Short notes

Toe regeneration in the neotropical frog *Allobates femoralis*

Eva Ursprung[1], Max Ringler[1], Robert Jehle[2] & Walter Hödl[1]

[1]*Department of Evolutionary Biology, University of Vienna, Austria*

[2]*School of Environment and Life Sciences, Centre for Environmental Systems and Wildlife Research, University of Salford, UK*

Toe-clipping is a standard method for marking and tissue sampling in amphibians, and in most adult anurans such marks are permanent. Here we document the consistent regeneration of toes in the aromobatid frog *Allobates femoralis* during a three-year population study. The emergence of new toe discs was observed after about two months. After one year the regrown toes had recovered to 65.6%/63.8% (males/females) of the size of unclipped toes and after two years they had attained 74.0%/69.0%. Whereas toe discs before amputation were white dorsally, all but one regenerated toe discs were dark. We did not detect any malformations or infections of the digits. Recapture rates of toe-clipped individuals were indiscernible from those of a nearby population where no toe clips were taken. We discuss a possible link between toe regeneration ability and life-history attributes.

Key words: amphibians, Aromobatidae, dendrobatoid, toe clipping

In studies of natural amphibian populations, toe regeneration is commonly reported from newts (Smith, 1978) and salamanders (Ott & Scott, 1999; Davis & Ovaska, 2001), whereas adult anurans are generally assumed to lack this ability (Halliday, 1996). Nevertheless, field studies occasionally report the regeneration of toes in anurans in an anecdotal way (Ovaska & Hunte, 1992; Jungfer & Weygoldt, 1999; Richter & Seigel, 2002), and often only juveniles or just a small proportion of adults are involved (Hoffman et al., 2008; Campbell et al., 2009; Fong et al., 2010).

Toe-clipping is a common, cost-efficient procedure of marking amphibians in the field (e.g. Donnelly et al., 1994; Schmidt & Schwartzkopf, 2010), and removed toes can also be stored as tissue samples for DNA analysis (Gonser & Collura, 1996). In species that have the capacity for restitutive regeneration, toe-clipping does not offer a permanent mark for demographic studies (Ovaska & Hunte, 1992; Jungfer & Weygoldt, 1999). Furthermore, lower recapture rates in toe-clipped animals (McCarthy & Parris, 2004; but see Lüddecke & Amézquita, 1999), as well as observations of infections and malformations of clipped toes (Golay & Durrer, 1994; Lemckert, 1996; Reaser & Dexter, 1996; Davis & Ovaska, 2001) have led to questioning of the ethics of toe-clipping in field studies (May, 2004). However, the extent of any negative impact may depend to a large extent on the species' morphology and behaviour (Lemckert, 1996; Funk et al., 2005; Liner & Smith, 2007), the number of toes clipped (McCarthy & Parris, 2004) and how clipping is conducted (Funk et al., 2005). While the use of buccal swabs with cotton buds, a tissue sampling method for DNA analysis, is easy only in larger species (Broquet, 2007), the lack of feathers, hair or sloughs in anurans offers no alternative to sampling methods that are to some extent invasive. In the present paper we document a case of consistent toe regeneration during a three-year population study on the aromobatid frog *Allobates femoralis*.

Allobates femoralis has a pan-Amazonian distribution with disjunct local populations. All activity in this species takes place on the forest floor (Hödl et al., 2004; Amézquita et al., 2009; Ringler et al., 2009). Our study population was located in a lowland rainforest near the Saut Pararé field camp (4°02´N, 52°41´W) in Les Nouragues nature reserve, French Guiana. The study plot comprised about 8.25 ha. Daily surveying took place from 15 January to 30 April 2008, from 15 January to 15 March 2009, and from 15 January to 20 March 2010, between 0900 and 1900. In all years we attempted to sample all males and females in the whole study plot. Although different years generally represent different generations due to low survival rates (Ursprung et al., 2011), the actual number of year-to-year survivors still enabled a comprehensive comparison of individuals of different age cohorts. Individuals were captured with plastic bags, recognized by distinct ventral coloration patterns, and sexed by the presence (males) or absence (females) of vocal sacs.

In the course of our study, the third toe of both hind limbs was removed from all newly encountered adult individuals using surgical scissors to cut the digit between the base and the first joint (Fig. 1b), and stored in 95% ethanol for molecular parentage analysis (Ursprung et al., 2011). Scissors were exposed to a flame to avoid DNA contamination between individuals, and no disinfectants or anaesthetics were used. We measured snout–urostyle length (SUL) as well as toe lengths of the individuals from 2010 (new encounters, one- and two-year recaptures) in the program ImageJ 1.43 (Abramoff et al., 2004), using digital photographs taken in front of a reference grid; repeated measurements revealed a precision of 0.15 mm on average.

From the first sampling period in 2008, 22 (15.3%) of the 144 registered males and eight (13.3%) of 60 registered females were recaptured in 2009, and six males (4.2%) and three females (5.0%) were again recorded in 2010. Of the 138 males and 64 females that were newly encountered in 2009, 12 males (8.7%) and 12 females (18.75%) were recaptured in 2010. A previous study on a population 11

Correspondence: Eva Ursprung, Department of Evolutionary Biology, University of Vienna, Althanstraße 14, A-1090, Vienna, Austria. *E-mail:* eva.ursprung@univie.ac.at

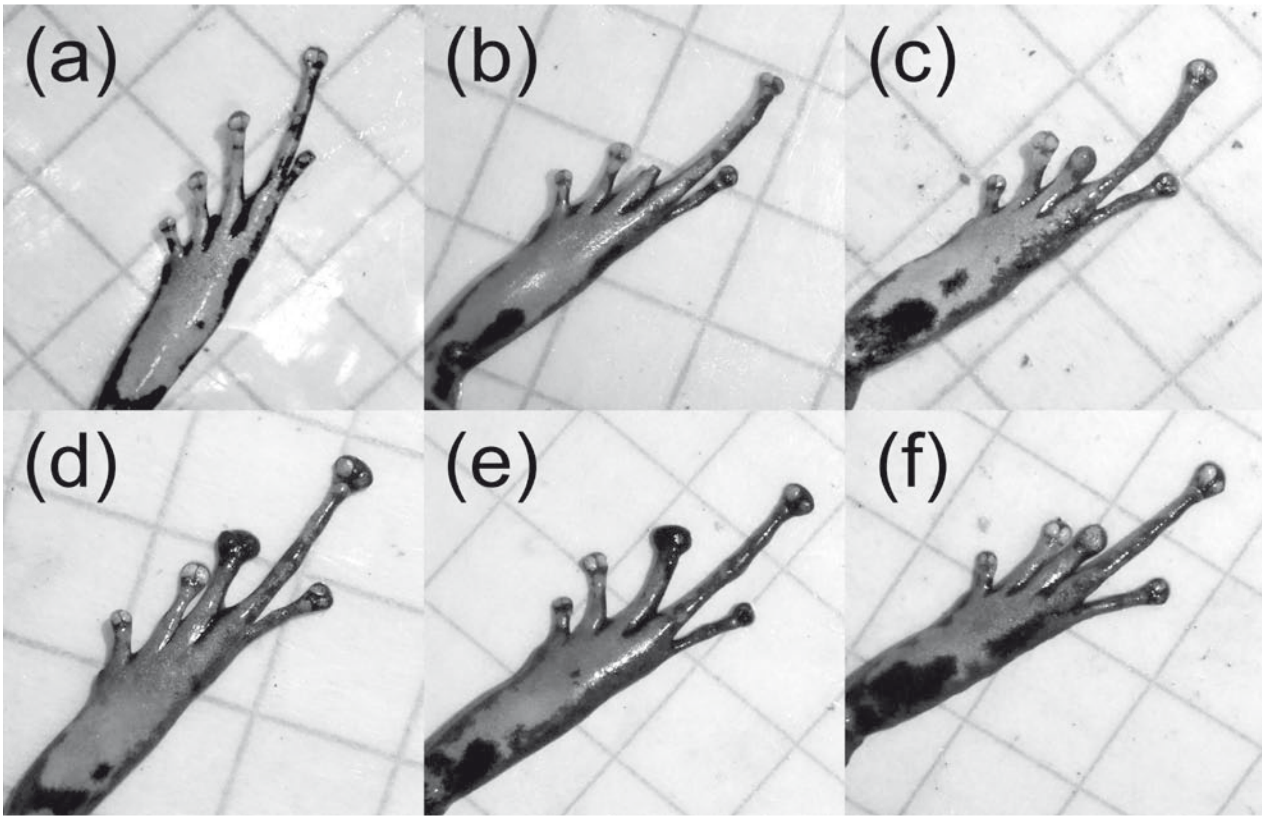

Fig. 1. Toes of *Allobates femoralis* (a) before clipping, (b) immediately after clipping, (c) after 6 weeks, (d) after 1 year, (e) after 2 years, (f) a toe-disc without colour change (remaining white instead of turning black).

km away from the site of the present study, where no toe clips were taken, revealed similar recapture rates (Ringler et al., 2009; one-year recaptures: 15.5/15.1% males/females; two-year recaptures: 2.4/2.1% males/females). We therefore have no evidence that toe clipping affected individual survival. Body size was slightly smaller in males than in females (mean SUL = 27.8/28.2 mm for newly encountered males/females), and increased with age in both sexes (males/females; one-year recaptures: 28.6/29.6 mm, two-year recaptures: 29.1/30.7 mm). The original toe length of the third toe of adult frogs was on average 4.07/3.9 mm for males/females (measurements from individuals in 2010). In all study years, we observed the regeneration of both clipped toes in all recaptured individuals after about two months (Fig. 1c). Regrown toes measured on average 2.67 mm (males) and 2.49 mm (females) in the individuals from 2009 recaptured after one year, while their length was on average 3.01 mm (males) and 2.69 mm (females) in the animals from 2008 recaptured after two years. Thus, regrown toes attained 65.6% and 74.0% (males), and 63.8% and 69.0% (females) of the average toe length of unclipped toes after one and two years, respectively. Whereas discs of unclipped toes are always white on the dorsal side (Fig. 1a), regrown toes possessed discs that were totally black (Fig. 1d,e), allowing for immediate identification of already marked individuals. Only in one case was one regenerated toe disc white (Fig. 1f). We found no malformations or infections of the clipped toes at any time.

This is the first field study that has documented the consistent regrowth of toes and toe discs over two years in adult anurans. Surprisingly, our findings differ from a study that suggested the lack of regeneration ability in another population of *A. femoralis* (Roithmair, 1992). Although removed digits did regenerate, recaptured individuals could be immediately identified in the field due to the reduced size of the clipped toe and a pronounced change in toe disc coloration. The subsequent comparison of individuals' belly patterns offered a virtually error-free means of individual identification (see also Ringler et al., 2009).

The capacity for regeneration and tissue repair differs widely across the main vertebrate groups (Sánchez Alvarado & Tsonis, 2006; Galliot et al., 2008). The fact that the capacity for gene expressions that are involved in growth and pattern formation progressively decreases with age (e.g. Endo et al., 1997; Yokoyama et al., 2000 for studies on *Xenopus*) might explain the higher prevalence of regeneration in larvae and metamorphs (e.g. Richards et al., 1975). Considering the high mutilation risk of larval amphibians by fish or invertebrate predators (Ballengee & Sessions, 2009; Bowerman et al., 2010), a high degree of regenerative ability appears to be especially advantageous at this stage. In adult amphibians, this ability is retained more widely only in urodeles and salamanders, while the actual mechanisms and reasons are not well understood (Sánchez Alvarado & Tsonis, 2006).

Despite its rarity, toe regeneration has been anecdotally reported in field studies from various anuran families, across a range of species with differing body size (e.g. Jungfer & Weygoldt, 1999; Richter & Seigel, 2002; Fong et al., 2010). The consistent toe regeneration in *A. femoralis* might be caused by the specific processes of cell proliferation and differentiation that allow for the lifelong growth of amphibians in general (Duellmann & Trueb, 1986), and the comparatively short life span of this species in particular. The difference in coloration between unclipped and regrown toe discs might in turn be due to the fact that differentiation and migration of pigment cells take place only in the early ontogenesis of amphibians (Erickson, 1993). However, quantitative and comparative as well as embryological studies of such possible effects remain to be conducted.

Finally, we would like to emphasize that at least for *A. femoralis* toe clipping has proved to be a very suitable method of tissue sampling for molecular analysis, due to the small amount of tissue needed and the high regenerative ability of this species.

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