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Perception over phenotype: opponent colour, not colour morph, shapes aggression in polymorphic lizards

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Research Article

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Abstract

Colour signals are widely assumed to convey information about competitive ability, yet their reliability remains debated, particularly in polymorphic species. We investigated the role of ventral colouration in aggressive interactions in two colour-polymorphic lizards, *Podarcis peloponnesiacus* and *Podarcis thais*. Using staged dyadic contests between size-matched, naïve males, we tested whether colour predicts contest outcomes and aggression levels, and whether experimentally manipulated colour influences behaviour independently of intrinsic traits.

We conducted 160 contests across intra- and interspecific pairings, including both natural and colour-manipulated treatments. Aggression was quantified using a weighted behavioural scoring system and analysed using GLM models. Morphological traits (snout–vent length and body condition) did not differ among colour morphs, confirming effective control of size asymmetries. Contrary to expectations, neither contestant nor opponent natural colour predicted aggression or contest outcome. Instead, species identity

was the primary determinant of aggression, with *P. peloponnesiacus* consistently more aggressive than *P. thais*.

However, we detected a context-dependent effect of perceived opponent colour. *P. peloponnesiacus* showed elevated aggression toward red opponents regardless of their intrinsic morph, whereas this effect was weak or absent in *P. thais* and disappeared in interspecific encounters. Experimental colour manipulation did not significantly alter aggression overall.

Findings suggest that colour doesn't function as an honest signal of competitive ability in this system. Rather, colour—particularly red—appears to influence behaviour via receiver perception. This highlights a mechanism where visual stimuli modulate aggression independently of signaler quality, with implications for the maintenance of colour polymorphism and the evolution of communication systems.

Keywords: colour polymorphism, sexual selection, aggression, *Podarcis*, honest signalling, contest behaviour

Significance Statement

Animals often use colour to advertise their fighting ability, but whether these signals are truly honest remains unclear. Working with two endemic, colour-polymorphic lizards of the Peloponnese, *Podarcis peloponnesiacus* and *Podarcis thais*, we staged 160 contests in which males competed over a limited heat source. We found that a male's colour did not predict who won or how aggressively he fought. What mattered was the species and, strikingly, the colour each male saw in his opponent: males of *P. peloponnesiacus* became more aggressive when facing a red rival, even when that redness was experimentally applied and did not reflect the rival's true identity. This shows that colour can shape animal contests through the viewer's perception rather than as a reliable signal of quality, offering new insight into why colour diversity persists in nature.

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Introduction

Colour signals play a key role in animal communication, particularly in the context of sexual selection, where they mediate both intersexual attraction and intrasexual competition. In many species, males use visual signals to convey information about their superiority, competitive ability or motivational state, thereby potentially influencing the outcome of aggressive encounters (Berglund et al. 1996). These signals may function either as honest indicators, accurately reflecting an individual's condition, or as dishonest signals, misleading competitors about their true fighting ability (Maynard Smith and Harper, 2003).

Colour polymorphism – the coexistence of two or more distinct, heritable colour morphs within a single population (Huxley 1955) – is particularly widespread in lizards (among other taxa; Stuart-Fox et al. 2021) and is often associated with specific morphological (Brock et al. 2020) or physiological traits (Huyghe et al. 2007) and behavioural strategies (Sinervo and Lively, 1996). Accordingly, in several lizard species, particular colour morphs have been linked to variations in aggression, dominance and reproductive success (Thompson and Moore, 1991; Martin et al. 1999).

Following Zahavi's Handicap Principle, red colouration is expected to signal increased aggression or superior competitive ability. Some findings across taxa remain consistent with this hypothesis (Evans and Norris, 1996; Pryke and Andersson, 2003; Hill and Barton, 2005). In certain systems, specific colour morphs reliably predict contest outcomes (summarised in Table 1), whereas in others, factors such as body size, prior residency or physiological condition appear to override any effect of colouration. In particular, it remains unclear whether colour signals act as reliable indicators of competitive ability (honest signals) or whether they can influence contest dynamics independently of intrinsic male quality. Distinguishing between these alternatives is crucial for understanding how colour polymorphism – and phenotypic diversity more broadly – is maintained within populations.

Table 1 Summary of research findings regarding the dominant colour morph in male-male contests across different lizard species. The third column indicates the prevailing colour morph (Red, Yellow, White, Blue or Black) or trait associated with a higher win rate

Paper	Species	Prevailing Colour Morph
Huyghe et al. 2007	*Podarcis melisellensis*	RED
Brock et al. 2022	*Podarcis erhardii*	WHITE
Mandavilli et al. 2025	*Podarcis erhardii*	WHITE
Hamilton et al. 2013	*Chlamydosaurus kingii*	RED
Abalos et al. 2016	*Podarcis muralis*	WHITE
Pérez i de Lanuza and Carretero 2018	*Podarcis muralis*	YELLOW
Sacchi et al. 2009	*Podarcis muralis*	SIZE / PRIOR RESIDENCY
Coladonato et al. 2020	*Podarcis muralis*	YELLOW
Sinervo and Lively 1996	*Uta stansburiana*	RED
Bastiaans et al. 2013	*Sceloporus grammicus*	RED
Thompson and Moore 1991	*Urosaurus ornatus*	BLUE
Bruinjé et al. 2019	*Tropidurus semitaeniatus*	BLACK
Healey et al. 2007	*Ctenophorus pictus*	RED
Stuart-Fox and Johnston 2005	*Ctenophorus vadnappa*	YELLOW
Yewers et al. 2016	*Ctenophorus decresii*	RED

Paper	Species	Prevailing Colour Morph
Stuart-Fox et al. 2006	*Bradypodion pumilum*	RED
Noble et al. 2021	*Eulamprus quoyii*	SIZE
Fernández et al. 2018	*Liolaemus sarmientoi*	RED

Study Species

The animals participating in this study belong to the Western and Eastern Peloponnese wall lizards: *Podarcis peloponnesiacus* (Bibron and Bory, 1833) and *Podarcis thais* (Buchholz 1960). Both are endemic lacertid lizard species of the Peloponnese and exhibit polymorphic ventral colouration with two discrete morphs. Males within populations of both species display white, red or intermediately coloured throats (Fig. 1), whereas females are not polymorphic.

The two species are distributed in the western and eastern Peloponnese, respectively (Fig. 2). A contact zone lies from northern to southern Peloponesse between Lakes Stimfalia and Doxa that syntopy has been observed (Kiourtsoglou et al. 2021), and limited interactions are suspected between bordering populations (Fig. 2). This zone therefore provides a strong rationale for investigating the effects of colour signals at the interspecific level.

Aims of the Study

In this study, we explored the effect of ventral colouration during pairwise agonistic intra- and interspecific interactions between size-matched males with no prior fighting experience, confronting each other in a neutral arena. We further tested whether throat colouration functions as a dishonest signal of dominance – i.e. whether the mere perception of a given colour can influence contest outcomes independently of the

bearer's actual quality – by experimentally altering natural colouration. Alternatively, if colouration acts as an honest signal, it is expected to correlate with underlying physiological or morphological advantages (e.g. strength, endurance or hormonal profile), in which case contest outcomes should reflect intrinsic male quality rather than visual perception alone.

Fig. 1 The colour morphs of male *Podarcis peloponnesiacus* (above) and *Podarcis thais* (below). (Photos by A. Panagiotopoulos)

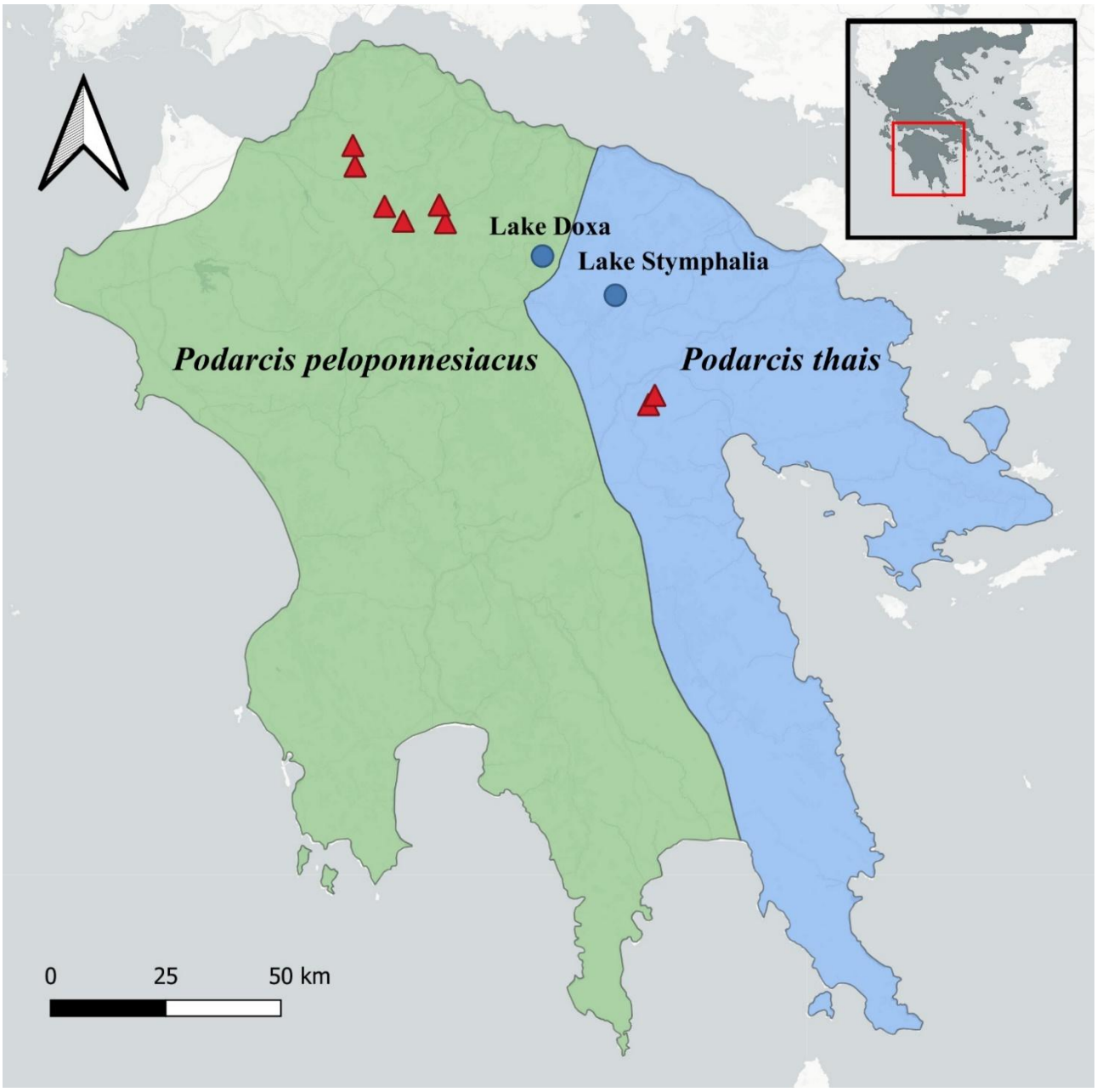


Fig. 2 Geographical distribution and sampling sites of the endemic species *Podarcis peloponnesiacus* (green) and *Podarcis thais* (orange) in the Peloponnese. Sampling spots are indicated by black dots in the northern parts of the species' ranges. Lakes Stymphalia (top) and Doxa (bottom) are marked in blue; the contact zone between the two species is located between them. The distribution map was generated using Q GIS (Version 3.34.12; QGIS Development Team, 2025; RRID:SCR_018507)

Materials And Methods

Sampling

Individuals were captured by noosing between April and May 2025 from neighbouring populations of each species to minimise interpopulation variation (Fig. 2). To reduce the likelihood of prior interactions between opponents, individuals were collected at a minimum distance of 40 m from one another (Bastiaans et al. 2013).

Following capture, snout–vent length (SVL) was measured using a digital caliper, and body mass was recorded with a precision balance. These measurements were used to calculate the Body Condition Index (BCI) (López et al. 2004; Abalos et al. 2016). The BCI was estimated as the residuals of a linear regression of log-transformed body mass against log-transformed SVL (Bonnet and Naulleau 1994) performed in R software (Version 4.4.2; R Core Team 2025; RRID:SCR_001905)

The final sample consisted of 40 adult males, 20 from each species. Within each species, 10 individuals with white and 10 with red throat colouration were selected, while individuals exhibiting intermediate or ambiguous colouration were excluded. All selected individuals met a predefined size criterion, with SVL ranging between 62.5 mm and 82.5 mm, targeting a mean SVL of approximately 73 mm. This constraint was applied to exclude juveniles and to minimise size asymmetries between potential opponents, thereby reducing confounding effects on contest outcomes (Sacchi et al. 2009).

Animal Housing and Maintenance

In the laboratory, lizards were housed individually in plastic terraria (21.5 × 31.5 × 17.5 cm) that were visually isolated using partitions to prevent visual contact between animals in adjoining enclosures. Each terrarium contained a soil substrate, as well as a stone or brick serving as a refuge. Temperature was set to mimic natural environmental conditions (15 °C at night and 26 °C during the day), and appropriate humidity levels were maintained by regularly spraying the terraria while providing water *ad libitum*.

Lizards were fed daily with three *Tenebrio molitor* larvae dusted with a vitamin supplement (Lucky Reptile "Pro Mineral + D3") (Adkins et al. 2003). Individuals were allowed to acclimate to laboratory conditions for one month prior to the experiments (Amo et al. 2004). Overall, animals remained in captivity for a limited period and were subsequently released at their exact capture locations immediately after the completion of the experiments.

Experimental Design

We staged 160 contests of 20 minutes each, including both intra- and interspecific pairings between *P. peloponnesiacus* and *P. thais*, categorised based on 16 colour combinations (same versus different throat colour) as shown in Table 2.

Table 2 Experimental design: colour combinations for staged contests. R = red morph; W = white morph. Painted individuals are shown in bold (e.g. **R**–**R**). Each combination was replicated 10 times (total N = 160 contests)

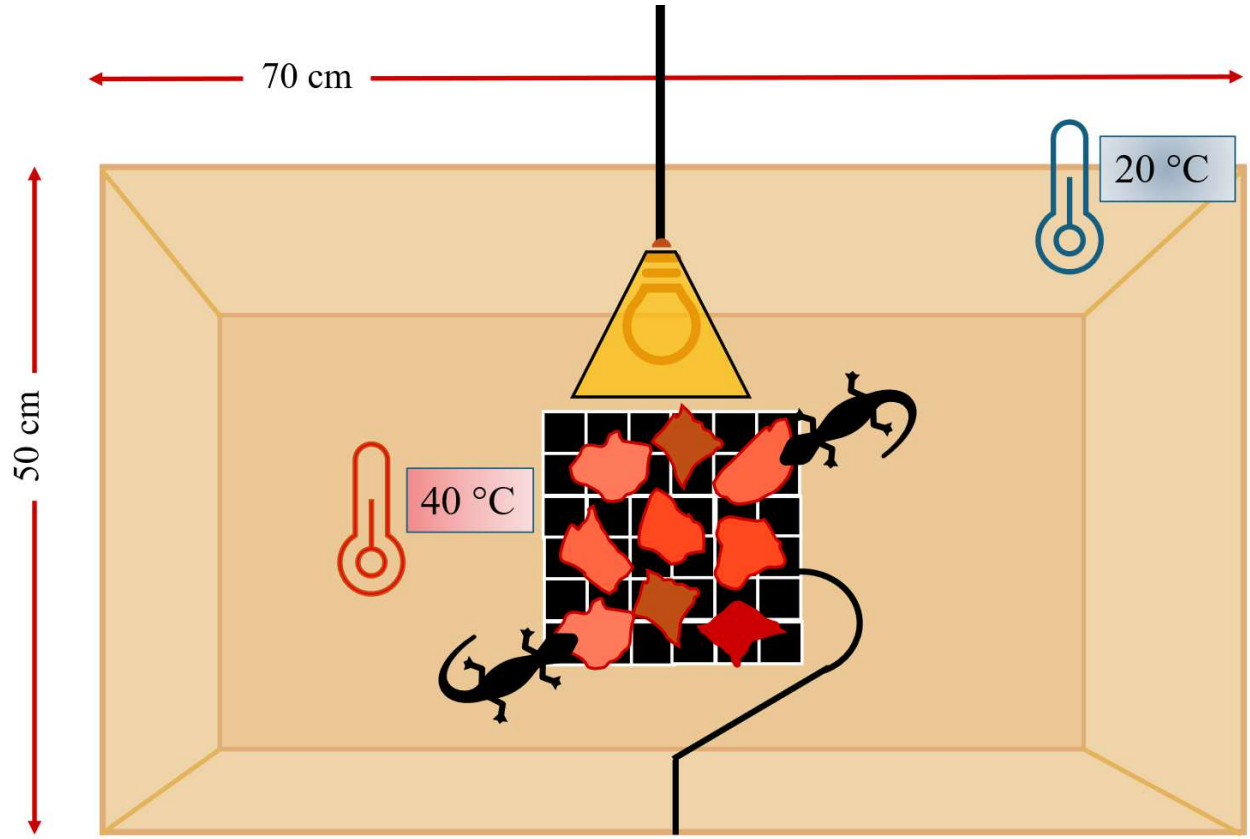
Species / Condition	**Comb 1**	**Comb 2**	**Comb 3**
Intraspecific – Natural			
P. peloponnesiacus	R–R	W–R	W–W
P. thais	R–R	W–R	W–W
Interspecific – Natural			
P. peloponnesiacus vs. *P. thais*	R–R W–W	R–W	W–R

Species / Condition	Comb 1	Comb 2	Comb 3
Intraspecific – Colour-modified			
P. peloponnesiacus (painted)	**R–R**	**W–R**	**W–W**
P. thais (painted)	**R–R**	**W–R**	**W–W**

To isolate the effect of visual signals on contest outcomes, throat colouration was experimentally manipulated in a subset of experiments using non-toxic, water-soluble paints: Testor's pearl red (Martin et al. 1999; Abalos et al. 2016) and Vallejo off white to simulate the alternative morph. Painting was performed one day prior to trials without anesthesia. Individuals were temporarily housed in clean, substrate-free terraria for 24 h to preserve colouration. After each trial, paint was gently removed using water. No skin damage or behavioural stress was observed.

Each male participated in eight contests against five different opponents, and contest order was randomised, with interspecific trials conducted at the end to minimise carry-over effects of prior experience and hierarchy formation (Stuart-Fox and Johnston 2005). To control for potential effects of body size, opponents were closely size-matched, with a mean SVL difference of 2 ± 1.5 mm (Baird 2013).

Contests were conducted in glass arenas (50 × 70 × 50 cm) containing a centrally heated basking site (~40 °C) created with a heat mat covered by a pile of schist rocks and a lamp fixed above it, while the surrounding area was maintained at ~18 °C to promote competition over the thermal resource (Fig. 3). Prior to each trial, contestants were placed in opposite corners of the arena without visual contact (using an opaque barrier) and acclimated separately for 5 minutes, after which barriers were removed simultaneously and interactions were recorded for 20 minutes. Experiments were conducted from 10:00 a.m. to 4:00 p.m. from late May through mid-July.



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164 **Fig. 3** Sketch of the experimental arena showing the heated central zone (basking site) and the peripheral
165 cool zone where the contests took place

166 Following each trial, individuals were removed from the arena, prioritising clear winners to prevent
167 resident effects (Sacchi et al. 2009), and the enclosure was thoroughly cleaned with 75% ethanol to
168 eliminate scent cues (Brock et al. 2022). All contests were video-recorded using a Turbo-X Action Camera
169 ACT-150 and analysed using BORIS software (Version 9.4.1; Friard and Gamba 2016,
170 RRID:SCR_025700). We constructed an ethogram to score the number of behaviours performed during
171 each contest, enabling qualitative and quantitative assessment. To ensure consistency, all behavioural
172 scoring was conducted by a single observer, and each video was analysed twice, focusing on each contestant
173 separately. It was not possible to score behavioural data blind, because the study relied on video recordings
174 in which the species identity and natural or experimentally manipulated throat colour of focal animals, were
175 clearly visible to the observer.

Table 3 Ethogram of contest behaviours exhibited by male *P. peloponnesiacus* and *P. thais*. Behaviours are grouped into seven categories: Aggression (physical agonistic actions), Boldness (non-aggressive exploratory actions towards an opponent), Chemosensory Behaviours (chemical signalling and detection), Visual Signals (visual displays for communication), Submission (avoidance and de-escalation behaviours), and Thermoregulatory Behaviour (time spent on the heated basking site). Descriptions and category assignments follow Abalos et al. (2016), Baird et al. (2004), Brock et al. (2022), Coelho et al. (2018), Font et al. (2012), Marshall and Stevens (2014), and Sacchi et al. (2009)

Behaviour	Description	Category
Grab	Bites opponent for >2 seconds	**Aggression**
Bite	Bites opponent for <2 seconds	(Behaviours involving direct physical interaction or threat, typically aimed at intimidating, displacing or dominating an opponent; Abalos et al. 2016; Brock et al. 2022)
Lunge	Hits opponent using the head with a fast, forward motion	
Chase	Rapidly follows opponent	
Push	Forceful displacement of opponent using the head	
Approach	Walks towards opponent	**Boldness**

Behaviour	Description	Category
Active Touch	Deliberate physical contact without aggressive intent (Sacchi et al. 2009)	(Behaviours reflecting willingness to engage with and investigate the opponent in a non-aggressive manner)
Passive Touch	Involuntary or accidental physical contact without aggressive intent	
Femoral Pore Rubbing	Rubs femoral pores on substrate	**Chemosensory Behaviours**
Mouth Wiping	Rubs the side of the mouth on substrate	(Behaviours associated with the production or detection of chemical cues used in communication)
Tail Display	Wriggles tail towards opponent (Brock et al. 2022)	**Visual Signals**
Foot Waves	Lifts foot in a circular, waving motion (Baird et al. 2004; Font et al. 2012)	(Behaviours used for visual signalling, potentially functioning in threat display, communication or distraction; Marshall and Stevens 2014)
Retreat	Rapid withdrawal to avoid contact	**Submission**
Hiding	Remains in a concealed, non-visible location (Coelho et al. 2018)	(Behaviours exhibited primarily by subordinate individuals to avoid conflict or reduce aggression)

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Colour Quantification

Colour was quantified using a standardised photobox setup (Runemark et al. 2010) with controlled LED lighting and a neutral background. Each individual was photographed at a fixed distance alongside a grey scale card for calibration.

Images were analysed in ImageJ (Version 1.54q; Schneider et al. 2012; RRID:SCR_003070). Background calibration was performed by normalising grey values to standard brightness (GBR ~129). Mean GBR values were then extracted from the throat and upper chest region, providing a quantitative measure of colouration. The same procedure was applied to painted individuals to quantify treatment colours.

Statistical Analyses

Biometric and Colour Data

Differences in snout–vent length (SVL) and body condition index (BCI) among colour morphs were tested using one-way ANOVA, both within and between species, including the required preliminary tests (Shapiro–Wilk for normality, Levene's for homogeneity of variances). When significant ($p < 0.05$), Tukey post hoc tests were applied. Colour measurements (GBR values) were analysed using Principal Component Analysis (PCA).

Contest Outcomes and Aggression

The outcome of the battles was determined initially assessing the basking period of each contestant in each battle according to Brock et al. (2022). However, there were no statistically significant differences between the contestants basking times so eventually contest winners were determined by scoring aggression behaviours based on behavioural intensity: Grab = 5 points, Bite = 4 points, Lunge = 3 points, Chase = 2

points, Push = 1 point. Total aggression scores were calculated per individual and per contest (McLean and Stuart-Fox 2015; Brock et al. 2022; Mandavilli et al. 2025) and the winner was the individual with the higher total score per battle. Differences in aggression scores were analysed using Kruskal–Wallis tests (Brock et al. 2022), while post hoc pairwise comparisons were conducted using the Mann-Whitney U test with Bonferroni correction to control for multiple comparisons. Analyses were conducted in PAST (Version 5.3; Hammer et al. 2001; RRID:SCR_019129).

Aggression and Colour Relationships

The relationship between aggression and explanatory variables was examined using Generalised Linear Mixed Models (GLMMs) in R with a negative binomial distribution (nbinom2). Models were fitted using *glmmTMB* (McGillycuddy et al. 2025) and validated with *DHARMa* (Harting 2022), including individual identity and contest ID (experiment type) as random effects. The Full model applied was: *Aggression score ~ Colour * Species + Opponent's Colour * Species + Opponent's Visible Colour * Species * Opponent's Species + Standardised BCI Difference * Type + (1 | Individual ID) + (1 | Battle Combination)*.

Effect of First Contest Outcome (Winner/Loser Effects)

To test for winner and loser effects, only intraspecific contests with clear outcomes were included. A binomial GLMM was used to model subsequent contest outcomes (win = 1, loss = 0), with first contest outcome, species, and their interaction as fixed effects, and individual identity as a random effect. The Full model applied was: *Outcome of following battle ~ Outcome of first battle * Species + (1 | Individual ID)*.

Effect of Colour Manipulation

To assess the effect of experimental colour manipulation, intraspecific contests were categorised as natural or manipulated. Aggression scores were analysed using a zero-inflated negative binomial GLMM to account for excess zeros, including individual identity and battle combination as random effects. The Full model applied was: *Aggression Score ~ Colour Modification * Species + (1 | Individual ID) + (1 | Battle Combination)*.

Results from all models were interpreted using estimated marginal means (emmeans) and visualised with ggplot2 (Wickham 2016). Statistical significance was set at $\alpha = 0.05$.

Behavioural Analysis

Recorded behaviours were coded and quantified from video data. Frequencies were calculated per behaviour and per behavioural category (e.g. aggression, boldness) for each species and colour morph, and analysed using χ^2 goodness-of-fit tests (Brock et al. 2022).

Results

Biometrics and Colour Quantification

The mean biometric measurements of the colour groups are presented in Table 4. Statistical analysis using one-way ANOVA revealed no significant differences in mean snout–vent length (SVL) (F_3=0.990, p = 0.407) or body condition index (BCI) (F_3= 0.656, p = 0.584) among individuals of different colour morphs (red/white) or species (*P. peloponnesiacus* and *P. thais*).

Table 4 Mean (± SD) snout–vent length (SVL) and body condition index (BCI) values for each colour morph group (red/white) of each species (*P. peloponnesiacus* and *P. thais*) participating in the experiment

Species	Colour Morph	n	SVL (mm) (Mean ± SD)	BCI (Mean ± SD)
P. peloponnesiacus	All	20	76.76 ± 2.36	1.00 ± 0.08
	Red	10	76.41 ± 2.35	1.02 ± 0.09
	White	10	77.12 ± 2.44	0.98 ± 0.06
P. thais	All	20	75.95 ± 2.76	1.00 ± 0.06
	Red	10	75.23 ± 2.68	1.01 ± 0.05
	White	10	76.67 ± 2.78	1.00 ± 0.08

The results of the digital analysis conducted in ImageJ showed that red morphs (both natural and artificial) exhibited low values in the Green and Blue channels relative to the Red channel, whereas white morphs displayed high and relatively similar values across all three channels. The first two components (PC1 and PC2) of the Principal Component Analysis (PCA) explained 98.74% of the total variance (91% by PC1 and 8% by PC2) in the colour data (SI, S. Fig. 1). This confirms that the empirically defined categories "red" and "white" correspond to genuinely distinct colour profiles and justifies their treatment as categorical variables in subsequent modelling analyses.

Contest Outcomes and Aggression Levels

Out of the 160 battles, 73 (46%) ended in a draw, and 64 of those involved at least one *P. thais* opponent. In *P. peloponnesiacus*, different-coloured encounters showed a tendency for white morph dominance; however, colour modification reversed this pattern, with white painted individuals of red colour morphs winning more often (Fig. 4). The non-parametric Kruskal-Wallis test along with the pairwise Mann-Witney test revealed a statistically significant difference ($p < 0.001$) between the groups (SI, S. Table 1).

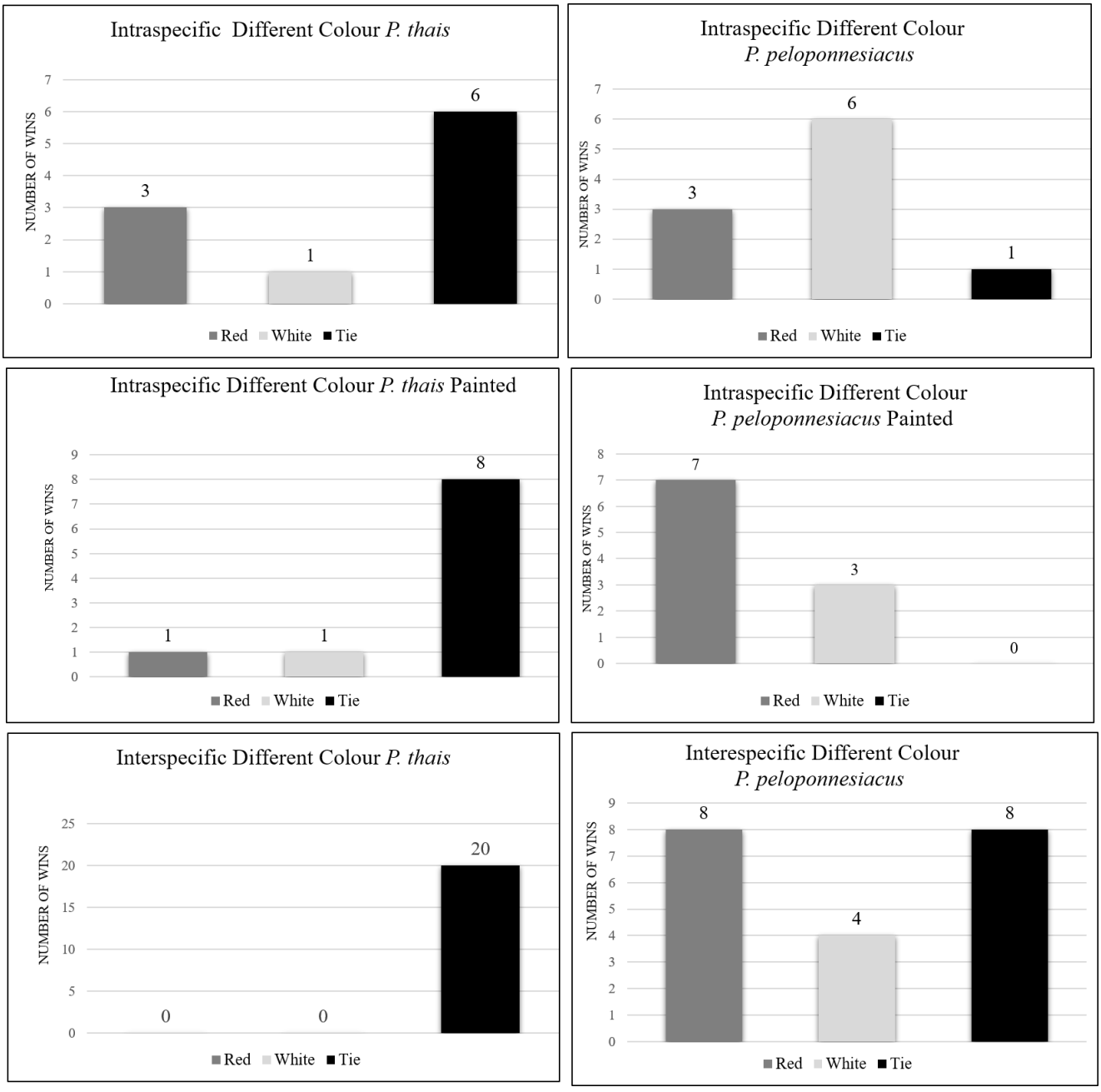


Fig. 4 Battle outcomes based on aggressiveness: Bar charts showing the outcomes of all types of differently coloured battles. From left to right: intraspecific battles for both *Podarcis peloponnesiacus* and *Podarcis thais*, battles with colour modification, and interspecific battles. Dark grey: Win of red morph; Light grey: Win of white morph; Black: Tie / No aggression

Scoring aggressive behaviours indicated a clear interspecific difference in aggression between the two species, but no intraspecific difference between the two colour morphs. χ^2 tests indicated a significant effect of opponent colouration in *P. peloponnesiacus*, with males exhibiting increased aggression towards natural red colouration. (SI, S. Table 2). Experimentally manipulated colouration produced the same effect, though not always statistically confirmed.

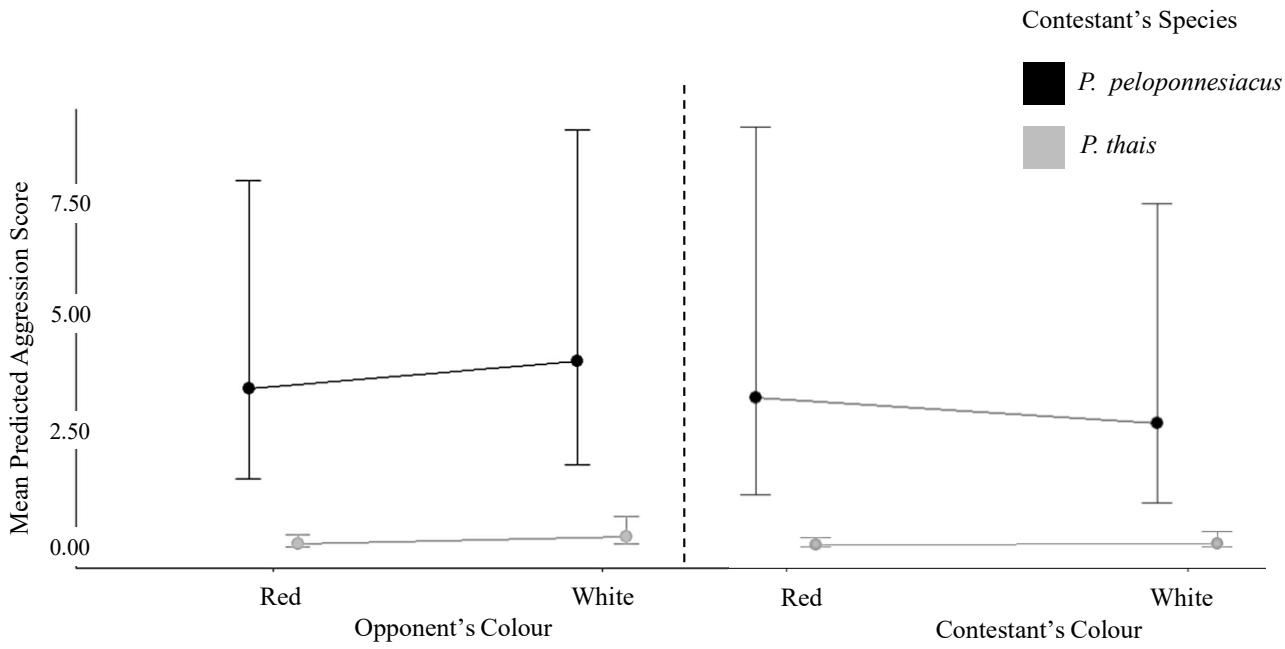
In contrast, *P. thais* exhibited overall significantly lower aggression, and the few statistically significant observations were associated with colour-manipulated trials, suggesting a potential increase in aggression when the opponent was, or had been altered to, white (SI, S. Tables 2-5).

In interspecific interactions, *P. peloponnesiacus* showed a significant reduction in aggression, but its aggression levels remained significantly higher than those of *P. thais*, where no statistically significant change in aggression was detected. Regarding submissive behaviours, *P. peloponnesiacus* markedly reduced such behaviours during interspecific interactions, whereas *P. thais* exhibited a clear increase. χ^2 tests also suggested that interspecific interactions lead to a significant increase in boldness and visual signalling behaviours compared to intraspecific ones.

GLMM results (Table 5) showed no correlation between a contestant's or opponent's natural colouration and aggression levels (Fig. 5), but confirmed that species identity is the primary determinant of aggression ($\beta = -5.55$, $p < 0.0001$). Aggression levels were significantly lower when individuals interacted with *P. thais* opponents (emmeans values: 3.00 ± 1.17 for *P. peloponnesiacus* vs 0.07 ± 0.04 for *P. thais*) ($\beta = -1.34$, $p = 0.029$).

Table 5 GLMM results: Aggression ~ Colour and Biometric Characteristics. Bold text corresponds to statistically significant values ($p < 0.05$)

Variable	Estimate (β)	SE	z	p
Intercept	1.829	0.589	3.106	**0.0019**
Colour (White)	−0.152	0.700	−0.217	0.828
Opponent's colour (White)	−0.157	0.386	−0.407	0.684
Opponent's colour seen by contestant (White)	−0.503	0.327	−1.542	0.123
Opponent's species (*P. thais*)	−1.341	0.616	−2.177	**0.029**
Species (*P. thais*)	−5.549	1.250	−4.440	**<0.001**
BCI Difference (standardised)	0.193	0.202	0.956	0.339
Colour (White) × Species (*P. thais*)	0.732	1.075	0.681	0.496
Species (*P. thais*) × Opponent's colour (White)	1.052	0.668	1.574	0.116
Opponent's colour seen (White) × Opponent's species (*P. thais*)	1.656	0.833	1.987	**0.047**
Species (*P. thais*) × Opponent's colour seen (White)	−1.020	1.618	−0.630	0.528
Species (*P. thais*) × Opponent's species (*P. thais*)	3.293	1.219	2.701	**0.007**
Species (*P. thais*) × BCI Difference	−0.555	0.397	−1.400	0.162
Species (*P. thais*) × Opponent's colour seen (White) × Opponent's species (*P. thais*)	−0.939	1.816	−0.517	0.605



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297 **Fig. 5** Effect of opponent's colour morph (left) and contestant's colour morph (right). Estimated aggression
298 levels with 95% confidence intervals (error bars) from the model's estimated marginal means. Black:
299 Contestant *P. peloponnesiacus*; Grey: Contestant *P. thais*

300

301 However, the model revealed two significant interactions:

302 (1) Between perceived opponent colour and opponent species ($p = 0.047$). Post-hoc comparisons based on
303 estimated marginal means, despite lacking statistical significance, revealed: (a) A higher aggression trend
304 of *P. peloponnesiacus* towards red conspecific opponents (from 3.23 to 5.34). (b) A reverse pattern towards
305 the opposite species, with slightly higher aggression against white *P. thais* opponents (from 1.34 to 4.41).
306 (c) *P. thais* showed slightly higher aggression toward red opponents overall, albeit at very low levels (Fig.
307 6).

308 (2) Between contestant species and opponent species ($p = 0.007$). Specifically: (a) *P. peloponnesiacus*
309 escalates aggression in conspecific interactions (mean ≈ 4.14), significantly higher than in any interaction

involving *P. thais* ($p < 0.0001$). (b) *P. thais* exhibits the lowest aggression when facing *P. peloponnesiacus* (mean ≈ 0.02, $p = 0.017$) (Fig. 7).

Body condition (BCI) did not significantly affect aggression, nor did it interact with species. A weak, non-significant trend suggested that *P. peloponnesiacus* individuals with higher relative BCI may be slightly more aggressive. The inclusion of random factors (individual and battle identity) explained substantial variance, and diagnostic checks confirmed a good model fit (SI, S. Fig. 2).

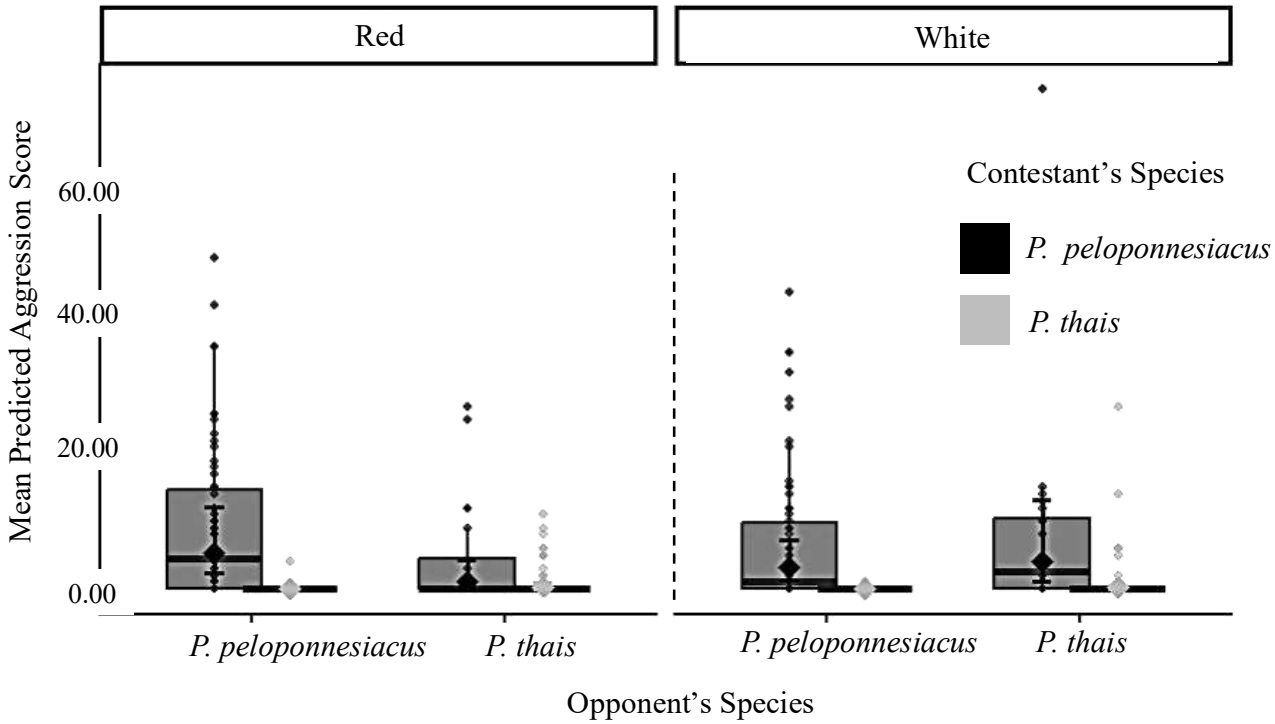


Fig. 6 Effect of the perceived colour of the opponent. Estimated aggression levels with 95% confidence intervals, analysing the interaction between species, opponent type, and the colour that the individual perceives in the opponent (natural or modified). Black: Contestant *P. peloponnesiacus*; Grey: Contestant *P. thais*

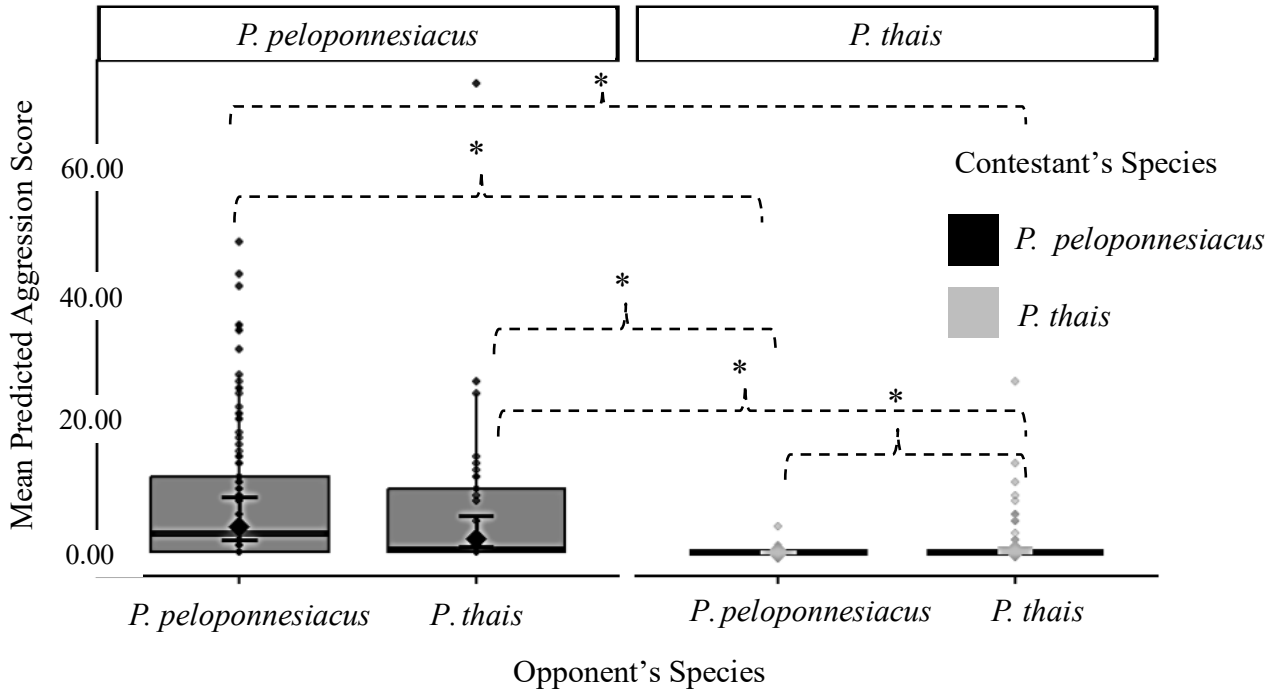


Fig. 7 Effect of opponent species on contestant aggression levels. Estimated aggression levels with 95% confidence intervals, analysing the interaction between contestant species and opponent species. **Black:** Contestant *P. peloponnesiacus*; **Grey:** Contestant *P. thais*

Effect of First Contest Outcome (Winner–Loser Effects)

The GLMM provided no evidence of a significant winner–loser effect under the conditions of this study (Diagnostic checks for model's fitness; SI, S. Fig. 3). The outcome of the first contest did not significantly affect the probability of winning in subsequent encounters for either species (Table 6). However, despite the lack of statistical significance, estimated marginal means suggested a consistent trend: individuals that won their first contest showed higher subsequent winning probabilities compared to those that lost, in both species (Fig. 8).

Table 6 GLMM results examining the winner–loser effect: impact of first contest outcome on the probability of winning a subsequent battle. Bold text indicates statistically significant values ($p < 0.05$)

Variable	Estimate (β)	SE	z	p
Intercept	−1.890	1.520	−1.244	0.214
First battle outcome (Win)	2.884	2.095	1.377	0.168
Species (*P. thais*)	−0.083	3.059	−0.027	0.978
First battle outcome (Win) × Species (*P. thais*)	−0.821	3.726	−0.220	0.826

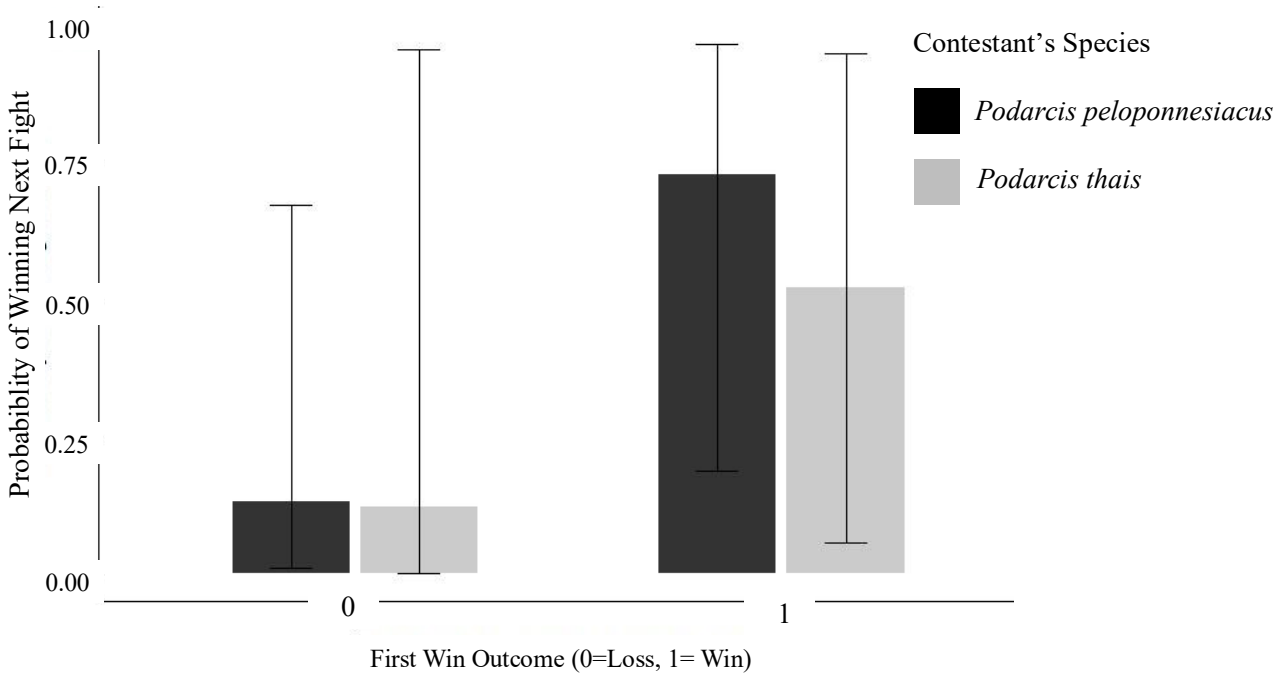


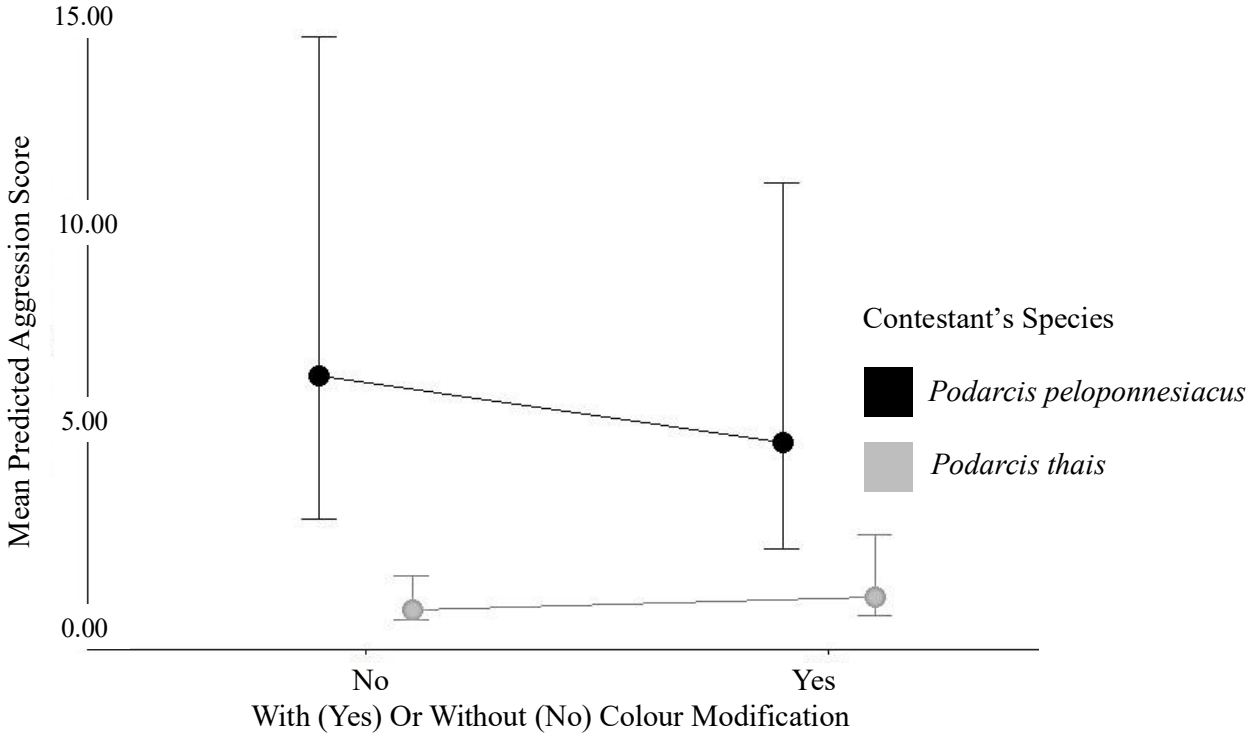
Fig. 8 Probability of winning the next battle: Bar chart showing how the estimated probability of winning a subsequent battle varies with the outcome of the first contest (win or loss), for each species. Error bars correspond to 95% confidence intervals of the model's estimated marginal means

Effect of Colour Modification

The results of the model examining the effect of artificial colour modification are presented in Table 7. The model indicated a non-significant effect on overall aggression levels ($p = 0.35$), and this effect did not differ between species, as the interaction term was also non-significant ($p = 0.12$) (Diagnostic checks for model's fitness; SM Fig 4). Although not statistically confirmed, model estimates suggested: (a) A decrease in aggression in *P. peloponnesiacus* (from 6.11 to 4.45) following painting. (b) A slight increase in aggression in *P. thais* (from 0.35 to 0.66) following painting (Fig. 9).

Table 7 Effect of colour modification on aggression levels: assessing the effect of artificially altering colouration on the experimental procedure. Bold text corresponds to statistically significant values ($p < 0.05$)

Variable	Estimate (β)	SE	z	p
Intercept	1.812	0.438	4.134	**<0.001**
Colour modification (YES)	−0.312	0.335	−0.931	0.352
Contestant's species (*P. thais*)	−2.839	0.621	−4.575	**<0.001**
Colour modification (YES) × Contestant's species (*P. thais*)	0.934	0.599	1.558	0.119



356

357 **Fig. 9** Estimated aggression with and without colour modification: Estimated means and error bars
358 corresponding to 95% confidence intervals of the model's estimated marginal means, comparing
359 experiments with and without artificial colour modification, separately for each species

360

361 **Behavioural Analysis**

362 The results from the χ^2 tests for the rest of the behavioural categories are also presented extensively in the
363 Supplementary Information, S. Tables 3-5. Submissive behaviours are consistent with the overall
364 asymmetry in contest outcomes between the two species with *P. thais* rising significantly the extremely low
365 levels of submission during the intraspecific battles at the interspecific ones against *P. peloponnesiacus*.
366 For the latter following what was previously indicated about aggression the opposite significant pattern was
367 detected against red opponents with submission rising against the white (natural or painted) colouration.

The χ^2 analyses for boldness behaviours revealed a weak and inconsistent pattern increasing such behaviours against red colouration in *P. peloponnesiacus* while both species increase significantly such behaviours during the interspecific battles. A similar pattern was observed for visual communication signals however *P. thais* exhibited consistently more visual signals in any type of contest. No tendencies regarding the chemosensory behaviours were detected considering the limited number of occurrences during the battles.

Discussion

In this study, we examined the role of ventral colouration in aggression levels and dominance of male *Podarcis peloponnesiacus* and *Podarcis thais*. Overall, our results – contrary to many previous studies (Sinervo and Lively, 1996; Hamilton et al. 2013; Yewers et al. 2016) – suggest that colour morphs do not function as signals of male quality predicting contest outcomes. Although a weak tendency for white individuals to prevail was observed in intraspecific battles of *P. peloponnesiacus*, consistent with earlier findings (Abalos et al. 2016; Pérez i de Lanuza et al. 2017; Brock et al. 2022; Mandavilli et al. 2025), this pattern was not supported by our statistical models. Instead, our findings indicate that species identity and the visual perception of opponent colouration (natural or modified) play a more important role.

Body size differences – minimised by design – did not predict contest outcomes, consistent with previous work showing that size effects emerge only beyond larger asymmetries (Thompson and Moore 1991; Stuart-Fox and Johnston 2005). In this study, we used the Body Condition Index (BCI) instead of SVL alone as a more reliable indicator of individual condition. The model suggested a non-significant trend that individuals in better body condition may exhibit increased aggression, in accordance with Sacchi et al. (2009); however, this requires further investigation. Similarly, prior contest experience did not have a statistically significant effect, although observed patterns were consistent with a winner–loser effect, also

noted by Stuart-Fox and Johnston (2005), indicating a potential influence of experience that should be explored with a larger sample.

Colour manipulation experiments did not significantly alter aggression levels or contest outcomes, as previously suggested by Stuart-Fox and Johnston (2005), although many other studies have demonstrated that colour modification alters battle outcomes (Thompson and Moore 1991; Olsson 1994; McGraw and Hill 2000). However, the imperfect replication of natural colouration by the paints used may have influenced this result.

The Response to Red Colouration

Despite the lack of a direct effect of colour morph on dominance, a key finding of this study was the tendency for male *P. peloponnesiacus* to increase aggression levels when facing opponents with red colouration, regardless of their natural morph. A similar, though less consistent, pattern was also observed in *P. thais*. This suggests that behavioural responses are driven by the perceived colour of the opponent rather than by intrinsic morph-related traits.

The interpretation of this pattern may be based on the deeply rooted evolutionary significance of red colouration as a signal of danger, threat, warning or tension in many vertebrates, which may have evolved through the repeated association of red with injury or with blood (red due to haemoglobin). In humans and other animals, red causes increased arousal, anxiety and readiness for action ("fight or flight"). In behavioural experiments with humans, exposure to red (versus other colours) elicits increased involuntary competitive anxiety or fear (Feltman and Elliot 2011; Ten Velden et al. 2012). In animals, classic studies such as Tinbergen's (1955) indicate that red acts as an aposematic signal influencing behaviour by causing avoidance or vigilance, as summarised by Guilford (1990), Schluter and Roper (1992) and, more recently, Chen et al. (2024).

Interspecific Interactions

In interspecific trials, *P. peloponnesiacus* consistently dominated *P. thais*, which exhibited low aggression and frequently displayed avoidance behaviours, revealing a clear prevalence of *P. peloponnesiacus*. These differences likely reflect species-specific behavioural strategies and may be linked to ecological or physiological factors (e.g. differences in testosterone peaks or reproductive timing). The reduced aggression of *P. peloponnesiacus* toward the less aggressive *P. thais* is an adaptive behaviour that avoids unnecessary costs, similar to that observed by Yewers et al. (2016) among different colour morphs of *Ctenophorus decresii*.

Importantly, the increase in aggression levels against red morphs was absent in interspecific encounters, suggesting that this mechanism functions primarily within species-specific signalling systems. However, both species significantly increased their use of visual signals in interspecific encounters, likely due to the "unfamiliar" nature of the opponent.

Limitations and Future Directions

Several factors should be considered when interpreting these results. First, the imperfect visual match between natural colouration and the applied paints may have reduced the strength of the colour manipulation effect. Second, the sample size per experimental category (n = 10) was relatively small, which may explain the marginal statistical significance of some key patterns (e.g. the response to red opponents). Third, lizards perceive ultraviolet (UV) light, and colour differences invisible to humans may be important for communication in these species (Fleishman et al. 1993). Future studies should therefore: (a) Increase sample sizes to achieve higher statistical power. (b) Use spectrally matched paints that replicate natural colouration more accurately, including UV reflectance. (c) Investigate the sensory and physiological basis of responses to colour signals (e.g. hormonal correlates, retinal sensitivity). (d) Examine population-level

variation in colour morph frequencies and behavioural strategies, particularly within the natural contact zone.

Conclusions

Overall, our findings indicate that colour polymorphism in *P. peloponnesiacus* and *P. thais* does not determine dominance in a simple or consistent manner across colour morphs. Instead, aggression appears to be shaped primarily by species identity and context-dependent responses to visual stimuli. The observed effect of red colouration – increasing aggression in the perceiver rather than signalling the quality of the bearer – highlights the importance of signal perception and suggests a previously unrecognised mechanism influencing aggressive behaviour. This study contributes to our understanding of the complex role of colour signals in lizard communication and opens new avenues for research on the evolutionary maintenance of colour polymorphism. Moreover, the clear behavioural divergence in male contests and dominance between two closely related species with overlapping distributions, observed in both intra- and interspecific interactions, highlights the need for further research into the ecology of these highly understudied species.

Supplementary Information

The online version contains supplementary material

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Author Contributions

All authors contributed to the study conception and design, material preparation, data collection and analysis. The first draft of the manuscript was written by Maria Eleni Gotsopoulou and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Declarations

Ethics Approval

Animal welfare and stress minimisation were prioritised throughout. Individuals were monitored daily, and contests were observed continuously to prevent injury (Martin et al. 1999). Experiments followed strict safety protocols, with immediate termination if bites exceeded 5 seconds (Brock et al. 2022) – a circumstance that never occurred. No deaths or serious injuries were recorded, and all animals were released

unharmed at their capture sites. The study complied with international guidelines and was conducted under official permit (ΥΠΕΝ/ΔΔΔ/39584/1349).

Competing Interests:

The authors have no competing interests to declare that are relevant to the content of this article.

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