Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Short Communication

Turk J Zool
(2017) 41:
© TÜBİTAK
doi:10.3906/zoo-1607-53

A case of limb regeneration in a wild adult *Podarcis lilfordi* lizard

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Received: 28.07.2016 • **Accepted/Published Online:** 01.08.2017 • **Final Version:** 00.00.2017

Abstract: We report here a case of spontaneous limb regeneration in a wild *Podarcis lilfordi* lizard from the Balearic Islands. The animal had lost a hind limb, which regenerated posteriorly into a tail-like appendage. Despite not representing a functional regeneration, the growth of this structure after limb amputation suggests that survival of the individual may have been favored by the less restrictive conditions prevailing in insular environments. Nevertheless, such cases are extremely rare in lizards, with no reported cases over the last 60 years.

Key words: Limb regeneration, *Podarcis lilfordi*, Lacertidae, islands, Balearics

Regeneration refers to the ability of an adult organism to restore injured or completely lost tissues and organs (Alibardi, 2010). In reptiles, successful regeneration is usually restricted to the replacement of the tail, mainly in lizards that perform tail autotomy (self-amputation) as a defensive strategy (Clause and Capaldi, 2006; Alibardi, 2010). When chased or seized by a predator, small-sized lizards, such as Lacertidae species, have the ability to deliberately release their tail, which keeps moving after being detached from the body. The intense movement of the shed tail distracts the attention of the predator, facilitating the lizard's escape (Higham et al., 2013). In evolutionary terms, this defensive mechanism, together with the role that the tail can play in locomotion (Cromie and Chapple, 2012), energy storage (Chapple and Swain, 2002), or intraspecific interactions as a status-signaling badge (Fox et al., 1990), likely favored tail regeneration throughout evolutionary time (Meyer et al., 2002; Clause and Capaldi, 2006). Indeed, tail regeneration is rare in large-sized species, such as in Varanidae lizards, where the ability to fight back allows them to not rely on tail autotomy as a defensive mechanism (Maginnis, 2006). In contrast with urodel amphibians, which are known to regenerate both functional tails and limbs (Bryant et al., 2002), even those lizards that successfully regrow the tail after it is damaged fail to achieve a functional restoration of lost limbs (Alibardi, 2010, 2017).

Lilford's wall lizard (*Podarcis lilfordi*) is a lacertid species endemic to the Balearic Islands. It is currently restricted to the Cabrera archipelago and the offshore islets of Mallorca and Menorca, as it has become extinct in the main islands (Salvador, 2014), likely due to the Neolithic introduction of allochthonous predators (Pinya and Carretero, 2011).

Remaining populations of *P. lilfordi* inhabit environments with low terrestrial predation pressure, frequently attaining high densities (up to 7815 individuals/ha, Pérez-Mellado et al., 2008). In such conditions, insular *Podarcis* individuals tend to be aggressive towards conspecifics, particularly adult males towards juveniles, with tail and toe amputation and even cannibalism tending to be common (Castilla and Van Damme, 1996; Cooper et al., 2015). Here we report a case of limb regeneration in one of these microinsular populations.

On 23 June 2014, during a lizard collecting campaign on Sa Dragonera islet, Mallorca (39°35′16″N, 2°19′42″E), we found an adult male (snout–vent length (SVL) >60 mm) of *P. lilfordi* with the left hind limb amputated up to the knee. Below the knee, the limb presented a regenerated appendage of approximately 10 mm in length, with scale rings, externally resembling a tail (Figure). Compared with the other conspecifics observed on the islet, this specimen seemed to have normal morphological features regarding apparent body mass, number of digits per limb, and general body structure, without any other anomaly

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Figure. The adult male of *Podarcis lilfordi* from Sa Dragonera, Mallorca (Balearic Islands), presenting a tail-like appendage in the left hind limb.

beyond the tail-like appendage. Another 50 individuals of the same species were sampled in the same site within a 1-h interval; despite regenerated tails being common across the population, none of them presented any evidence of limb regeneration.

Generally, limb amputation in reptiles is followed by cicatrization of the limb stump's surface, leading to a relatively short scar (Alibardi and Toni, 2005; Alibardi, 2010, 2017; Vitulo et al., 2017). The strong inflammatory reaction of the wound impedes the establishment of mesenchymal cells on the stump, which are essential in the formation of the regenerative blastema. The early stages of limb healing have been demonstrated by experimental amputation (either in adult and embryonic lizards) to be similar to those of a tail regeneration. However, in the case of the limbs the later stages rarely produced a blastema (Bellairs and Bryant, 1968). In a few cases where an initial blastema cone grows, mesenchymal cells rapidly differentiate into fibrocytes, giving place to short outgrowths or, in rare cases like this, to a tail-like appendage. In fact, a few similar cases have been reported for other lacertid species (*Podarcis muralis*, Guyénot and Matthey, 1928; *Lacerta agilis*, Weiss, 1930; *Takydromus takydromoides*, Okada, 1945) and have even been induced by experimental amputation in *P. muralis* (Guyénot and Matthey, 1928). However, the occurrence of these cases in the literature is so sparse over time that their observation can be considered certainly rare, the current example being the first to be reported in more than 60 years.

Under the insular conditions of the population, with few terrestrial predators and high densities, higher intraspecific competition would be expected (Whittaker and Fernández-Palacios, 2007; for lacertids, see Castilla and Van Damme, 1996; Carretero, 2004; Raia et al., 2010; Cooper et al., 2015). Thus, the amputation of the limb was most likely suffered by the lizard when it was still a juvenile and was likely due to an episode of conspecific aggression, triggered either by dominance behavior or a cannibalism attempt, which has already been recorded in this species (Salvador, 1986).

Against expectations, limb loss did not appear to have compromised the survival or the body condition of the individual. Despite it not being a functional regenerated limb, the simple presence of the tail-like appendage may aid in its locomotor performance. Moreover, the lizard was completely active and surrounded by adult conspecifics of both sexes when found at 1730 hours, one of the peaks of the species' bimodal activity in summer (Salvador, 1986), and no locomotor impairment was observed, since the atypical appendage was only noticed after the capture of the specimen.

According to the review by Alibardi (2010), among amniotes, lizards are the only group in which both successful and unsuccessful regeneration of tails and limbs, respectively, takes place. He hypothesized that, unlike the tail, losing a limb is more prone to have fatal results due to a loss of locomotor performance in subsequent predation events. This author speculates that this has impeded limb regeneration from being positively selected for in evolutionary history.

The representativeness of this observation in the context of the lizard insular syndrome (Novosolov et al., 2012) remains unclear. Certainly, this individual may have benefited from relaxed predation pressure on the island, increasing its survival probabilities while the limb regenerated. However, similar observations are also reported for continental populations of lacertids (Guyénot and Matthey, 1928; Weiss, 1930; Okada, 1945). On the other hand, amputation without regeneration is much more common in lizards, in both insular and continental populations. More detailed studies are needed to elucidate the exact ecological conditions and embryological mechanisms promoting limb regeneration in reptiles (Bellairs and Bryant, 1985).

Acknowledgments
The field work was supported by the project FCOMP-01-0124-FEDER-008929 PTDC/BIA-BEC/101256/2008 of FCT (Fundação para a Ciência e a Tecnologia, Portugal). We wish to thank the Servei de Protecció d'Espècies, Conselleria de Medi Ambient, Govern de les Illes Balears for permits (ref.: 32/2014) to study *Podarcis* in the Balearic Islands.

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