

Red coloration in juvenile spiny-footed lizards, *Acanthodactylus erythrurus*, reduces adult aggression

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In many animal taxa, coloration is a visual signal used for communication among conspecifics, for example between age classes. Juvenile coloration has been hypothesized to reduce aggression from adults in some species, in what is called the aggression avoidance hypothesis. Spiny-footed lizards are good subjects for testing this hypothesis, as juveniles develop conspicuous red coloration on their hind limbs and tails that fades in adulthood. To test the influence of juvenile coloration on adult aggressiveness, we conducted videotaped encounters in captivity between adults of both sexes and juveniles with their natural red coloration, or experimentally painted either red or white on their natural red parts. Then we recorded the number of times juveniles were bitten and attacked. In unpainted juveniles, no significant relationship was found between juvenile coloration (brightness, red chroma or hue) and adult aggressiveness. However, juveniles painted red were bitten less than those painted white when number of times bitten was controlled for number of times attacked. This result supports the aggression avoidance hypothesis, as an escalation from low-intensity (attacking) to high-intensity aggression (biting) was less probable towards red juveniles. The presence of red coloration in juveniles caused the reduction in adult aggression, while small natural variations in this red colour did not seem to have any further effect. Juvenile red coloration in this species might indicate age or sexual immaturity to adults.

Keywords: aggression avoidance, between-age class communication, female mimicry, juvenile coloration, spectrophotometry, staged encounter, status signalling

Animal communication involves a wide variety of signals (Bradbury & Vehrencamp, 2011). One of them is coloration, which serves, among other functions, for intraspecific communication between age classes in several taxa (e.g. Barrios-Miller & Siefferman, 2013; Hawkins, Hill & Mercadante, 2012). Intraspecific communication based on coloration is commonly studied under the paradigm of sexual selection, but much less is known about the signalling function of juvenile coloration (but see Bailey, 2011; Barrios-Miller & Siefferman, 2013; de Ayala, Saino, Møller & Anselmi, 2007; Tringali & Bowman, 2012; van den Brink, Henry, Wakamatsu & Roulin, 2012). Conspicuous juvenile coloration is not very common (Cott, 1940), but is present in some species and may, for example, modulate parental investment in offspring feeding and defence (Barrios-Miller & Siefferman, 2013; de Ayala et al., 2007), or indicate age (Hawkins et al., 2012; Kemp, 2006). One of the main hypotheses explaining the evolution of conspicuous coloration in juveniles is the aggression avoidance hypothesis, which states that juvenile coloration reduces adult aggression towards juveniles, and is supported by studies in several animal groups (Clark & Hall, 1970; Hill, 1989; Ochi & Awata, 2009). Despite this support, the effects of juvenile coloration on adult aggressiveness are still under debate (Cooper & Greenberg, 1992; Hawkins et al., 2012).

Lizards are good subjects for testing the aggression avoidance hypothesis, as juveniles of several species have conspicuous coloration that fades when they reach sexual maturity (Carpenter, 1995; Cooper & Greenberg, 1992; Hawlena, Bochnik, Abramsky & Bouskila, 2006). This hypothesis was first proposed in lizards by Clark and Hall (1970), who suggested that juveniles develop such coloration to show aggressive adult males that they are not competitors, and are therefore attacked less. It has been suggested that this mechanism is beneficial to both adults and juveniles, as it reduces the possibility of the former attacking their own offspring and lessens adult

aggression on the latter with the associated stress and/or risk of injury. The few previous studies analysing the role of juvenile lizard coloration in interactions between age classes have used different methods and reached different conclusions, even within the same species. Clark and Hall (1970), for example, found fewer attacks from *Plestiodon (Eumeces) fasciatus* adult males towards conspecific juveniles with blue tails compared to juveniles with autotomized tails, whereas other authors (Cooper & Vitt, 1985) did not find such differences, either in the same or in other species. However, experiments comparing adult aggression towards juveniles with and without tails do not seem the best procedure to examine the effects of juvenile tail coloration on adult behaviour. Another technique used to study the adaptive value of juvenile coloration is to experimentally paint the coloured patches that are presumed to reduce adult aggression. In some species, paint has been used to cover typical juvenile colour patches and no significant differences in adult aggression towards painted and unpainted juveniles were found (Cooper & Vitt, 1985; Husak, McCoy, Fox & Baird, 2004). However, in other species, subadult males painted to mimic juveniles/females were more tolerated by adult males than unpainted ones (Werner, 1978). In this case, painted subadult males were probably recognized as adult females and not as juveniles, because adult males approached them in the typical way they approach adult females, while juveniles are usually chased away (Werner, 1978). The disparity of results found in the few studies that have attempted to test the aggression avoidance hypothesis in lizards makes necessary more research on the factors driving the evolution of juvenile conspicuous coloration in this taxon.

Two main types of aggression may be seen in lizards: attacking (fast direct approach), which can be considered low-intensity aggression, and biting, which can be considered high-intensity aggression. Although biting may sometimes cause lethal

injuries, more often it just increases the probability of mortality, for example, by causing open wounds that are susceptible to infection, or the loss of part of the tail, thus depriving the individual of an antipredator mechanism and fat stores (Arnold, 1984; Arnold, 1988; Bauwens, 1981; Wilson, 1992). The loss of the tail may be even more important for juveniles than for adults, as the former will need to invest resources in tail regeneration that will not be available for somatic growth (Bateman & Fleming, 2009). Furthermore, attacks (without biting) might also reduce juvenile survival rate, for example, if subordinate individuals are forced to live in suboptimal habitats (Carothers, 1981), or if escaping from attack involves loss of foraging opportunities (Cooper, 2000; Cooper, Pérez-Mellado, Baird, Baird, & Caldwell, 2003; Pérez-Cembranos, Pérez-Mellado & Cooper, 2013). Therefore, if a signal of age or sexual immaturity can deter adult aggression, it will increase juvenile survival and thus individual fitness, and will be evolutionarily selected.

The spiny-footed lizard is a good model organism for testing the aggression avoidance hypothesis, as juveniles of this species have conspicuous red coloration on their hind limbs and tails that fades in adulthood. Aggression avoidance could be an explanation for juvenile coloration in this species, because juveniles and adults coexist (Seva Román, 1982), and although juveniles usually occupy more open areas than adults (Seva Román, 1982), there are frequently encounters between individuals of both age classes in which juveniles flee from adults (personal observation). In the present study, we tested the aggression avoidance hypothesis in spiny-footed lizards through an experimental approach, specifically examining (1) whether the number of times unmanipulated juveniles were attacked and bitten by adults of both sexes was related to red colour parameters (brightness, red chroma and hue), and (2) whether the number of attacks and bites by adults of both sexes differed between juveniles painted red

(imitating juvenile coloration) or white (imitating adult coloration). According to the aggression avoidance hypothesis, redder juveniles were expected to be attacked and bitten less. Aggressiveness was also expected to be higher in adult males than in adult females, as encounters between age classes were conducted during the mating period, when male lizards are assumed to be more aggressive (Baird, Timanus & Sloan, 2003).

METHODS

Study Species

The spiny-footed lizard is medium-sized (snout–vent length (SVL) and total length up to around 80 and 230 mm, respectively; Carretero & Llorente, 1993; Seva Román, 1982), although sizes can vary between populations. In populations in central Spain, this species reaches sexual maturity when males are 58–65 mm SVL and females 60–66 mm SVL (Bauwens & Díaz-Uriarte, 1997), during their second spring. At our study site, the mating period runs from May to June; newborn lizards appear in mid-August and are active until November. At the beginning of April, these lizards become active again, and they do not reach sexual maturity until the following spring. These observations agree with those described for other populations in central Spain (Castilla, Barbadillo & Bauwens, 1992; Pollo & Pérez-Mellado, 1990). Therefore, all individuals can be divided into three rough age categories, hereafter referred to as hatchlings (from hatching until the first winter), juveniles (from first to second winter) and adults (from the second winter onwards).

Coloration in this species undergoes ontogenetic changes. Juveniles develop red coloration on the rear part of their hind limbs and the ventrolateral part of their tails (Carretero & Llorente, 1993; Seva Román, 1982). Juvenile males lose the red colour at the end of their second summer, whereas juvenile females retain it through adulthood (Seva Román, 1982). In the second spring, when animals of both sexes reach sexual maturity, sexual dichromatism becomes evident: while males show white coloration on the rear part of their hind limbs and the ventrolateral part of their tails, females retain red coloration in these body parts until they are gravid, when they lose their red colour and become pallid yellow, nearly white (Cuervo & Belliure, 2013; Seva Román, 1982). It remains unclear whether adult females, after the reproductive season is over, regain their red coloration.

Captures and Captivity Conditions

A total of 47 adults (21 females and 26 males) and 49 juveniles were captured, using a fishing pole with a loop of dental floss at the end, from April to June 2010 and 2011 in Chapinería, southwestern Madrid Region, Spain (40°22'N, 4°13'W). The area is a Mediterranean oak forest with meadows, where oaks, *Quercus ilex*, and lavender, *Lavandula stoechas*, dominate vegetation patches surrounded by open areas. Lizards were placed in individual cloth bags (23 x 28 cm) in the shade immediately following capture to prevent overheating. After a maximum of 6 h from capture, they were transported by car to the Alcalá University Animal Research Centre, Madrid, for the experimental study. During transport, which never took longer than 1 h, lizards were kept in their individual cloth bags at a temperature of around 22 °C. All lizards looked

healthy when they arrived at the laboratory. Lizards were then placed in individual terraria (42 x 26 cm and 19 cm high) with a thin layer of sand on the bottom and cardboard shelter (egg cartons). Olfactory, but not visual, contact between individuals was possible, through a mesh covering the top of the terrarium. The light cycle was 12:12 h light:dark. Room temperature was 25 °C and a bulb hanging over the edge of each terrarium provided a temperature gradient for thermoregulation. Lizards were supplied with food (two mealworms, *Tenebrio molitor*, dusted with vitamins) once a day, including the day of arrival at the laboratory, and water ad libitum. No lizard was kept in captivity for more than 79 days (mean + SD = 43.47 + 17.16 days, $N = 96$). We checked daily whether animals looked healthy and the food supplied had been eaten. All animals were released after the study in exactly the same places where they had been captured. All of them behaved normally when released.

Experiment in Captivity

We classified lizards as juveniles or adults according to their SVL, considering individuals with SVL over 63 mm as adults (Bauwens & Díaz-Uriarte 1997; Castilla et al., 1992). Mean + SD SVL for adults and juveniles was 74.6 + 3.9 mm (range 67–82 mm, $N = 47$) and 48.7 + 4.8 mm (range 39–63 mm, $N = 49$), respectively. Adults were sexed according to the base of the tail, which is much wider in males than in females (Blasco, 1975), but juveniles could not be sexed because sexual dimorphism in juveniles of this species is not evident in spring. All adult females captured were considered to be sexually receptive because they all showed the typical red coloration of

sexually receptive females in this species (Cuervo & Belliure, 2013) and there were no copulation marks or oviductal eggs in any of them.

Lizards were kept in their individual terraria at least 3 days before trials for acclimatization. We videotaped (JVC GZ-MG680 camera) encounters, each lasting 30 min, between lizard pairs (one adult and one juvenile) in a neutral trial terrarium (77 x 55 cm and 43 cm high) with a thin layer of sand on the bottom and no shelter (see Ethical Note). Both lizards were placed in the trial terrarium at the same time to avoid the effects of prior residency on the outcome of encounters (Cooper & Vitt, 1987; Olsson & Shine, 2000). Trials were performed from 1000 to 1700 hours local time, within the species' period of activity (Busack, 1976). A bulb hanging over the middle of the trial terrarium provided light. The temperature in the trial terrarium was recorded in some encounters to ensure that it was within the activity range for the species (mean + SD = 32.9 + 2.1 °C, range 29.7–35.3 °C, $N = 63$; Belliure, Carrascal & Díaz, 1996). No lizard was involved in more than one trial per day to prevent prolonged or excessive stress. All encounters took place during the mating period (from mid-April to the end of June) in both years.

Two kinds of interactions between an adult of either sex and a juvenile were designed based on juvenile coloration: painted (hereafter, painted trials) and unpainted (hereafter, natural colour trials). In the painted trials, naturally red parts of juveniles were painted either white (adult-like coloration) or red (juvenile-like coloration) with nontoxic paint (Satin Paint, La Pajarita, Manises, Spain). Paint colour partially overlapped with the range of natural coloration this species develops, although juveniles included in this study did not reach the maximum red chroma observed in the species (Fig. 1). We attempted to mimic the reddest coloration found in juveniles of this species with red paint and to mimic adult coloration with white paint (see Appendix Fig. A1 for

reflectance spectra). The use of lizard models instead of real juveniles was not appropriate in this study because adult aggression may depend heavily on juvenile behaviour, as aggressive and submissive behaviours usually elicit different responses from the opponent (e.g. Van Dyk & Evans, 2008).

Four 45 min pilot tests were carried out to determine the optimal trial length, allowing some aggressive behaviour to happen while keeping it as short as possible to minimize lizard stress. According to these tests, 30 min seemed to be an appropriate length for trials because lizards started moving within the first 10 min, the number of times that juveniles were attacked and bitten within 30 min ranged from two to 16 and zero to one, respectively, and the level of aggressiveness was acceptable, i.e. juveniles were not injured when bitten and could flee when attacked. We tried to minimize the number of lizards involved in the trials but allowing a sample size sufficient to achieve large statistical power with at least medium effect size (for instance, a two-tailed t test with $\alpha = 0.05$ reaches large power (e.g. 0.80) with medium effect size (e.g. 0.50) when $N = 128$, and in our aggression comparison between juveniles painted red and white, $N = 124$; see Results). A total of 216 interactions were recorded, but 13 videos were discarded as one of the animals remained immobile (this might imply a strong reaction to manipulation or captivity) and one video was discarded because of an error in juvenile colour measurement. Therefore, 202 interactions were used for the study: 124 painted trials and 78 natural colour trials. Some individuals (41 juveniles and 33 adults) were used in both painted and natural colour trials, but each adult–juvenile pair was used only once. Mean \pm SD difference in SVL between adults and juveniles within pairs was 26.2 ± 5.3 mm (range 14.5–39.0 mm, $N = 202$). For each trial, we counted (on video recordings) the aggressive behaviours that adults directed at juveniles, specifically attacking (fast direct approaches; low-intensity aggression) and biting (high-intensity

aggression). The number of times that juveniles were attacked (mean + SD = 3.27 + 5.03, $N = 202$, range 0–27) or bitten (mean + SD = 0.50 + 1.22, $N = 202$, range 0–8) was not generally large. Moreover, attacking and biting never lasted for more than 5 and 2 s, respectively, and biting never caused visible bleeding or injuries, so no interaction had to be prematurely stopped. Juveniles always fled (never counterattacked) when attacked or bitten.

In the painted trials, each adult was recorded from two to five times. Each trial consisted of an interaction with a juvenile of one of the two colour treatments (white or red). All adults took part in at least one trial with each colour treatment and participated in approximately the same number of trials per colour treatment. There were 63 trials with juveniles painted red (29 trials with 16 different adult females and 34 trials with 19 different adult males) and 61 trials with juveniles painted white (28 trials with 16 different adult females and 33 trials with 19 different adult males). A total of 45 juveniles were used in this experiment, 34 of which were painted both white and red, but always presented to different adults in every trial, so the same adult–juvenile pair was never recorded twice. The first colour treatment presented to an adult was chosen at random, but ensuring a similar number of adults in the first trial with red ($N = 19$) and white ($N = 16$) treatments. We also randomized the first colour treatment applied to juveniles that were used in both red and white treatments, while ensuring a similar number of juveniles in the first trial were painted red ($N = 24$) and white ($N = 21$). As juveniles were randomly assigned to the red or white treatment and most of them were included in both, a significant difference in SVL or in natural colour parameters (brightness, red chroma and hue) between experimental treatments was not expected, and indeed did not occur (Student's t test: $t \leq 0.51$, $P \geq 0.610$ in the four tests).

In the natural colour trials, we measured the reflectance of hind limbs and tails of juveniles after each interaction as a measure of the colour shown by the juvenile (see Colour Measurements below). Each adult was recorded from one to three times. A total of 35 trials were recorded for 20 different adult females and 43 trials for 25 different adult males. We used 45 juveniles for these trials, of which 33 were used twice, but always with adults of different sex.

Colour Measurements

Colour measurements are described in detail elsewhere (Cuervo & Belliure, 2013). Briefly, we quantified the spectral properties of juvenile coloration after each natural colour trial by taking reflectance readings (with a USB 2000 spectrometer and a DT-MINI-2-GS tungsten halogen light source, Ocean Optics, Dunedin, FL, U.S.A.) in the range of 320–700 nm (Whiting et al. 2006). Four body regions (the rear part of both hind limbs and the ventral part of the tail around 1 cm and 2.5 cm from the cloaca) were measured three times each. Then, reflectances at 1 nm intervals in the range studied were calculated using AVICOLOR software (Gomez, 2006). From the reflectance data, three colour parameters were calculated as follows: brightness as the mean reflectance between 320 and 700 nm, red chroma as the sum of reflectances between 630 and 700 nm divided by the sum of reflectances between 320 and 700 nm, and hue as the wavelength at which the maximum reflectance was recorded (Montgomerie, 2006). Although measurement error was relatively high, repeatability of the three colour parameters in each body region was statistically significant in all cases (repeatability according to Lessells & Boag (1987); brightness: $0.638 \leq r \leq 0.919$; red chroma: $0.860 \leq r \leq 0.896$; hue: $0.612 \leq r \leq 0.666$; $F_{78,158} \geq 5.70$, $P < 0.001$ in the 12 tests), so we

calculated the means for each body part. Likewise, as measurements in the four body regions were positively correlated (Pearson correlations; brightness: $0.535 \leq r \leq 0.878$; red chroma: $0.454 \leq r \leq 0.801$; hue: $0.235 \leq r \leq 0.547$; $N = 79$, $P \leq 0.038$ in the 18 tests), they were all unified in single brightness, red chroma and hue values for each individual (the mean of the four body parts), and these values were then used for further analyses (Cuervo & Belliure, 2013).

Statistical Analyses

We analysed whether juvenile natural colour or the experimental treatment (red- or white-painted tail) had an effect on the number of times juveniles were attacked or bitten using mixed-effect models, one of the best methods when the same individual appears in more than one experimental trial (Briffa et al., 2013). Specifically, we used generalized linear mixed models (GLMMs) with a Poisson error distribution. GLMMs always included the following terms: juvenile SVL, because opponent size may influence lizard behaviour (Arnott & Elwood, 2009); sex of the adult, as adult males were expected to be more aggressive than adult females (Baird et al., 2003); number of days the adult had been in captivity before the trial, as this might affect adult behaviour; adult identity as a random factor to control for possible differences between adults (apart from sex); and juvenile identity as another random factor to control for possible differences between juveniles (apart from SVL or coloration). In addition, juvenile colour parameters (brightness, red chroma and hue) measured immediately after each trial were included in analyses of natural colour trials, and experimental treatment (red or white) and also the interaction between experimental treatment and adult sex were

included in analyses of painted trails. Because the number of times bitten was positively related to times attacked in both natural colour and painted trials (natural colour trials: $\beta + SE = 0.184 + 0.044$, $Z = 4.20$, $N = 78$, $P < 0.001$; painted trials: $\beta + SE = 0.090 + 0.023$, $Z = 3.91$, $N = 124$, $P < 0.001$), all analyses of the number of times bitten were repeated including times attacked as another predictor. A backward stepwise procedure was used in all GLMMs, retaining only terms associated with P values below 0.10 in final models. We also checked whether the use of Akaike's information criterion (Burnham & Anderson, 2002) to select final models yielded qualitatively identical results regarding the variables of interest (juvenile coloration and the experimental treatment), and this was indeed the case (the whole set of best models for every analysis according to Akaike's information criterion can be found in Appendix Tables A1–A6).

We checked whether juvenile painting itself had any effect on adult behaviour by comparing the times attacked and bitten, and times bitten controlled for number of times attacked between naturally red juveniles and those painted red. The predictors included in the GLMMs with Poisson error distribution were juvenile SVL, type of juvenile (painted red or naturally red), number of days the adult had been in captivity before the trial, adult sex and the interaction between the type of juvenile and adult sex. We also included adult and juvenile identity as random factors.

All statistical analyses were carried out using R (R Development Core Team, 2014). GLMMs were implemented using the lme4 package (Bates, Maechler, Bolker & Walker, 2014). All statistical tests were two tailed and the significance level was 0.05.

Ethical Note

This study was conducted following the ASAB/ABS (2006) and ASIH (2004) guidelines for the treatment of animals in behavioural research, and complied with the laws of Spain and the Madrid Autonomous Region. Permission to capture and keep spiny-footed lizards in captivity was given by the Madrid Autonomous Region Environment Department (permit number 10/163269.9/10 in 2010 and 10/315072.9/11 in 2011).

Capture methods, captivity conditions and release procedures used in this study seemed to be appropriate as determined by our previous experience with this species both in the field and in the laboratory (Belliure & Carrascal, 2002; Belliure et al., 1996; Cuervo & Belliure, 2013). The capture method used in this study (noosing) is appropriate for small lizards (Fitzgerald, 2012) and has been used with other lizard species with no apparent detrimental effect (e.g. Healy, Uller & Olsson, 2007; López, Hawlena, Polo, Amo & Martín, 2005). We observed no adverse effects of noosing and transport to the laboratory, and all individuals were in good condition during both housing and experimental procedures. When animals arrived at the laboratory, they were gently handled to measure their size and weight. This first handling of the individuals also served to detect any abnormality in physical condition or appearance that could indicate an individual was not in good health. An endpoint criterion for the experimental trials (Morton & Hau, 2010) was established a priori: trials should be terminated if attacking lasted more than 15 s, if biting lasted more than 5 s, or if biting caused injury or bleeding. As these levels of aggression never happened, there was no need to interrupt any trial. None of the lizards studied showed any sign of stress or pain (all of them behaved and fed normally) after each experimental trial. No shelter was provided in the experimental arena because juveniles were expected to act always as subordinates

and might have retreated to the shelter, thus preventing any aggressive interaction taking place and making it impossible to perform the experiment.

When lizards were released in the field after the study, they always behaved normally, i.e. they immediately fled to hide under the vegetation and, after a short time (seconds or a few minutes), began basking or searching for food. Neither captivity nor the experiment seemed to affect lizard survival negatively, as the percentage of individuals recaptured in 2011 (17%) was similar to recapture rates reported for *Acanthodactylus* lizards in other field studies (e.g. Rehman, Ahmed & Fakhri, 2002). Although the study was conducted during the mating season, no adverse consequences for the studied population were detected, possibly owing to the small proportion of lizards captured and to the resilience of this species to the extraction of individuals (Busack & Jakšić, 1982). In fact, a reduction in capture rate during the study or in the following seasons was not noticed.

RESULTS

Natural red coloration of juveniles was not significantly related to adult aggression, as no colour parameter was retained in the final models explaining the number of times attacked or the number of times bitten (either absolute or relative to times attacked). Times attacked was not significantly related to any of the predictors considered, whereas times bitten (both absolute and relative to times attacked) increased as adults spent more time in captivity prior to the trials (GLMMs; $Z \geq 2.12$, $N = 78$, $P \leq 0.033$ in the two tests).

Juveniles painted red or white in painted trials did not differ in the number of times attacked, as none of the predictors was retained in the final model (GLMM including experimental treatment as the only predictor: $\beta + SE = 0.198 + 0.147$, $Z = 1.35$, $N = 124$, $P = 0.178$). When number of times bitten was analysed, we found that adult males were biting more than adult females (Table 1), but juveniles painted red or white did not differ in the number of times bitten (Table 1). However, if number of times attacked was included in the model, juveniles painted red were bitten less than those painted white, and adult males were still biting more than adult females (Table 2, Fig. 2). The fact that the interaction between experimental treatment and adult sex was excluded from the final model when times bitten was controlled for times attacked (Table 2) implies that the reduction in adult aggressiveness towards red-painted juveniles occurred in adults of both sexes.

The number of times juveniles painted red and unpainted (i.e. naturally red) were attacked and bitten did not differ significantly, as no predictor was retained in the final models (GLMMs including experimental treatment as the only predictor: $Z \leq 0.680$, $N = 141$, $P \geq 0.496$ in the three tests), suggesting that the paint itself did not significantly affect adult behaviour.

As we found significant differences in the number of times juveniles painted red and white were bitten relative to times attacked, and given that both groups showing red coloration were bitten a similar number of times, we explored whether juveniles showing natural red coloration were also bitten less than juveniles painted white while simultaneously controlling for times attacked. We repeated the same analysis used to compare juveniles painted red and white, but using the natural colour group instead of the one painted red. As expected, juveniles showing natural red coloration were bitten less than juveniles painted white (final GLMM including type of juvenile, number of

times attacked, adult sex and time in captivity; type of juvenile: $\beta + SE = 1.325 + 0.494$, $Z = 2.68$, $N = 139$, $P = 0.007$; all other predictors: $Z \geq 2.51$, $N = 139$, $P \leq 0.012$).

DISCUSSION

In this study, we found that adult spiny-footed lizards were less aggressive towards red juveniles in painted trials: juveniles painted red were bitten less than those painted white when controlling for times attacked. Although red-painted juveniles were not attacked or bitten less than those painted white, once a juvenile was attacked, its probability of being bitten was lower if it was painted red, implying a difference in adult aggressiveness depending on juvenile colour. Therefore, our results support the aggression avoidance hypothesis, i.e. that juvenile red coloration reduces aggression by adults. Similar results were found in a different lizard species when adult male aggression was compared between subadult males with or without juvenile/female coloration (Werner, 1978), although subadult males with juvenile/female coloration were probably recognized as adult females, whereas red-painted juveniles in our study were probably recognized as juveniles (see below for the possible mechanisms behind the reduction in aggressiveness). Other studies, however, have not found a reduction in aggression towards conspicuously coloured juveniles (Cooper & Vitt, 1985; Husak et al., 2004), suggesting that conspicuous juvenile coloration may have different functions in different lizard species.

This study enabled us to test the importance of the presence/absence of red juvenile coloration (painted trials) and of the natural variation in red juvenile coloration (natural colour trials) to adult aggressiveness. The combination of three different results

suggests that the probability of juveniles being bitten (once they have been attacked) is reduced simply by displaying red coloration, regardless of small variations in the expression of the signal. First, red juveniles (both naturally red and painted red) were bitten less than those painted white when controlling for times attacked. Second, naturally red juveniles and those painted red were bitten a similar number of times. Third, there was no significant relationship between natural red colour expression and times bitten. There was an escalation in intensity of aggression (i.e. an increase in the number of times bitten once the juvenile had been attacked) towards juveniles painted white, possibly because adults considered adult-like juveniles real adults and thus potential competitors, at least for some period of time and despite their smaller size (Laubach, Blumstein, Romero, Sampson & Fofopoulos, 2013; Martín & Forsman, 1999; Rohwer, 1977). In contrast, both naturally red juveniles and those painted red showed the typical red coloration of their age class, so adults behaved as they normally do with juveniles showing subordinate behaviour which does not imply real competition for resources, i.e. without resorting to high-intensity aggression. Thus, the presence of red coloration in juveniles reduced the probability that low-intensity adult aggression (attacking) escalated into high-intensity aggression (biting).

Body size was expected to have an effect on adult behaviour because it is used by reptiles to assess fighting ability, with larger individuals being stronger competitors than smaller ones (Andersson, 1994; Olsson & Shine, 2000). Body size is usually correlated, among other features, with the ability to cause injury (Arnott & Elwood, 2009). In our study, juveniles of different sizes were used in the trials and juvenile SVL was therefore included in our statistical models. However, no significant effect of juvenile body size on adult aggression was found in any statistical test for either of the two types of trials (painted and natural colour trials). The reason juvenile body size had

no significant effect on the number of times attacked or bitten may be the considerable difference in body size between the juvenile and the adult in each encounter, which was always more than 14 mm SVL and usually more than 20 mm SVL (see Experiment in Captivity). Juvenile fighting ability was probably always perceived by adults as modest, and small variations in juvenile size would not affect that perception.

Adult males in this study were more aggressive towards juveniles (biting more) than adult females. This result was expected because trials were recorded during the mating season, when adult male lizards are usually more aggressive to defend territories or to gain access to females (Baird et al., 2003). Home ranges have been defined for age and sex classes in this species (Seva Román, 1982), but the possible defence of these areas has not yet been studied, so it is not known whether this species is territorial. In reptiles, territorial species (in which aggressive encounters are common) have developed stereotyped aggressive displays to assess the fighting ability of the opponent, and thereby avoid escalation of aggression unless necessary (Whiting, Nagy & Bateman, 2003). Although this kind of behaviour has not been described for spiny-footed lizards, and no such display was observed during the trials, our results suggest that adult males are aggressive (at least more than adult females) towards possible competitors during the mating season. In any case, aggressive behaviour in lizards may be influenced by many different factors other than territoriality, such as population density (Manteuffel, 2001) or food availability (Simon, 1975), probably often mediated by circulating levels of steroid hormones (e.g. testosterone; Moore, 1987).

This study tested the hypothesis that red coloration in juvenile spiny-footed lizards was associated with a reduction in aggression by adults, and found results consistent with the hypothesis. Two mechanisms have been hypothesized to explain the reduction in aggressiveness associated with juvenile coloration, the status-signalling

hypothesis (Rohwer, 1977) and the female mimicry hypothesis (Rohwer, Fretwell & Niles, 1980). These mechanisms have been studied mainly in birds (e.g. VanderWerf & Freed, 2003; Vergara & Fargallo, 2007), but have not generally been investigated in other taxa. In both hypotheses, the ultimate function of the colour signal is aggression avoidance, but the underlying mechanism is different: whereas female mimicry implies that adult males cannot distinguish between females and juveniles, status signalling suggests that juvenile coloration announces subordination and is not necessarily mimetic. Female mimicry might be possible in this system, as red coloration is present in juveniles and in sexually receptive females (Cuervo & Belliure, 2013; Seva Román, 1982), but a peculiarity of this and other lizard species is that males looking for mating opportunities try to bite females in order to hold them down and ensure copulation (e.g. Bosch & Zandee, 2001). Therefore, it would be a poor strategy for juveniles to mimic receptive females, as this might increase rather than reduce aggression by adult males. Although our experimental design does not allow us to discriminate between these two mechanisms, current knowledge on the ecology of the species would be more in accordance with the status-signalling hypothesis. The fact that the reduction in adult aggressiveness towards red-painted juveniles occurred in adults of both sexes (also in females) provides further support for the status-signalling hypothesis in this species.

To summarize, our results support the aggression avoidance hypothesis, suggesting that red coloration in juvenile spiny-footed lizards reduces aggression from adults. The mere presence of this colour signal in juveniles, regardless of small variations in its expression, informs adults of both sexes about age or sexual immaturity (and thus possibly subordination).

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Table 1

GLMM explaining the number of times juveniles were bitten in painted trials ($N = 124$)

	$\beta + SE$	Z	P
Intercept	-2.512 + 0.745	-3.37	< 0.001
Experimental treatment	0.210 + 0.598	0.35	0.725
Adult sex	1.510 + 0.725	2.08	0.037
Time in captivity	0.053 + 0.027	1.95	0.051
Experimental treatment * Adult sex	-1.306 + 0.751	-1.74	0.082

Juvenile SVL was removed from the final model after a backward stepwise procedure (see Statistical Analyses for details of the test).

Table 2

GLMM explaining the number of times juveniles were bitten in painted trials while simultaneously controlling for the number of times attacked ($N = 124$)

	$\beta + SE$	Z	P
Intercept	-2.196 + 0.511	-4.30	< 0.001
Number times attacked	0.155 + 0.032	4.86	< 0.001
Experimental treatment	-1.021 + 0.337	-3.03	0.002
Adult sex	0.966 + 0.465	2.08	0.038

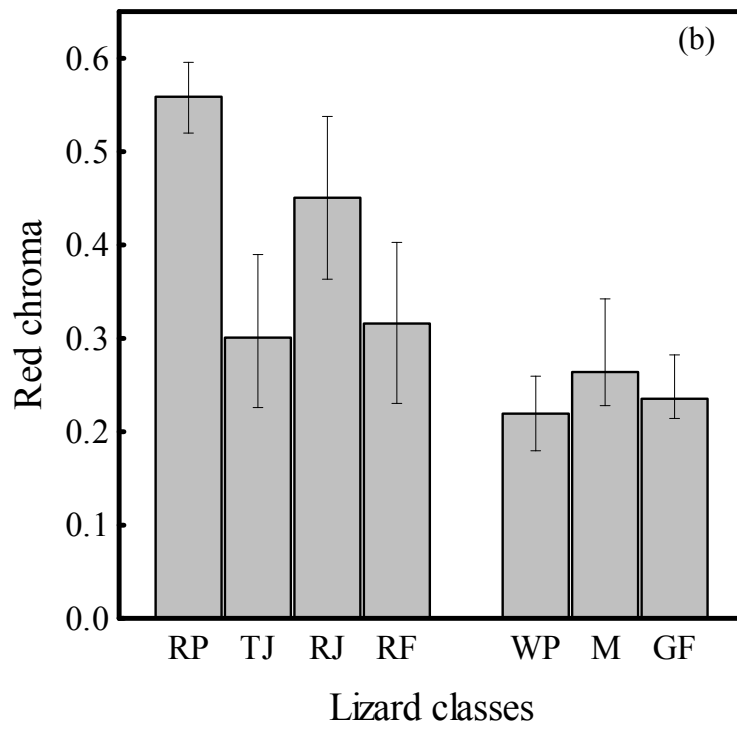
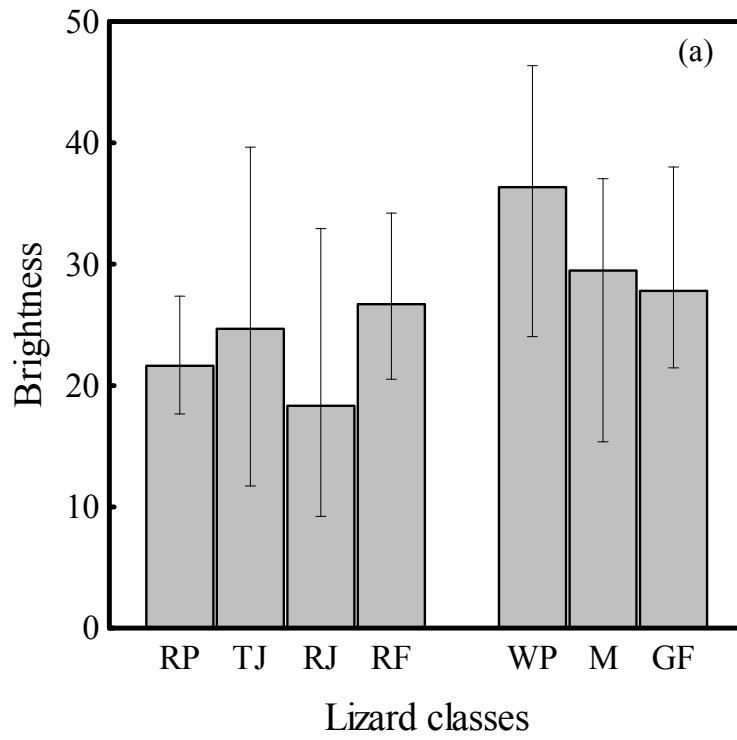
Juvenile SVL, time in captivity and the interaction between experimental treatment and adult sex were removed from the final model after a backward stepwise procedure (see Statistical Analyses for details of the test).

Figure captions

Figure 1. Mean, maximum and minimum (a) brightness, (b) red chroma and (c) hue of hind legs and ventral tail in spiny-footed lizards and of the paints used for red and white experimental treatments. Red paint (RP; $N = 12$) and white paint (WP; $N = 12$) were measured on the lizard. Lizard classes: TJ = trial juveniles (all juveniles used in the natural colour trials, $N = 78$); RF = receptive females (all females used in painted or natural colour trials, $N = 20$); RJ = reddest juveniles (sample of the reddest juveniles of this species, $N = 12$); M = males ($N = 5$); GF = gravid females ($N = 20$). Classes RJ, M and GF include individuals not used in this study and are shown for comparative purposes. All colour measurements were taken on day of capture except for TJ, which were taken immediately after natural colour trials.

Figure 2. Mean \pm SE relative number of times juvenile spiny-footed lizards painted red or white were bitten (controlled for number of attacks) by adults of both sexes in painted trials. Relative numbers of times bitten were residuals from a GLMM including number of attacks as the only predictor (see Statistical Analyses and Table 2).

Figure 1



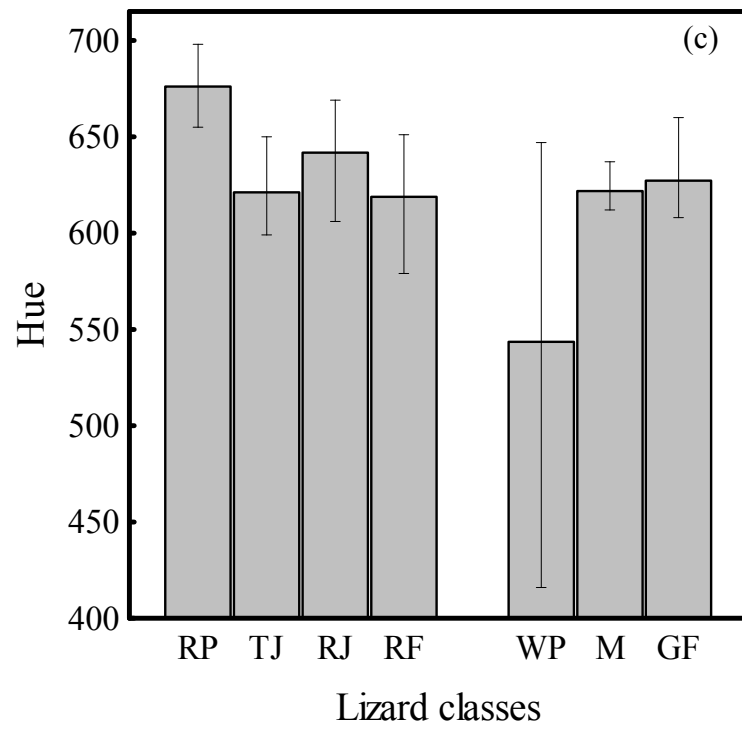
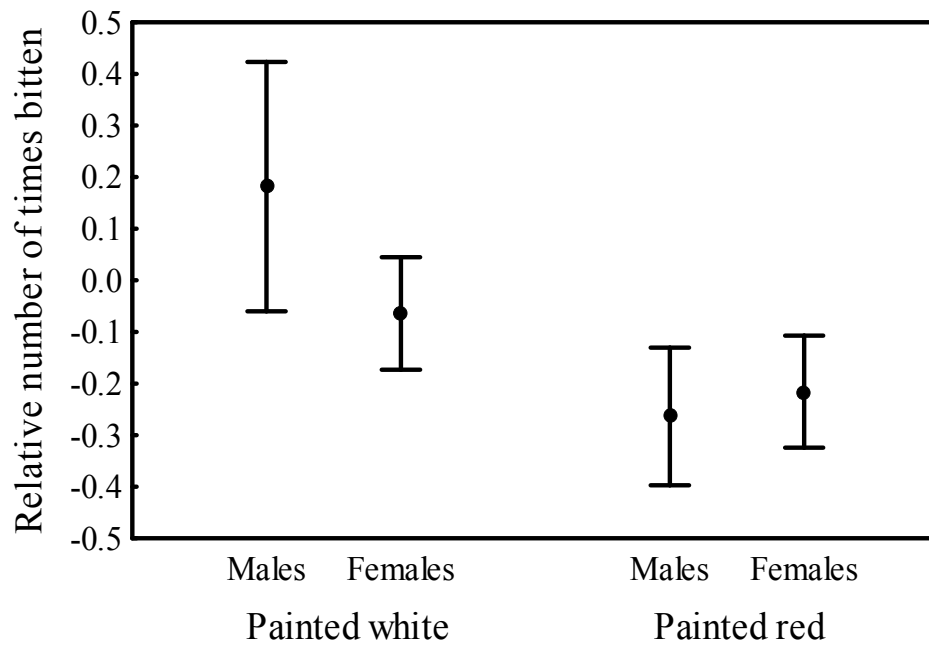


Figure 2



APPENDIX

Table A1

Best models explaining the number of times juvenile spiny-footed lizards were attacked by adults in natural colour trials using Akaike's information criterion (AICc)

Model	AICc	Δ AICc
SVL	365.45	0.00
SVL + time in captivity	365.68	0.22
Hue + SVL	365.87	0.42
Hue + SVL + time in captivity	366.42	0.96
No predictor	366.48	1.03
Adult sex + SVL	366.91	1.45
Time in captivity	366.94	1.48
Red chroma + SVL	367.12	1.67
Adult sex + SVL + time in captivity	367.25	1.80

Only models differing by less than 2 AICc units from the model with the smallest AICc are shown. Adult and juvenile identities are included as random factors in all models.

Table A2

Best models explaining the number of times juvenile spiny-footed lizards were bitten by adults in natural colour trials using Akaike's information criterion (AICc)

Model	AICc	Δ AICc
Time in captivity	109.35	0.00
SVL + time in captivity	110.01	0.66
Hue + time in captivity	111.17	1.82

Only models differing by less than 2 AICc units from the model with the smallest AICc are shown. Adult and juvenile identities are included as random factors in all models.

Table A3

Best models explaining the number of times juvenile spiny-footed lizards were bitten by adults while simultaneously controlling for the number of times attacked in natural colour trials using Akaike's information criterion (AICc)

Model	AICc	Δ AICc
Time in captivity	94.59	0.00
Brightness + time in captivity	95.40	0.81
No predictor (apart from times attacked)	96.30	1.71
Adult sex + time in captivity	96.43	1.85
Hue + time in captivity	96.49	1.90

Only models differing by less than 2 AICc units from the model with the smallest AICc are shown. Adult and juvenile identities are included as random factors in all models. Number of times attacked is also included in all models.

Table A4

Best models explaining the number of times juvenile spiny-footed lizards were attacked by adults in painted trials using Akaike's information criterion (AICc)

Model	AICc	Δ AICc
No predictor	586.18	0.00
Experimental treatment	586.64	0.46
Time in captivity	587.88	1.70

Only models differing by less than 2 AICc units from the model with the smallest AICc are shown. Adult and juvenile identities are included as random factors in all models.

Table A5

Best models explaining the number of times juvenile spiny-footed lizards were bitten by adults in painted trials using Akaike's information criterion (AICc)

Model	AICc	Δ AICc
Experimental treatment + adult sex + time in captivity + experimental treatment * adult sex	250.89	0.00
Experimental treatment	251.40	0.51
Experimental treatment + adult sex	251.90	1.01
Experimental treatment + adult sex + time in captivity	251.91	1.02
Experimental treatment + time in captivity	252.03	1.14
Experimental treatment + adult sex + experimental treatment * adult sex	252.19	1.30

Only models differing by less than 2 AICc units from the model with the smallest AICc are shown. Adult and juvenile identities are included as random factors in all models.

Table A6

Best models explaining the number of times juvenile spiny-footed lizards were bitten by adults while simultaneously controlling for the number of times attacked in painted trials using Akaike's information criterion (AICc)

Model	AICc	Δ AICc
Experimental treatment + adult sex	232.83	0.00
Experimental treatment + adult sex + time in captivity	233.63	0.80
Experimental treatment + adult sex + experimental treatment * adult sex	234.70	1.87

Only models differing by less than 2 AICc units from the model with the smallest AICc are shown. Adult and juvenile identities are included as random factors in all models. Number of times attacked is also included in all models.

Figure A1. Mean, maximum and minimum reflectance spectra of (a) red paint ($N = 12$) and natural red coloration of some of the reddest juvenile spiny-footed lizards ($N = 12$), (b) red paint and natural red coloration of juveniles used in the natural colour trials ($N = 78$), (c) red paint and natural red coloration in sexually receptive females used in painted and/or natural colour trials ($N = 20$), (d) white paint ($N = 12$) and natural white coloration in adult males ($N = 5$), and (e) white paint and natural white coloration in gravid females ($N = 20$). See Colour Measurements and Fig. 1 legend for further details.

Figure A1

