

## Intraspecific variation in the egg-wrapping behaviour of female smooth newts, *Lissotriton vulgaris*

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**Abstract.** In many species of the Salamandridae family, females provide parental care by carefully wrapping plant material around their eggs. As this behaviour has been shown to have a large effect on offspring survival, variation in this trait is expected to be low. Detailed investigations are, however, lacking. In the present study, we analyzed the consistency of egg-wrapping behaviour in two time periods within a breeding season in female smooth newts (*Lissotriton vulgaris*). We found a surprisingly low proportion of wrapped eggs of around 47% during the first period (when males were present) and an almost doubled ratio of around 92% in the second period (when males were absent). Also, the variation between individuals was significantly lower in the second period than in the first one. Furthermore, the bigger the females were, the more the proportion of wrapped eggs increased day by day within the first period; however, this relationship did not fully explain the observed difference between the two periods. Our results suggest that parental care in smooth newts can be influenced by the presence of mating partners and body size, and provide the first empirical evidence for within-individual variation in egg-wrapping in a salamandrid species.

**Keywords:** consistency in behaviour, egg-wrapping, *Lissotriton*, parental care.

### Introduction

Parental care increases offspring survival and has been documented in a wide variety of animal taxa including amphibians (Duellman and Trueb, 1994; Crump, 1995, 1996; Bruce, Jaeger and Houck, 2000; Summers, McKeon and Heying, 2006). For example, female newts in many species of the Salamandridae family carefully wrap leaves of submerged aquatic plants around their eggs (Díaz-Paniagua, 1989; Griffiths, 1996; Norris and Hosie, 2005). Previous studies suggested a row of benefits that may arise from egg-wrapping behaviour: it may provide protection against invertebrate predators and adult newts (Miaud, 1993; Oriazola and Braña, 2003), mechanical damage (Ward and Sexton, 1981), toxic pollution (Ortiz-

Santaliestra, 2007) and harmful UV radiation (Marco et al., 2001). By facilitating embryonic survival, this behaviour is likely to increase female reproductive success and therefore individuals can be expected to maximize their egg-wrapping efficiency.

On the other hand, wrapping each egg separately requires time and energy (Díaz-Paniagua, 1989; Miaud, 1994a): e.g., the mean time per egg deposition was estimated to be 284 s in small marbled newts, *Triturus marmoratus pygmaeus* (Díaz-Paniagua, 1989), and as in some species females lay 100-400 eggs during one breeding season, egg-wrapping can be considered costly for the performing individual. Consequently, individual differences and/or environmental factors may influence and create variation in the ability to maximize the proportion of wrapped eggs. For example, body size may influence egg-wrapping efficiency if larger females are more skilful – similarly to what was found in small marbled newts by Díaz-Paniagua (1989), where larger females were more efficient in egg-laying than smaller ones as they failed to deposit their eggs less often and wasted less time with each attempt. Also,

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females may adjust their wrapping behaviour to the net benefit they can obtain from egg-wrapping under given circumstances, e.g., invest less into egg-wrapping in habitats where or when potential predators are less abundant. Differences in wrapping behaviour between newt species have been documented before (Miaud, 1994b; Oriazola and Braña, 2003; Norris and Hosie, 2005), but despite its potential significance, the consistency of egg-wrapping within a species and within individuals has never been investigated in detail.

In order to explore between- and within-individual variation in egg-wrapping behaviour, we observed egg-wrapping frequency in female smooth newts in two time periods. Both periods belonged to the first part of the species' reproductive season (i.e., March–April) when most of the reproductive activity takes place, but differed in the presence of a mating partner. The opportunity for re-mating has been found to affect females' reproductive success in Montandon's newt, *Triturus montandoni* (Osikowski and Rafinski, 2001), but its potential effect on parental behaviour is unknown. Using the data obtained from these periods, we tested whether (i) the presence of males or (ii) individual characteristics (i.e., body size, population origin or duration of captivity) had any influence on egg-wrapping in female smooth newts.

## Materials and methods

The Smooth newt (*Lissotriton vulgaris*) is the most widespread newt species in Europe (Gasc et al., 1997) and breeds in still and slowly moving shallow waters, small ponds and temporary pools. Breeding starts in early spring and lasts for up to three months. During that period, females deposit their eggs continuously, up to 400 in total, typically wrapping leaves or other plant material around them (Nöllert and Nöllert, 1992).

We captured smooth newts using underwater traps in five ponds in the Pilis Mountains, Hungary (three ponds at 47°42'N, 19°02'E and two at 47°43'N, 19°00'E) during 13–21 March 2009. At the site of capture, we measured snout-to-vent length (SVL) of the animals with a plastic ruler ( $\pm 1$  mm) and assigned each female to a male. After transporting them to the laboratory, we housed each female-male pair separately in transparent plastic aquaria measur-

ing 50 × 30 × 40 cm, filled with approx. 18 litres of aerated reconstituted soft water (RSW; American Public Health Association, 1985). We kept the animals under a 12:12 photoperiod and 20–25°C ambient temperature (only varying between night- and day-periods within days, but not between days), and fed them ad libitum with live *Tubifex* spp. Each container contained a sponge filter, a horizontally placed clay flowerpot, and living *Elodea* threads (purchased commercially) attached to the pot as egg-laying substrate. Pairs started mating within a few days after capture. We collected deposited eggs together with the *Elodea* leaves they had been attached to every day during two periods: between 25–31 March (Period 1), when females were kept together with their mates, and also between 9–11 April (Period 2), when females were kept alone (on 1 April we removed all males from the housing containers and released them at the site of capture). At the start of each period, new plant threads were placed into the aquaria (and replaced later again if necessary) to ensure the continuous availability of leaves for egg-laying. During both periods, we counted the number of wrapped and unwrapped eggs laid by the females. Eggs were classified as wrapped if at least one leaf was folded back on them, and unwrapped if they were laid uncovered on the surface of a leaf or stem. Out of 35 females collected for a kin discrimination experiment (Tóth, Hoi and Hettyey, unpublished data), we analyzed the egg-wrapping behaviour of 19 individuals that met the following criteria: (i) they laid eggs each day during both periods, (ii) they were kept together only with one male during their captivity, (iii) they were caught in one of the three ponds (47°42'N, 19°02'E), from which 94.3% of the females originated.

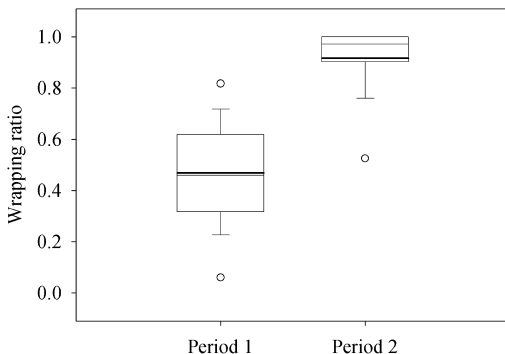
Statistical computations were performed in the R computing environment (R Development Core Team, 2009). We calculated the proportion of wrapped eggs for each individual (number of wrapped eggs divided by the total number of eggs laid) on each day, and used it in two different analyses. First, we compared individuals' average proportion of wrapped eggs between the two periods by using paired tests. Second, we analyzed proportion of wrapped eggs on seven consecutive days in Period 1 (when between-individual variation in average proportion of wrapped eggs was greater – see Results) by fitting linear mixed effect models (LMs; 'lme' function of the 'nlme' package; Pinheiro and Bates, 2000). Into these models, we included females' identity as a random factor, in which significance of the fixed factors is assessed against variation in proportion of wrapped eggs within females. Such random factor designs are equivalent to repeated measures models in R (Pinheiro and Bates, 2000; Faraway, 2006). Because preliminary analysis showed significant autocorrelation in our longitudinal data, we also added a first-order within-group correlation structure with a continuous time covariate to the random term ('corCAR1' correlation class, 'nlme' package). We included proportion of wrapped eggs as the dependent variable, and used population origin (factor), SVL (covariate), duration of captivity (days spent in captivity until the start of the observation period; covariate) and their interaction with the day of the observation ('day'; covariate) as candidate explanatory variables. By including 'day' as a fixed predictor, we could test how wrapping ratio may

change over time within Period 1 and examine whether high proportion of wrapped eggs in Period 2 can be extrapolated from its increase with days in Period 1. We used backward removal procedure for model selection (Grafen and Hails, 2002). We also calculated effect sizes (Garamszegi, 2006) for the predictors in the final model as the proportion of variance explained by each trait, i.e.,  $\eta^2$  and its 95% confidence interval (Cohen, 1988). All tests were two-tailed with a 5% significance level.

## Results

The proportion of wrapped eggs differed significantly between the two periods: females wrapped their eggs more frequently in Period 2 than in Period (Period 1:  $0.47 \pm 0.04$  [mean  $\pm$  SE], Period 2:  $0.92 \pm 0.03$ ; Sign-test:  $S = 19$ ,  $P < 0.001$ ; fig. 1), even though they also laid significantly more eggs in Period 2 (Period 1:  $14.68 \pm 0.94$  eggs/day/individual, Period 2:  $18.61 \pm 1.53$  eggs/day/individual;  $t$ -test:  $t_{18} = 2.50$ ,  $P < 0.05$ ). On the other hand, variance in average proportion of wrapped eggs between individuals was significantly greater in Period 1 than in Period 2 (Levene-test:  $F_{1,38} = 5.15$ ,  $P < 0.05$ ), indicating a lower level of consistency in egg-wrapping between individuals during the first period.

We found a significant effect of the day  $\times$  SVL interaction on the frequency of egg-wrapping in Period 1: the bigger the females were, the more the proportion of wrapped eggs



**Figure 1.** The proportion of wrapped eggs in the two sampled periods. Boxes represent the interquartile range, medians and means are marked by simple and bold horizontal lines, respectively. Whiskers extend to 10th and 90th percentile, open circles are outliers.

increased with days (table 1, fig. 2). Other predictors (i.e., population origin, duration of captivity) had no significant effect on the proportion of wrapped eggs either by themselves (population origin:  $F_{2,15} = 0.23$ ,  $P = 0.794$ ; duration of captivity:  $F_{1,16} = 1.08$ ,  $P = 0.315$ ) or in interaction with day (day  $\times$  population origin:  $F_{2,110} = 0.54$ ,  $P = 0.587$ ; day  $\times$  duration of captivity:  $F_{1,111} = 0.03$ ,  $P = 0.858$ ).

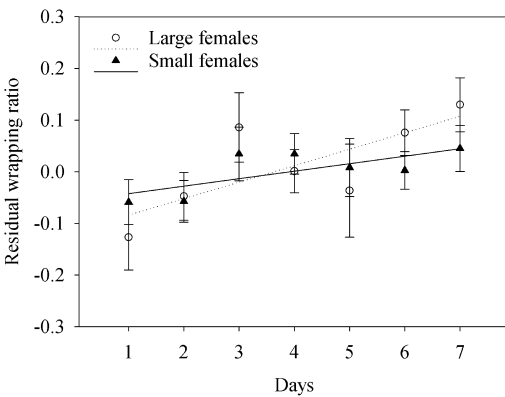
## Discussion

Although previous studies did not report variation in egg-wrapping behaviour in smooth newts (e.g., Bell and Lawton, 1975; Norris and Hosie, 2005), we found inconsistent performance of such behaviour both at the within- and the between-individual levels. In the first investigated time period, females laid many of their eggs (20-94%) without wrapping them. This is an unexpected result, since unwrapped eggs have been reported to have reduced survival probabilities compared to that of wrapped ones (e.g., Miaud, 1993, 1994b; Marco et al., 2001; Ortiz-Santaliestra, 2007), so females were expected to wrap all or most of their eggs during the entire reproductive season.

The proportion of wrapped eggs differed between the two sampled periods: average proportion of wrapped eggs almost doubled, and the variation between individuals became significantly lower in the second period. Currently we have no clear explanation for this difference. It is possible that females were less skilful in their attempt to wrap each egg at the start of their reproductive season, but wrapping may increase with experiences with the progress of the season. Such a learning process could be responsible for the greater variation in wrapping behaviour within the first period as well. Alternatively, females may have invested less time and energy in egg-wrapping during Period 1, because a mating partner was continuously present, and eggs became more valuable for females in Period 2, because their re-mating opportunities vanished due to the removal of

**Table 1.** Parameters of the final LMM with individual identity ('id') as random factor and with continuous within-group correlation structure. Within- and between-individual random effects are indicated by standard deviations (SD) and their 95% confidence intervals (CI); their values suggest similar variation in wrapping ratio both between days and among females. The confidence interval on the first-order correlation parameter ( $\Phi$ ) indicates that it is significantly different from zero, i.e., the proportions of wrapped eggs measured on consecutive days were correlated with each other.

Model parameters	Estimates [CI]	df	<i>F</i>	$\eta^2$ [CI]	<i>P</i>
<b>Fixed effects</b>					
day	−0.507 [−0.951, 0.064]	1, 112	2.24	0.02 [0, 0.10]	0.138
SVL	−0.040 [−0.115, 0.034]	1, 17	0.36	0.02 [0, 0.24]	0.555
day × SVL	0.014 [0.002, 0.034]	1, 112	5.46	0.05 [0, 0.14]	<0.05
<b>Random effects</b>					
Between-individual SD	0.166 [0.102, 0.269]				
Within-individual SD	0.205 [0.171, 0.246]				
Correlation parameter ( $\Phi$ )	0.315 [0.130, 0.587]				



**Figure 2.** Residual proportion of wrapped eggs of large (i.e., larger than the median SVL in females; >37 mm,  $N = 9$ ; open circles and dotted line) and small ( $\leq 37$  mm,  $N = 10$ ; black triangles and solid line) females on seven consecutive days in Period 1. Please note that we only created these body size categories to demonstrate the relationship between the proportion of wrapped eggs and the day × SVL interaction; in the LMM body size was incorporated as a covariate. Means are shown  $\pm$ SE.

males. On the other hand, newts are known to eat their own eggs both in the field (Bell and Lawton, 1975; Denoël and Andreone, 2003) and in the lab (Miaud, 1993, 1994b), thus the difference between the two periods may originate from the presence/absence of potentially cannibalistic males and from the fact that unwrapped eggs are more likely to be targeted during egg-cannibalism (Miaud, 1993; Gabor, 1996). However, we propose that if males' egg-cannibalism had been a major influential factor, the proportion of wrapped eggs would have been higher in the first period, which is the opposite of what we

found. It is nonetheless possible that cannibalistic foraging by males modified the observed proportion of wrapped eggs in some females, which may have resulted in the greater apparent between-female variation in average proportion of wrapped eggs in Period 1. Another possible explanation is that as the abundance of invertebrate predators increases as the season progresses, and females wrap their eggs at a higher frequency only when their eggs are more at risk due to such predators.

When we examined egg-wrapping on seven consecutive days during the first period, we found that the proportion of wrapped eggs increased with days depending on the size of females. This result is in concordance with a previous finding on small marbled newts, showing that larger females were more skilled in egg-laying (Díaz-Paniagua, 1989), and suggests that larger females in smooth newts may have higher reproductive success not only because they can lay more eggs (Verrell, 1986), but also because they can additionally increase hatching success by wrapping them. This advantage of large females may originate from experience gathered during preceding reproductive seasons (although age and body size do not correlate strongly in females of this species [Verrell and Francillon, 2009]), or large females are able to manipulate plant material more efficiently simply because of their larger body size. The results of the detailed analysis of Period 1 also suggest that the increase in wrapping ratio with

days cannot fully explain the differences between the two periods: females' wrapping efficiency greatly improved to Period 2 irrespective of their body size. Consequently, it is likely that the presence/absence of mating partners affected the proportion of wrapped eggs in our study.

In this study, we provided the first empirical evidence for within-individual variation in egg-wrapping in smooth newts. We also showed that this parental behaviour can be influenced by various factors in a complex manner. We propose that future studies are needed to better understand the evolutionary background of variation in this interesting form of parental care.

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