Loss of caudal autotomy during ontogeny of Balkan Green Lizard, Lacerta trilineata

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Loss of caudal autotomy during ontogeny of Balkan Green Lizard, *Lacerta trilineata*

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Tail loss is an effective antipredator strategy in many lizards. After loss the tail continues to thrash vigorously and may distract predators away from the escaping lizard. However, autotomy imposes energetic and survival costs (loss of lipid reserves, reduction of reproductive output, impairment of locomotor performance). Autotomy may have been lost when costs exceed benefits, while a substantial reduction or full loss may occur during ontogeny. The Balkan green lizard, *Lacerta trilineata* is a skilful sprinter despite its robust structure. Predation was simulated in a total of 83 individuals (48 juveniles and 35 adults). All juveniles shed their tail readily while none of the adults autotomized their tails. Post-autotomy duration of movement and levels of involving metabolites in shed tails were measured. No differences were found on comparison to other Greek lacertids. These findings suggest that autotomic ability is lost ontogenetically in *L. trilineata* while post-autotomy energetics seems to be a conservative character.

**Keywords:** tail loss; ontogeny; lacertids; post-autotomy energetics

**Introduction**

Caudal autotomy is an effective defensive tactic, common in lizards (Bellairs and Bryant 1985), in which the animal sheds its tail when attacked by a predator (Arnold 1988; Cloudsley-Thompson 1994). Post-autotomy tail movement facilitates escape in two ways: the shedding enables lizards to break away from predators that have grasped them by the tail (Arnold 1984), and the vigorous movement of the detached appendage distracts the predator from the escaping individual (Vitt et al. 1977; Daniels et al. 1986) simultaneously increasing handling time of the shed tail and providing enough time for a successful escape (Dial and Fitzpatrick 1983; Medel et al. 1988).

Despite the immediate benefit of tail loss, this particular tactic comes with many costs (Arnold 1988; McConnachie and Whiting 2003). The tail constitutes the primary lipid reserve for many species and thus caudal autotomy reduces energy stores (Daniels 1984; Doughty and Shine 1998; Doughty et al. 2003). Furthermore, tailless individuals have been reported to show reduced social status (Fox and Rostker 1982; Martin and Salvador 1995; Salvador et al. 1995) and decreased running speed (Ballinger et al. 1979; Punzo 1982; Martin and Avery 1998; Cooper et al. 2004). Finally, tail loss has a strong impact on reproductive output (Dial and Fitzpatrick 1981; Fox and McCoy 2000; Chapple et al. 2002), and on territorial behaviour (Fox et al. 1990; Martin and Salvador 1993a, 1993b; Salvador et al. 1996).

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Tail movement after autotomy is supported by anaerobic metabolism (Dial and Fitzpatrick 1983). Anaerobiosis is widespread in reptiles (Bennett and Dawson 1972; Gleeson and Bennett 1982; Lambrecht et al. 1991) and the main substrate is glycogen, which is oxidized into lactic acid (Gleeson 1982; Gleeson and Dalessio 1989). Lactate concentration in tails after thrashing is much higher than in intact tails (Dial and Fitzpatrick 1983) and thus lactate production can be used as an index of thrashing activity (Meyer et al. 2002).

The balance between benefits and costs plays an important role in the performance of autotomy. When cost exceeds its likely benefits, tail loss should be avoided. Arnold (1984) maintains that absence or substantial reduction of tail loss is a derived feature, which has been lost several times in several lineages. The most obvious way is the vertebral fusion of the sections across the fracture plane (Arnold 1988).

In the course of a study of autotomy in Greek lacertids (Pafilis 2003) a spectacular difference was noticed in the percentages of tail breakage between juveniles and adults of the Balkan green lizard, *Lacerta trilineata* Bedriaga, 1886. The fragmentary character of these observations and the small sample size needed an experimental study. Hence, by inducing autotomy in lizards (Perez-Mellado et al. 1997), a confirmation of the initial observations was attempted, which suggested a possible ontogenetical modification of the ability to shed the tail. Secondly, variation in post-autotomy tail activity of *L. trilineata* was compared to other Greek lacertids in order to detect any divergences due to alterations (if any) in tail loss ability. Thirdly, previous conclusions concerning the conservative character of postautotomy energetics (Pafilis et al. 2005) were tested.

### Materials and methods

#### Study species

The Balkan green lizard (*Lacerta trilineata*) is distributed in the Balkan Peninsula and Asiatic Turkey and is also present on many Aegean and Ionian Islands, and on Crete. Specimens used in this study (n=83) were collected in northern Greece (Pindos and Paiko mountains) and in Peloponnese (Mt Ziria).

*Lacerta trilineata* is a robust lizard with an average snout to vent length (SVL) of over 16 cm, and tail twice the body length or more, ranking as the biggest lacertid in the Balkan Peninsula. The lizards become sexually mature in their second spring (Nettmann and Rykena 1984). For the purposes of these experiments lizards with SVL smaller than 8 cm were considered juveniles.

All the animals were collected in the wild during the non-reproductive period (October–November), and in accordance with Greek National Law (Presidential Decree 67/81). They were subsequently held at the laboratory facilities of the Biology Department at the University of Athens. Animals were housed singly in glass terraria (90 cm × 50 cm × 50 cm) with a substratum of sand and stones as hiding places. The lizards were held at 25°C under a controlled photoperiod (12 h L: 12 h D) using fluorescent lights for a period of at least 4 weeks before the experiments took place in order allow adjustment to conditions in captivity. Additional incandescent lamps (60 W) allowed animals to thermoregulate behaviourally for 8 h per day. Animals had access to water *ad libitum* and were fed every other day with
mealworms, except for the last two days before an experiment, when they did not receive food.

Additional to the predation simulation experiment, the tails in 138 museum specimens (Herpetological Collection of the A. Koenig Zoological Research Institute and Museum, Bonn) were examined. When a broken or regenerated tail was detected, its presence was recorded and both SVL and TL of the lizard were measured (specimen codes available upon request from the authors).

**Predation simulation**

Since autotomy is often temperature dependent (Brattstrom 1965; Bustard 1968; Daniels 1984) each animal was allowed to attain its preferred body temperature for approximately 1 h before each experimental trial. A specially outfitted terrarium (1 m × 20 cm × 25 cm) providing a temperature gradient ranging was used. The gradient ranged from 17°C to 55°C, with two incandescent heating lamps (100 W and 60 W) at one end, and two ice bags at the other (Van Damme et al. 1986).

Predation simulation was carried out according to the protocol designed by Perez-Mellado et al. (1997), which closely resembles a predator’s attack. The lizards were placed in a terrarium (50 cm × 20 cm × 25 cm) covered with a rough cork mat, which allowed them to maintain good traction. To simulate the bite of a predator, a pair of calipers was used (the diameter of the tail in the position of pressure application was measured and the apparatus was then closed around it till the initial movement was halved) to grasp the tail a distance of 20 mm from the cloaca. Each trial lasted a maximum of 15 s and if by that time autotomy had not occurred, the lizard was returned to its terrarium. Once a tail was shed, the time from the moment of autotomy to cessation of all movement was measured (“exhausted” tails) and it was then placed in a container with liquid N₂. In an alternative treatment group, tails were placed immediately after shedding and without any movement, into liquid N₂ in order to determine rapidly baseline concentrations of lactate and other components at time zero (“resting tails”).

**Tissue lactate determination**

To ensure that the tail would remain frozen during muscle tissue removal (and thus metabolites would be preserved), a special round aluminum table was used, immersed by its shaft in liquid N₂ (see Pafilis et al. 2005).

Muscle tissue (approx. 150 mg) was homogenized (1:3 w/v) with 10% ice-cold perchloric acid in a cold pestle on ice. The homogenate was centrifuged at 4°C and 5000 rpm for 10 min. The supernatant was then neutralized with 0.5 M Tris/0.5 M KOH and subsequently centrifuged at 4°C and 10,000 rpm for 10 min. The pellet was discarded and the supernatant was used for the estimation of total lactate concentration according to the method described by Hohorst (1965). Lactate concentration was expressed as mg lactate/mg tissue.

**Tissue lipid determination**

Extraction of total lipids was performed by homogenizing muscle tissue (30–40 mg) with 1.5 ml of a mixture containing two volumes of chloroform and one volume of
absolute methanol. The homogenate was then centrifuged at 4°C and 3000 rpm for 10 min. The pellet was used for protein analysis (see below), and the supernatant was used for the determination of total lipid concentration, using an appropriate kit (Chromatest) according to the method described by Alexis et al. (1985). A mixture of olive oil and corn oil (2:1 v/v) was used as the standard.

**Tissue protein determination**

Determination of total protein levels was performed using the Biuret method (Layne 1957). Briefly, the pellet of centrifugation obtained from the lipid analysis (see above) was dissolved with 0.5 ml of 0.1N NaOH and incubated at 37°C for 30 min with occasional vortexing. Fifty µl of the sample was diluted with 950 µl of H2O, and 4 ml of the Biuret Reagent were added. The mixture was incubated for 30 min at room temperature and the absorbance was read at 550 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech). Bovine serum albumin (0.5 mg/ml–10 mg/ml) was used as a standard.

**Statistical analysis**

The non-parametric Mann–Whitney U test was used to compare the differences in the physiological traits between resting and exhausted tails. Differences in the various traits between the different species were examined with analysis of variance (ANOVA). This analysis was made using the PDSINGLE program from PDAP software (PDAP version 6.0, Garland et al. 2002), which performs conventional analysis of variance on traits among different species that are related with star phylogeny (Garland et al. 1993, 1997; Brashares et al. 2000).

**Results**

**Predation simulation**

In all trials 100% of juveniles shed their tail readily. In contrast, none of the adults performed autotomy (0%). While tail loss occurred in juveniles during the first 5 s, in adults no autotomy was recorded even after 15 s. Instead, adult lizards adopted an active attitude against the calipers (biting or coiling around it).

As for museum specimens, in 138 specimens, 15 broken tails (10.86%) were found to have subsequently been regenerated. From these 15 individuals only 3 were juveniles (SVL less than 8 cm). The ratio tail length/SVL was 2.1 ± 0.34 cm for lizards with intact tails (range=1.16–2.63 cm) while for adult individuals with regenerated tails it was 1.38 ± 0.25 cm and for juveniles with regenerated tails it was 0.42 ± 0.12 cm. These data demonstrate that in the case of the adult lizards with regenerated tails, autotomy had occurred when they were juveniles since regeneration was complete.

**Post-autotomy tail movement**

Average duration of tail thrashing was 5.49 min (range=2.38–9.4 min, Table 1). No statistically significant difference (ANOVA: F$_{1,5}$=2.34, P>0.05) was found when the duration of post-autotomy tail thrashing for *L. trilineata* was compared to the values for other lacertids (Figure 2 in Pafilis et al. 2005).
Lactate accumulation

Accumulation of lactate in exhausted tails was higher than in resting tails (Table 1) (Mann–Whitney U test, \( U = 5.00 \), \( P < 0.05 \)). No significant difference (ANOVA: \( F_{1,5} = 0.89, P > 0.05 \)) between \( L. \) trilineata and other lacertids (Table 2 in Pafilis et al. 2005) was detected.

Lipid and protein concentration

The concentration of proteins and lipids did not differ between exhausted and resting tails (proteins: Mann–Whitney U test, \( U = 43.5, P > 0.05 \); lipids: \( U = 6.0, P > 0.05 \)) (Table 1). Compared to other lacertids (Table 3 in Pafilis et al. 2005), no difference was found for both lipids (ANOVA: \( F_{1,5} = 3.51, P > 0.05 \)) and proteins (ANOVA: \( F_{1,5} = 0.986, P > 0.05 \)).

Discussion

According to the results of this study, adults of \( L. \) trilineata are not capable of tail loss, in contrast to juveniles. In all cases where predation simulation was applied, autotomy occurred in younger individuals while none of the adults shed their tail. These findings support the idea that tail-shedding ability has been lost ontogenetically. Ease of autotomy has been reported to decrease in the case of insular species subjected to low predation (Arnold 1988, and references therein; Perez-Mellado et al. 1997). Previous authors have pointed out that the tendency for restriction or loss of autotomy in lizards is attributed to ontogenetical changes.

Being an adaptive feature (Gould 1983; Arnold 1988), autotomy is amenable to both evolutionary and ecological effects. It is probable that the reason for autotomy loss in \( L. \) trilineata is recent, since the phylogenetic history of European lacertids is well known (Arnold 1973, 1989, 1993; Harris et al. 1998). The causes for this uncommon (among lacertids) ontogenetical change must be sought in the specific characteristics of \( L. \) trilineata. Thus, energy use (as shaped by the greater longevity and massive clutches of the species), the large body size of adults, and the locomotory characteristics must be the more important reasons.

Since many lizards store fat in their tails, loss of the tail involves loss of energy reserves, which may have severe consequences for the reproductive potential of the

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>Lactate</th>
<th>Lipid</th>
<th>Protein</th>
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<tbody>
<tr>
<td>Exhausted tails</td>
<td>5.49</td>
<td>1.93±0.35; 45; 163.6±32.8; 13; 282.1±41.8; 38;</td>
<td>(2.38–9.46)</td>
<td>(102.6–204.85)</td>
</tr>
<tr>
<td>Resting tails</td>
<td>–</td>
<td>0.89±0.04; 3; 191.9±7.6; 3; 282.5±25.29; 3</td>
<td>(0.85–0.93)</td>
<td>(186.5–197.3)</td>
</tr>
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Note: N: number of individuals; mean: mg/g tissue except from time where the unit is minute; SD: standard deviation; numbers in parentheses: range. All values are given as mean ± SD; N; (range).
individual (Dial and Fitzpatrick 1981; Fox and McCoy 2000; Chapple et al. 2002). Males use energy in social and territorial behaviour while females exploit fat reserves to produce eggs (Vitt and Cooper 1986). Karasov and Anderson (1984) reported that 90% of the energy necessary for egg production is derived from stored lipids and not from daily food intake.

Females of *L. trilineata* have a high reproductive output, laying seven to 14 eggs, while clutch sizes of over 20 eggs are not uncommon (Arnold and Ovenden 2004). Egg size is 14–22 mm width and 9–14 mm height, and SVL of the offspring varies from 3.5 to 5 cm (Arnold and Ovenden 2004). In order to produce such massive clutches, comprising numerous large eggs, *L. trilineata* females cannot afford to shed the tail, so valuable in energy terms. Loss of the tail, especially during the breeding season, could cause females to produce smaller clutches of eggs, or no eggs at all. Hence restriction or loss of ability in this case offsets the high costs of caudal autotomy.

Moreover, energy stored in the tail is crucial for survival during unfavourable periods of the year (Avery 1974; Daniels 1984; Vitt and Cooper 1986). *Lacerta trilineata*, like many other lizards, hibernates (Nettmann and Rykena 1984), especially mountainous populations like those studied here. Loss of caudal lipids has a negative effect on the ability of lizards to survive (Avery 1970; Jameson 1974; Bauwens 1981). Given the longevity of *L. trilineata*, 10–20 years according to Nettmann and Ryhena (1984), hibernation is a repeated and important feature of its life cycle. Autotomy reduction may be the solution for securing precious energy reserves. This idea is further supported by the fact that only large species (SVL over 15 cm) show great longevities (20 years for *L. lepida* Daudin, 1802, around 17 years for *Gallotia simonyi* (Steindachner, 1889)) while the majority of lacertids have shorter lives (two to seven years) (Arnold and Ovenden 2004).

Tail loss may decrease locomotor performance in lizards (Martin and Avery 1998; Chapple and Swain 2002). *Lacerta trilineata* is a skilful runner and climber (Nettmann and Rykena 1984) and the tail has an active (Vitt et al. 1977) role. Any possible modifications in locomotory potential, a crucial fitness trait (Huey and Pianka 1981), would have a serious impact on the overall performance of the lizard.

Speed has been correlated with size and growth in lizards (Van Damme et al. 1998; Aerts et al. 2000). The small size of *L. trilineata* juveniles prevents them from escaping by flight, an important element for living in open areas where predation pressure is intense (Arnold 1987; Mayer et al. 1990). In order to compensate for high predation risk, young individuals of *L. trilineata* are more prone to caudal autotomy, like juveniles of other species (Cooke 1979).

Antipredatory tactics may change ontogenetically (Greene 1988). In some iguanids, skinks and teids, for instance, juveniles are able to shed the tail readily while as adults they lack the ability, or show reduced performance since they lose fracture planes during ontogeny. Ontogenetic loss of autotomy occurs more often in large species that can display more active defence (Arnold 1984). Adult individuals of *L. trilineata* may be considered large among European lacertids, a small-body size family in general. It is not unusual to find males of a total length of almost 0.5 m in mainland Greece. Malkmus (1982) reported that the closely related *L. lepida*, the largest lacertid in Europe, with SVL up to 20 cm, rather than fleeing, often turns against the predator, trying to bite it. That same behaviour was observed in the laboratory predation simulation: adult individuals either turned and attempted to bite the caliper or they remained still. It seems that tail loss ability tends to weaken
ontogenetically in *L. trilineata*, finally to be replaced by other defence means (e.g. biting and clawing).

Examination of regenerated tails in museum specimens provides indicative information (see Arnold (1984) for collecting bias). In respect of this, museum measurements were used as an additional element, though regenerated tails may be the result of accident or predatory attack. The low percentage (10.86%) found in individuals of *L. trilineata* gave the same impression of restricted autotomy. Tails, in cases where tail shedding had occurred, were fully regenerated (most of them had tails twice the length of the body), indicating that tail loss had occurred when the lizards were juvenile.

Three possible biases that may alter autotomic performance under the laboratory conditions must be stressed. First, captive lacertids tend to tolerate a degree of handling while the same stimulus would cause an immediate tail shedding in the wild (Arnold 1984). Lizards that were tested were kept in captivity for substantial periods of time and hence might have lost the tendency to react instantaneously. Second, tail shedding occurs in lacertids readily if the predator grasps the escaping animal but if the tail is pulled or twisted by the predator then it can be detached only with difficulty (Arnold 1984). In the experimental procedure followed, lizards were first placed carefully in the trial terrarium, and then pressure was applied to the tail of the motionless individual. Furthermore, in most cases, adults, in their effort to bite the calliper, coiled their tail around it. Third, the tension of the simulated stimuli might not be sufficient to reach the threshold for autotomy. It is possible that the strength of the stimulus applied in the experiments was minor in comparison to the pressures that lizards experience in the wild.

Since only juveniles shed the tail in response to predation simulation, comparison of postautotomy movement and physiological traits refer only to this category. Average duration of tail movement for other species varies from 0.8 min (*Eumeces fasciatus* (L., 1758), Vitt and Copper 1986) to 5–5.2 min (*Scincella lateralis* (Say, 1823), Dial and Fitzpatrick 1983; *Hemidactylus mabouia* (Moreau de Jonnès, 1818), Meyer et al. 2002). As far as lacertids are concerned, times recorded for *L. trilineata* are within the range reported for other Greek species (6–8 min, Pafilis et al. 2005) while appearing longer than the values reported for western Mediterranean species (Perez-Mellado et al. 1997). Differences from previous studies may be attributed to differences in methodology, for instance the definition of “resting” tails, and to small sample sizes.

Average lactate concentrations for exhausted tails are in accordance with previous studies (Bennett and Licht 1972, and references therein; Meyer et al. 2002; Pafilis et al. 2005). This fact was more or less predictable since lactic acid is generally believed to be the cause of movement cessation (Gleeson 1996). Lipid and protein levels were similar to the respective values in other lacertids (Pafilis et al. 2005). In general, concentrations did not differ before and after tail thrashing (Table 1). Metabolism of those macromolecules is a time-consuming process and thus cannot provide the necessary amount of energy for tail movement after shedding.

An obvious discrepancy in the evaluation and interpretation of the present results concerning physiological metabolites is related to the lack of a negative control (no data for tails of adult individuals). As mentioned above, none of the experimental trials in adults led to caudal shedding and thus no tissue was available for biochemical analysis. It was decided not to remove the tail manually, since scientific work involving cruelty to animals is not acceptable (see Association for the Study of Animal Behaviour 2006).
In summary, caudal autotomy in *L. trilineata* is believed to have been lost ontogenetically. Fully-grown adults are capable of alternative antipredatory tactics that render the costly strategy of tail loss redundant. Moreover, the costs of autotomy become intolerable for individuals that have to invest in reproduction, territoriality and augmented locomotory challenges. The present results reinforce the prediction that postautotomy energetics is a conservative character, at least among the members of the same family (Pafilis et al. 2005). The examination of autotomy in similar species (e.g. *L. lepida*) and the morphological approach to the effect (fusion of vertebrae) would provide essential conclusions.

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