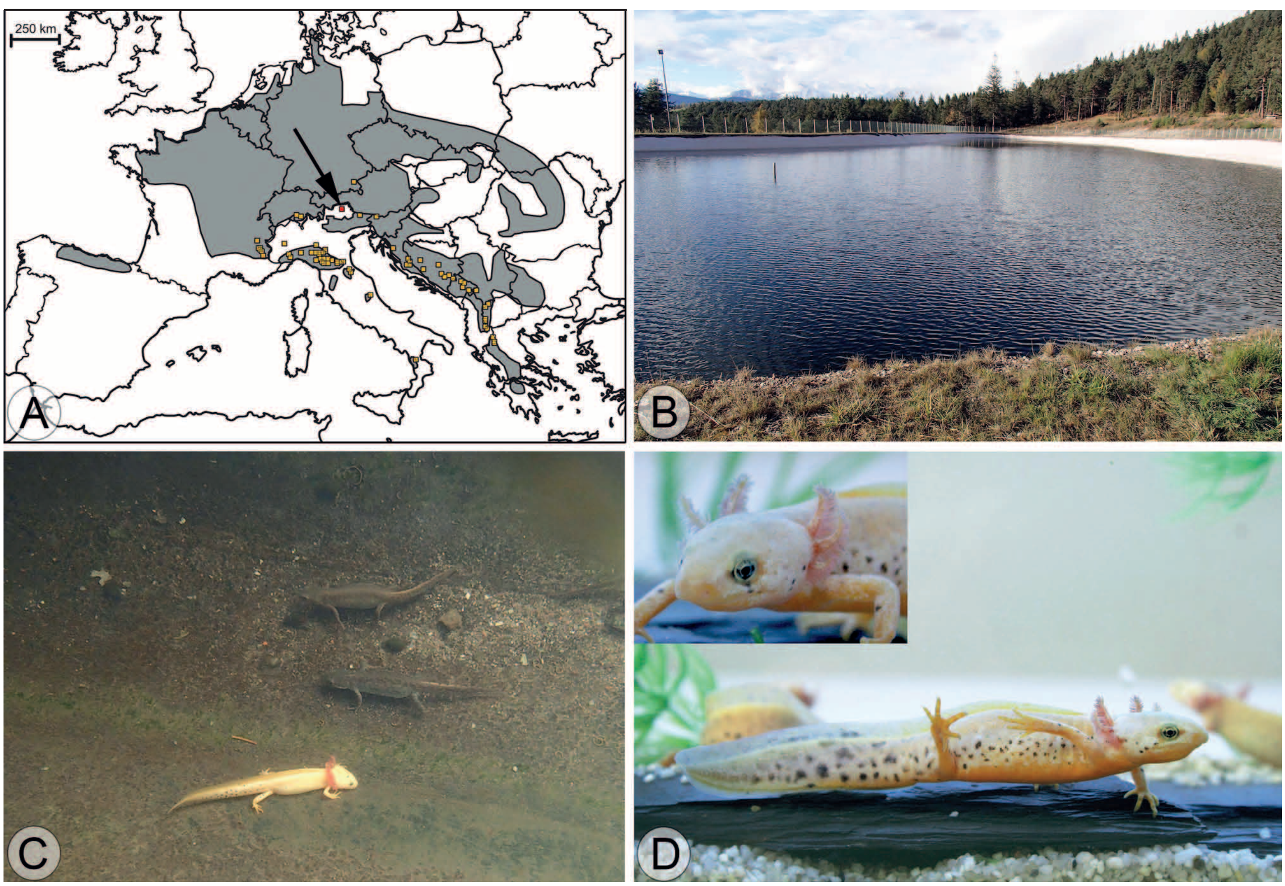

Figure 1. (A) Map of Central Europe showing the distribution of *Ichthyosaura alpestris* (grey areas), locations of known neotenic populations (orange squares: distribution pattern from Denoël et al. 2001), and the location of the neotenic-leucistic subpopulation reported here (red square) highlighted by an arrow. (B) The collection site was an artificial irrigation reservoir with an insulating bottom of white pond liner. Only a small section of the bank sported gravel. (C) One neotenic-leucistic and two "regular" forms of *I. alpestris* in their natural habitat in one of the few dark spots close to the bank. (D) Lateral view of one neotenic-leucistic individual and close-up of the head (insert). Note the well-developed external gills, tailfin, and unusual colour pattern: head, trunk, limbs, and tail are yellowish white with black spots distributed dorsally and laterally while the ventral body is coloured orange. (A) is modified after Denoël et al. (2001a).

Italian Alps represents one of the northernmost ones yet, and is furthermore from a region where this phenomenon had not been documented before.

Previous studies have shown that paedomorphism is genetically controlled in many salamander species (Semlitsch & Wilbur 1989, Voss & Smith 2005). Consequently, a genetic predisposition for paedomorphism is very probable in *I. alpestris* (Denoël et al. 2001a), i.e., in paedomorphs inhabiting and breeding in temporary pools. When these pools dry out, paedomorphic newts will metamorphose and become terrestrial, just like regular adult *I. alpestris*. Once the pond is refilled with water, paedomorphic newts will reappear in these populations. However, the process of metamorphosis is not reversible and new paedomorphs will be recruited from the next generations of larvae. The most apparent mechanism to explain the reappearance of paedomorphic newts in these ponds is an inherited genetic predisposition that is phenotypically expressed under suitable environmental conditions (Denoël & Joly 2000).

Except for the stream that feeds the irrigation pond of the study site, no permanent body of water was found within approximately 1,000 m from the reservoir, and *I. alpestris* are unlikely to reproduce or permanently live in fast-flowing streams (Thiesmeier & Schulte 2010). By contrast, some small temporary pools were present within < 500 m of the study site and these are regularly used as breeding waters by *I. alpestris* (Heiss pers. obs.). It seems likely that the irrigation pond was originally invaded from neighbouring pool populations by individuals with a possible genetic predisposition to paedomorphism.

In *I. alpestris*, both metamorphs and paedomorphs display the same courtship pattern (Denoël 2002, 2003b), and both morphs are sexually compatible. Mate choice is not biased toward paedomorphs or metamorphs and intermorph breeding is frequent, facilitating geneflow between the two morphs (Denoël et al. 2001b, Denoël 2003b). Denoël (2003b) suggested that the absence of sexual isolation does not favour sympatric speciation in newts. While this is probably the case in general, the effects of leucism in the population reported herein might have an effect on mate choice, although this is not yet known. Given that besides chemical cues (Treer et al. 2013), colour patterns do matter in male mate choice in *I. alpestris* (Himstedt 1979), a leucistic subpopulation of neotenic Alpine Newts might be subject to a certain degree of sexual isolation, especially in combination with unique environmental conditions (light-coloured bottom of the reservoir). In theory, sexual and environmental selection might favour neotenic *I. alpestris*, which might have the potential to stabilize such a population in the end, given the relative stable environmental conditions. However, a winter inspection on 16 November 2014 revealed that the irrigation pond had been pumped empty for maintenance, leading to mass extinc-

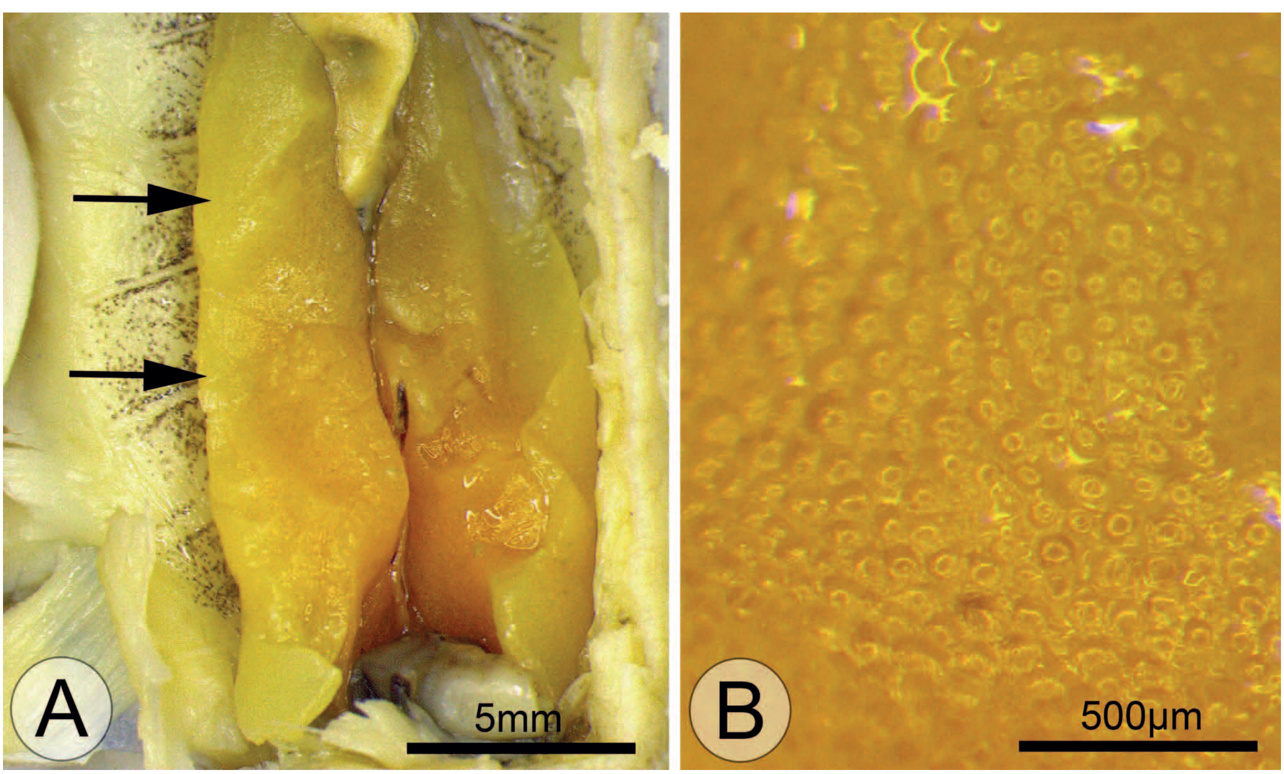

Figure 2. Stereo micrographs showing aspects of the abdominal cavity of neotenic and leucistic *Ichthyosaura alpestris* (SMNS 16344) in ventral view. (A) Overview of the abdominal cavity after removal of the digestive tract and associated structures. Note the enlarged ovaries (right ovary indicated by arrows). (B) The surface of the right ovary at higher magnification with well-visible oocytes.

tion of the resident amphibians. No live *I. alpestris* could be found on this occasion. Once the irrigation pond is refilled with water, metamorphosed Alpine Newts will most probably re-invade and use it as a breeding site. Future surveys will reveal whether leucistic neotenic *I. alpestris* reappear or whether it remains a remarkable but sadly temporary phenomenon of phenotypic plasticity.

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