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Unexpected Heterochrony in the Edible Frog, *Pelophylax esculentus* (Linnaeus 1758), and Pallas' Spadefoot, *Pelobates vespertinus* (Pallas 1771), in Eastern Ukraine

Mykola Drohvalenko*, Anna Fedorova, and Eleonora Pustovalova

Zoology and Animal Ecology Department, V.N. Karazin Kharkiv National University, Svobody Square 4, Kharkiv, Ukraine (m.drohvalenko@karazin.ua)
(Drohvalenko: https://orcid.org/0000-0002-3442-1394; Fedorova: https://orcid.org/0000-0002-5133-7928; Pustovalova: https://orcid.org/0000-0002-8396-4045)

Metamorphosis is a crucial process in anuran ontogenesis, through which the larva (tadpole) develops its adult morphology. During the climax stage, the tadpole skeleton, internal organs, and skin restructure, the tail is totally resorbed, and the tadpole loses weight (mostly due to significant water loss) (Brown and Cai 2007). It is quite important that these processes progress quickly and be coordinated in order to shorten the highly vulnerable period of the meta-

Fig. 1. Metamorphs from the Iskiv Pond in Ukraine and identified as diploid (left) and triploid (right) Edible Frogs (*Pelophylax esculentus*) on the basis of morphology (mainly coloration) and via microsatellite analysis. Photographs by Anna Fedorova.

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morph stage. Herein we report unusual asynchronically developed metamorphs of two anuran species in the same month in the same region.

On the nights of 7–8 July 2021, during an annual survey of the *Pelophylax esculentus* complex in the Iskiv Ravine Pond, Ukraine (49.627°N, 36.282°E) (Drohvalenko et al. 2019), we caught two distinctive metamorphs (Fig. 1). Based on skull maturity both were at Gosner stage 45 (Gosner 1960), but both individuals retained long and developed tails, which typically would have started to resorb shortly after stage 41 when forelimbs appear and the head starts to transform (Shi 2000). Despite being at the developmental level of an early metamorph, these individuals also had advanced developmental features. Snout-vent lengths (SVL) were 28.2 mm and 29.7 mm and tails were approximately 1.5–2 times SVL length (not measured precisely). Typical size for post-metamorphs of other Ukrainian species of *Pelophylax* is only 17–20 mm in *P. lessonae* and 18 mm for *P. ridibundus* (Terentiev 1950; Pysanets 2014; Tkachenko 2019). Average SVL of artificially reared *P. ridibundus* and *P. esculentus* post-metamorphs is only 20.6 mm (unpublished data). Berger and Berger (1992) also reported similar sizes for froglets during long-term monitoring of wild frog populations in Poland. Overall posture and leg proportions of these individuals resembled juveniles more than metamorphs, and dorsal and hip coloration was unusually developed, allowing us to identify them as *P. esculentus* (Plötner 2005). We confirmed the morphological identification using microsatellite analysis (following Christiansen 2005) and found that one of the individuals was diploid and the other triploid.

We also observed two similar cases in *Pelobates vespertinus*. A large metamorph with a mature body (SVL = 30 mm) and a long, developed tail was caught in Koriakiv Pond (49.615°N, 36.311°E) on 7 July 2021 (Fig. 2a). We caught another very similar but much smaller (around 14 mm) individual in a floodplain lake of the Udy River (Kharkiv City; 49.962°N, 36.152°E) on 17 July 2021 (Fig. 2b). Members of the genus *Pelobates* are well-known for having large larvae that at times exceed froglet size, but the first metamorph fits the typical size range for a froglet *P. vespertinus* (10–30 mm), whereas the second metamorph appeared to be stunted (Pysanets 2014).

The phenomenon of both giant tadpoles and metamorphs in *Pelophylax* has been documented throughout the entire range of the *Pelophylax esculentus* complex (from France and northern Europe to central Asia). The total size of some giant tadpoles exceeded 100 mm, and SVL of giant *Pelophylax* metamorphs from Poznań (Poland) reached 28.9–32.8 mm (Borkin et al. 1981). Covaciu-Marcov et al. (2003) described giant *Pelophylax* tadpoles from thermal waters. Guex et al. (2001) reported gigantic tadpoles with well-developed coloration occurring in *Pelophylax esculentus* progeny.

Despite the number of works, the actual causes of tadpole gigantism remain unknown, as do "the mechanisms governing the environmental regulation of metamorphosis" (Shi 2000). Iskiv Pond has dried up many times in the last few years, and several algal blooms have occurred; this decreases the possibility of overwintering (having led to overgrowth) by tadpoles. The *Pelophylax* breeding season here started relatively late in 2021 (June, typically begins in May); consequently, prolonged development was precluded. The breeding season for *Pelobates* in the region remains poorly known. Ponds drying up could accelerate tadpole development, but might not interfere with accordance (Denver et al. 1998). Internal factors, such as hormone (thyroid, prolactin, and steroids) deficiencies also may have been linked with the beginning and duration of metamorphosis (Denver 1997; Hayes 1997; Brown and Cai 2007; Vitt and Caldwell 2014).

However, we observed neither gigantic larvae (for which the term "arrested development" is often used (Rot-Nikcevic and Wassersug 2004)) nor giant froglets. Since we observed overlapping traits of metamorphic and post-metamorphic animals, we refer these cases to the convergence of sequence heterochrony and growth heterochrony, like that described for species of *Pseudis* by Fabrezi et al. (2010) and Fabrezi (2011).

Fig. 2. The metamorphs of Pallas' Spadefoot (*Pelobates vespertinus*) from the Koriakiv Pond (left) and a floodplain lake of the Udy River (right) in Ukraine. Photographs by Mykola Drohvalenko.

Linking the causes of heterochrony with ontogenetic disturbances in hybrid animals is tempting. Both individuals were hybrids, and the Iskiv Pond hemiclonal system is unique in that the reproduction mode remains unclear (Shabanov et al. 2020). The hybrid zone between *Pelobates fuscus* (Laurenti 1768) and *P. vespertinus* also occurs in this region (Dufresnes et al. 2019a, 2019b). We were unable to determine if the observed *P. vespertinus* were hybrids but the *Pelophylax* individuals were hybrids although they differed in ploidy.

Heterochrony in European anurans is uncommon, despite multiple records of larval gigantism. Considering the number of factors that influence growth, development, and metamorphosis, explaining the occurrence of these factors in the same year and region is difficult. Such variances in larval ontogenesis could be the result of external environmental factors or internal (genetic or hormonal) abnormalities. Developmental plasticity (including heterochrony) is among the adaptive processes that may have driven the past evolution of anurans (Roelants et al. 2011). As such, we cautiously suggest that such mechanisms could be adaptive in contemporary populations. Consequently, observations of heterochrony are of considerable interest and should be thoroughly documented.

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