

Interactions between Males of the Lizard *Liolaemus tenuis*: Roles of Familiarity and Memory

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Abstract

For territorial organisms, recognition of familiar individuals can reduce the frequency and intensity of aggressive encounters ('dear enemy' phenomenon), stabilize social systems, and reduce the cost of territory maintenance. Here, we investigated the behavioural events displayed during contests between familiar and unfamiliar individuals in the lizard *Liolaemus tenuis* (Liolaemidae), a species in which males are territorial. The behaviours recorded were attack, warning, evasion, and submission, and the latencies to the first aggressive (attack or warning) behaviour. Additionally, we assessed the ability of individuals to remember a familiar conspecific after a period without social interaction. Individual males reduced and delayed aggressive behaviour directed towards socially familiar individuals compared with unfamiliar ones. These results suggest that males distinguished between familiar and unfamiliar conspecific males and are in agreement with the 'dear enemy' phenomenon. Other behaviours were similar in the contests between familiar and unfamiliar individuals. Recognition of familiar conspecifics was lost after 20 d without social interactions. This may be relevant for interactions with floater males or with neighbours that lose their territory and subsequently attempt to fight for their ex-neighbour's territory.

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Introduction

Recognition of individuals, classes or groups of conspecifics is a widespread phenomenon in animals (e.g. Ydenberg et al. 1988; Wise et al. 1993; Temeles 1994; Vache et al. 2001). Numerous advantages are associated with this recognition, such as avoidance of incest (Burda 1995), pair bond maintenance (Wunderle 1978), and reduction of aggression towards familiar conspecifics (Fox and

Baird 1992). The latter is of particular relevance for territorial species and is called the 'dear enemy' phenomenon (Jaeger 1981; Ydenberg et al. 1988). This allows a reduction of the energy spent by each individual during a contest (Jaeger 1981; Ydenberg et al. 1988) and a stabilization of the social systems (Gosling 1982; Glinski and Krekorian 1985; Hojesjo et al. 1998). Additionally, increased aggression towards unfamiliar conspecifics, such as floaters or immigrants, occurs when these individuals threaten the territory or space of a resident (Maynard Smith 1982; Ydenberg et al. 1988; Temeles 1994). Thus, aggression and social familiarity are inversely related in the 'dear enemy' phenomenon (Whiting 1999), and depend on two major cognitive processes, learning and memory (Temeles 1994; see Ydenberg et al. 1988; Getty 1989 for details). Animals need to learn and remember individuals, classes or stimuli to discriminate between familiar and unfamiliar characteristics, but the discrimination can disappear unless reinforcement of the relevant stimuli occurs (Karavanich and Atema 1998).

Reduced aggression towards familiar conspecifics has been reported in several lizard species, including *Dipsosaurus dorsalis* (Glinski and Krekorian 1985), *Crotaphytus collaris* (Fox and Baird 1992), *Platysaurus broadleyi* (Whiting 1999) and *Ctenosaura similis* (Hanley et al. 1999). Additionally, *C. collaris* showed longer latency to the first aggression in familiar contests as compared with unfamiliar contests (Fox and Baird 1992).

Liolaemus tenuis (Liolaemidae) is mainly a tree-dwelling lizard species, with sexual dimorphism and a polygynous system in which males tolerate multiple females within their territories (Donoso-Barros 1966). Males compete for territories, and probably have a hierarchical organization, as a better territory provides more opportunities to mate (e.g. more females; Manzur and Fuentes 1979). Territories of the tree-dwelling individuals usually have well-defined boundaries with little or no overlap (Manzur and Fuentes 1979), contrary to the territories of males that live on walls, which have unclear limits (Donoso-Barros 1966; R. Trigoso-Venario, pers. obs.). In addition, part of the male population comprises non-territorial floaters who can replace resident males if the latter are removed or leave the territory (Manzur and Fuentes 1979). The territoriality and possible hierarchical organization of *L. tenuis* males make this species a suitable model to test the 'dear enemy' phenomenon.

In the current study, we tested whether males of *L. tenuis* can recognize other conspecific males and how this eventual recognition modifies the interaction between opponents. We hypothesized that males should discriminate between familiar and unfamiliar individuals. The main predictions are that aggressive behaviour (i.e. attacks and warnings) should occur at a lower frequency and latency to the first aggressive behaviour should be longer during interactions with familiar as compared with unfamiliar individuals, whereas escape and submissive behaviours should occur at a lower frequency. Finally, because differential response towards familiar and unfamiliar conspecifics may be reduced or even lost with increasing time of social isolation between contestants (Karavanich and Atema 1998), we also tested whether individual recognition is lost after a period during which familiar individuals are restrained from interacting socially.

Methods

Thirty males (snout vent length: mean \pm SE = 55.8 ± 2.6 ; range = 49.8–60.1 mm; body mass = 4.7 ± 0.5 ; 3.7–5.5 g) were captured during Apr. 2001 at Río Clarillo Natural Reserve and its surroundings ($33^{\circ}51'S$, $70^{\circ}29'W$; 850 m.a.s.l.), 45 km southeast of Santiago, Chile.

The lizards were transported to laboratory facilities and placed in a thermoregulated indoor vivarium, with a glass roof. Animals were placed individually in plastic enclosures ($37 \times 30 \times 14$ cm) covered with thin plastic mesh. Each enclosure contained a layer of sand, a small plastic bowl with water, and a rock used both as shelter and basking site. The enclosures were not cleaned or modified until the lizards were returned to the field to allow the lizards to remain in an experimental arena that would resemble a 'territory'. Lizards were fed mealworms daily; these were supplemented with vitamins once per week. The vivarium was exposed to a 13h : 11h L : D illumination regime and the room temperature varied from $13^{\circ}C$ at night to $35^{\circ}C$ at midday, similar to typical hot weather conditions of central Chile in the autumn.

After 20 d of habituation to the experimental conditions, lizards were randomly grouped in pairs and housed in a new clean enclosure (but otherwise identical to their own enclosures). For their identification, individuals of each pair were marked with a small dot of water-soluble paint on their backs. Pairs remained together for 2 d to allow social familiarization. Under natural conditions males interact at high proximity; cage size was determined with this fact in mind. After the familiarization period, each lizard was relocated in its own enclosure for 1 d. Thereafter experiments were performed at two consecutive stages.

Stage 1: Lizards were placed in an experimental enclosure (see below for details), and their behaviour during confrontation with conspecifics was filmed with a digital video camera. Every individual was randomly submitted to interactions with its familiar (F) opponent (F1 treatment), that is, the lizard with which it was previously paired, or with a randomly chosen unfamiliar (U) opponent (U1 treatment), that is, an unknown individual. Individual lizards remained alone in their own enclosures for 1 d between treatments. Experiments during stage 1 lasted 3 d, after which lizards remained in their own enclosures for 20 d (a period similar to the initial habituation period), until the beginning of the next stage.

Stage 2: The same treatments as in stage 1 were performed. Each lizard was confronted randomly with the previous familiar opponent (F2 treatment) or with the same unfamiliar opponent (U2 treatment) of stage 1. In this case, 'unfamiliar' indicates that animals spent less than 1 h together. Stage 2 also lasted 3 d, and at the end of these experiments the lizards were returned in good conditions to the places where they had been collected.

The experimental enclosures were similar to those used for lizard maintenance, but lacked the rock and the water bowl and had a removable opaque acrylic wall dividing it into two equal halves. At the beginning of the experiment,

the enclosure wall was in place, and each lizard was introduced in one half of the enclosure. After 10 min of acclimatization, the wall was removed and the behaviour of both individuals was filmed for 20 min. Acclimatization and recording times were chosen on the basis of pilot experiments (R. Trigoso-Venario, pers. obs.). The temperature during experiments was maintained at around 37°C, corresponding to the temperature that *L. tenuis* chooses in a thermal gradient under laboratory conditions (Labra 1998).

The number of times that different behaviours were performed was recorded. These behavioural activities were: (1) Attacks, the total number of effective or failed bites. (2) Warning behaviour, corresponding to visual displays, either head-bobbing or push-ups. The latter are repeated raisings of the head and the anterior region of the body by pushing against the floor with the forelegs. Most push-up events consist of three rising acts. Head-bobbings are repeated vertical movements of the head, similar to push-ups. As both displays have similar warning functions (Wirringhaus 1990; Carpenter 1995), they were pooled. (3) Evasive or escape behaviour, which included movements away from the opponent, as a response to the latter's behaviour (Wirringhaus 1990). (4) Submissive or stress behaviour was assessed by tail vibration counts (Wirringhaus 1990; Mason 1992), corresponding to rapid horizontal movements of the tail, mainly at the tip.

Latency to the first observed aggressive behaviour was also recorded. It was defined as the time elapsed between the beginning of each trial and the first attack or warning behaviour exhibited (both considered collectively as aggressive displays). In all the trials, lizards responded within 3–16 min.

Fifteen individuals were chosen randomly as the focal individuals, and all the behavioural activities performed in the interaction with opponents in each treatment were recorded. For each behaviour, a matrix of 15 rows (focal individuals) and four columns (opponent and stage combinations) was obtained. Behavioural frequencies of occurrence and latency to the first aggression were analysed separately using two-way ANOVAs for repeated measures for both factors on $\log(x + 1)$ transformed data. Tukey test was used for post hoc comparisons (Zar 1984). Stage 1 tested the occurrence of the 'dear enemy' phenomenon, and stage 2 the effect of memory.

Results

Attack frequency did not differ between stages, opponents or their interaction (ANOVA, $p > 0.05$ in each case; Table 1). In contrast, warning behaviour was affected by stage and by the interaction between stage and opponent (Table 1). Overall, these behavioural variables were performed at lower frequencies during stage 1 than stage 2 (Table 2), and they were performed less frequently in F1 compared with the other treatments [F2 (Tukey test, $p = 0.009$), U1 ($p = 0.025$) and U2 ($p = 0.004$)]. No differences were detected among F2, U1 and U2 treatments (Tukey test, $p > 0.05$ in each case). Evasive/escape and submissive/stress behaviours were only affected by stage (Table 1). Both

Table 1: Results of repeated-measures ANOVA testing for effects of opponents (familiar vs. unfamiliar), stage (1 vs. 2), and their interaction (df = 1,14), on different behavioural variables and the latency to the first aggressive behaviour (attack or warning) recorded during interactions between *Liolaemus tenuis* males. F indicates the value of the ANOVA result, and p the probability

Variable	Opponent F (p)	Stage F (p)	Opponent × stage F (p)
Attack	1.25 (0.28)	2.10 (0.169)	1.70 (0.21)
Warning behaviour	2.32 (0.15)	17.35 (0.001)	8.82 (0.01)
Evasive behaviour	0.01 (0.95)	14.64 (0.002)	1.07 (0.32)
Submissive/stress behaviour	1.02 (0.33)	6.27 (0.03)	2.14 (0.17)
Latency to the first aggressive behaviour	0.36 (0.56)	21.08 (0.0004)	14.44 (0.02)

Table 2: Frequency of occurrence of four behavioural variables and latency to first aggressive behaviour during the different treatments involving interactions between males of *Liolaemus tenuis* (see Methods for a detailed description of treatments and behaviours). Data are presented as $\bar{x} \pm SE$

Variables	Stage 1		Stage 2	
	Familiar (F1)	Unfamiliar (U1)	Familiar (F2)	Unfamiliar (U2)
Attack	0.3 ± 0.1	0.6 ± 0.4	5.9 ± 4.1	0.9 ± 0.5
Warning behaviour	6.6 ± 2.5	20.2 ± 8.4	33.5 ± 8.8	24.9 ± 5.9
Evasive/escape behaviour	3.4 ± 1.0	4.6 ± 1.1	13.4 ± 5.0	12.6 ± 3.5
Submissive/stress behaviour	1.6 ± 1.0	4.2 ± 1.7	5.0 ± 1.3	5.7 ± 2.4
Latency to first aggressive behaviour ^a	13.6 ± 1.3	7.1 ± 1.5	4.9 ± 0.9	7.2 ± 1.5

^a Latencies were recorded in minutes elapsed until the first observed aggressive behaviour.

behavioural activities occurred more frequently during the second stage. Warning was the most frequently observed behaviour, followed by evasive/escape behaviour (Table 2).

Latency to the first observed aggressive behaviour (attack or warning) was affected by stage and by the interaction of both factors (Table 1). Latency was longer in stage 1, and it was significantly longer during F1, compared with the other treatments [F2 (Tukey test, $p = 0.001$), U1 ($p = 0.023$) and U2 ($p = 0.035$)]. No differences were detected among F2, U1 and U2 treatments (Tukey test, $p > 0.05$ in each case).

Discussion

Our results suggest that males of *L. tenuis* discriminate between familiar and unfamiliar males following a period of social familiarization. Lizards exhibited fewer warning displays and longer latency to aggressive behaviour during contests with familiar male conspecifics than with unfamiliar ones. However, the frequency of occurrence of attacks, evasive/escape, and submissive/stress behaviours were not affected by familiarity. Thus, on the one hand, our predictions in relation to the 'dear enemy' phenomenon were partially supported. On the other hand, as predicted, memory played a key role in this discrimination since preclusion of interaction between familiar individuals for 20 d led to an increase in warning displays and shorter latency to the first aggressive behaviour. In this case, familiar individuals reacted to each other as if they were unfamiliar individuals. Thus, social separation reduces familiarity in *L. tenuis*, in agreement with the war of attrition model (Ydenberg et al. 1988).

Brief interactions (20 min) with an unfamiliar individual in the first stage did not affect the interactions with the same individual 20 d later; that is, unfamiliar individuals did not become familiarized during the brief contest to which they were exposed. This inability to remember briefly encountered individuals may be particularly relevant when considering that, under natural conditions, there is a population of non-territorial floaters who periodically make short visits to other males' territories in attempts to usurp them. Based on the current results, short encounters between floaters and territorial males will not induce familiarization between them, and each attempt by a floater to gain a territory will result in the same level of aggression from the territory resident. This does not contradict the 'dear enemy' phenomenon as floaters do not meet frequently with territorial males and hence a floater is never considered a familiar individual (Ydenberg et al. 1988). Animals often require multiple interactions over an extended period of time to establish normal social relationships with individuals with whom they will interact on an ongoing basis (Stamps and Krishman 1994, 1995).

Some behavioural differences between stages could be a consequence of the effect of time spent in captivity. There is a positive relationship between stress level and the time an animal has spent in captivity (Moore et al. 1991). Perhaps this is the main reason why evasive/escape and submissive/stress behaviours were more frequent in the second stage, and results for these variables may be unrelated to the type of opponent.

The low level of attack behaviour and the fact that they were observed only in 28% of all contests may be because of the low levels of testosterone whilst in captivity (Moore et al. 1991), a factor known to reduce overall aggression (e.g. Marler and Moore 1989; Marler et al. 1995). Additionally, the lack of differences in the frequency of attacks between familiar and unfamiliar individuals could be explained by the high cost of eventual injuries in relation to the value of the disputed resource. Given that interactions were performed in a neutral arena without a valuable resource (e.g. females) present, 'softer' or less risky aggressive behaviour such as warning displays would pay off more than attacks, as is in fact

shown by the comparatively higher frequency of warning behavioural acts exhibited than of attacks (Table 2).

In summary, we showed that males of *L. tenuis* are able to discriminate between familiar and unfamiliar conspecific males under laboratory conditions. This should reduce the cost of interactions with familiar opponents, in agreement with the 'dear enemy' phenomenon. Nevertheless, familiarity is lost after a period without social interactions. Future research should focus on ascertaining the relevance of these results to natural conditions, as well as on the mechanism determining the capacity to react differently towards a familiar individual (see López and Martín 2001). Discrimination between 'familiar' and 'unfamiliar' classes of individuals, habituation to stimuli of familiar individuals, or individual recognition could be in operation, although the latter seems more likely in *L. tenuis* given its hierarchical social system (Manzur and Fuentes 1979) and its ability to show self and conspecific discrimination (Labra and Niemeyer 1999).

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