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Negative density-regulated contest performance promotes conflict resolution in a tree lizard

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Abstract

1. Territoriality is a behavioural adaptation resulting from intraspecific competition and reflects the strategy of how individuals share limited resources in the environment. As a consequence, territorial contests and levels of aggressiveness are strongly influenced by population density.

2. The positive relationship between population density and the intensity of intraspecific competition may appear straightforward. However, empirical evidence regarding this association remains inconclusive. This ambiguity may be attributed to studies that have predominantly focussed on specific fight-associated traits while overlooking potential interactions between multiple phenotypic characteristics and population dynamics.

3. To examine the effects of population density and other ecological factors on the variation in fight behaviour as well as fight-associated morphology and performance. We conducted population surveys and behavioural experiments using the Swinhoe's tree lizard across eight populations.

4. Our findings revealed that males from higher density populations tended to engage in less intense fights and exhibited a weaker resident advantage in fights, which coincided with our findings on lower rates of injuries, weaker bite forces and smaller body sizes (and vice versa). Male-specific variation in fight behaviour, morphology and performance along a density gradient suggests different evolutionary equilibria in territoriality influenced by local costs and benefits.

5. Our study supports the significant role of negative density dependence as a fundamental regulator of eco-evolutionary dynamics. The observed phenotypic variation emphasizes the importance of ecological and social factors in shaping ontogenetic growth and life-history strategies. Our findings provide a basis for future investigations into pace-of-life syndromes and shed light on how phenotypic adaptation may shape population structure.

KEYWORDS
density dependence, intraspecific competition, phenotypic covariation, phenotypic variation, territoriality

1 | INTRODUCTION

Territoriality represents a behavioural adaptation that arises from the allocation of limited resources among individuals within a population (Deitloff et al., 2014; Kaufmann, 1983; Raffestin, 2012; Toobaie & Grant, 2013). This behaviour is closely linked to population density (Knell, 2009; Lamb et al., 2017), with increased density often resulting in heightened competition among organisms when resources in the environment remain relatively consistent (Knell, 2009; Kokko & Rankin, 2006; Tomkins & Brown, 2004). However, understanding the density-dependent effects on territorial behaviour presents challenges for empirical studies (Moczek, 2003) due to the intricate relationship involving fight-associated behaviour, morphology and performance. Animals employ flexible behavioural tactics (Benelli, 2013; Mitani & Rodman, 1979) and a variety of armaments (such as status badges, weapon-shaped structures, and specific behaviours) to resolve territorial conflicts (Arnott & Elwood, 2009). These traits are influenced by different selection forces and trade-offs (Camerlink et al., 2015; Kilgour et al., 2018), making the density-dependent effects less straightforward to comprehend.

We present five hypothetical associations concerning the relationship between population density and aggressive interactions (Figure 1A–E). These associations encompass positive (Donihue et al., 2016; Wegener et al., 2019), negative (Knell, 2009; Pomfret & Knell, 2008), unimodal (Jones, 1983), bimodal (Mayer et al., 2020) and no clear associations (Niemelä et al., 2012). These associations can be explained by the evolutionary equilibrium based on the costs and benefits of aggressiveness, which correspond to variations in population density. For instance, a positive association may arise when the benefits of being aggressive increase disproportionately with higher population density, outweighing the costs. This could
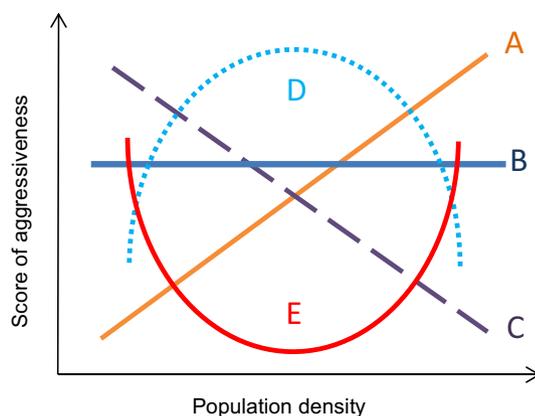
occur due to increased social interactions, allowing more dominant individuals to monopolize a greater quantity and higher quality of resources (e.g. mates and habitats) within denser populations (Abesamis & Russ, 2005; Holbrook & Schmitt, 2002). While many of these associations have empirical support, their diverse and occasionally contradictory directions underscore the complexity and absence of a general theory predicting how density-dependent selection drives the evolution of territoriality (O'Neil et al., 2020; Stamps & Buechner, 1985). Nonetheless, understanding such dynamics would significantly advance our comprehension of the role of behavioural mechanisms in population regulation.

In addition to population density, the distribution and quality of resources, such as food availability and habitat composition, are also key factors determining the effectiveness of territory defence (Grant & Guha, 1993; Jensen et al., 2005). The resource dispersion hypothesis suggests that if resources are scattered but abundant in the environment, territorial individuals may form groups that share spatially distributed resources but are defensible from one another in an economical manner (Macdonald & Johnson, 2015). This implies that animals may invest more in defending sites with valuable resources (Grant & Guha, 1993; Jensen et al., 2005; Monaghan & Metcalfe, 1985) and/or when resources are concentrated in a manner that facilitates easier defence (Grant & Guha, 1993; Jensen et al., 2005). As a result, variations in the distribution and availability of these resources can influence where and how individuals establish their territories (Diego-Rasilla & Perez-Mellado, 2003; Kokko & Rankin, 2006; Macdonald & Johnson, 2015).

In this study, we investigate the role of population density in driving the evolution of territorial behaviour in Swinhoe's tree lizard (*Diploderma swinhonis*). Specifically, we addressed (i) how density affects fight outcome and intensity, (ii) how density affects injury rates and (iii) how fight-associated morphology and performance vary with density. We conducted surveys to assess population density, habitat composition, food resources and dietary composition across eight studied lizard populations. We conducted behavioural experiments in each of the populations and measured multiple traits (e.g. snout-vent length [SVL], bite force and injury rates) associated with individual fight performance. Territorial fights in Swinhoe's tree lizards often involve a series of aggressiveness displays, ranging from noncontact displays to physical combat. The unique characteristics of this species make it an ideal study system for addressing the proposed research questions.

FIGURE 1 Five hypothetical relationships between density and aggressiveness. The level of aggressiveness would increase (A), remain constant (B), or decrease (C) with population density. The level of agressiveness may also reach the highest level in medium density population with population density (D), or reach the lowest level in medium density population (E).

2 | MATERIALS AND METHODS

2.1 | Study species and populations

We conducted the study from March to October of 2013–2014 and 2018–2020. The tree lizard, *Diploderma swinhonis* (Figure S1), is an endemic species inhabiting the forest and scrub environments on

the mainland and adjacent islands of Taiwan. We studied a total of eight lizard populations. Six populations are on the mainland island (from the north to the south): Taipei (25°01′04.1″N 121°33′08.6″E), Taoyuan (25°00′09.3″N 121°19′41.9″E), Yilan (24°36′51.8′N 121°51.8.″E), Taichung (24°08′44.3″N 120°33′25.5″E), Hualien (23°36′54.2″N 121°25′05.4″E) and Kenting (22°08′29.70″N, 120°42′11.77″E). The two additional populations were distributed on two adjacent islands sitting to the east of the mainland of Taiwan: Green Island (22°39′33″N, 121°29′15″E) and Orchid Island (22°3′18″N, 121°32′41″E) (Figure 2a).

Diploderma swinhonis is the largest *Diploderma* species in Taiwan, with a SVL range of 65–80mm (Huang, 2007; Lin & Lu, 1982). Males compete for territories, each of which has an area extending from the centre tree of the territory (Huang, 2007; Lin & Lu, 1982). Currently, it is still an unsolved question whether all adult males hold territories. Given that we aimed to investigate how territorial behaviour shifts with population density. In this study, we only used males who displayed territorial behaviour as focal individuals and focussed on how they defended their territories when encountering an intruder. Whether a male lizard has its territory and is a territory owner is very easy to determine in the field, as they often display territorial behaviour in their territory tree (e.g. push-ups). Territorial behaviours can be frequently observed during the reproductive period (March to October). The lizard is sexually dimorphic; males are characterized by a larger body size and a bright yellow band on each side of their bodies.

2.2 | Estimation of male density and sex ratio

We estimated the male densities of the eight populations by adopting the quadrat method (Gleason, 1920). We conducted a density survey for each of the populations, and each survey lasted for 5 days. During each survey, we set up a 1250 m^2 experimental quadrat and captured all of the lizards within this area with lassos. The surveys were conducted on sunny days, with air temperatures ranging from 30 to 33°C. We recorded the number of lizards within each quadrat, sex, SVL (nearest 0.01 mm, using digital callipers, CD-6ASX; Mitutoyo Corporation), body mass (nearest 0.1 g, using an electronic balance, Scout Pro SPU202; Ohaus Corporation) and the number of wounds on the lizards. After the measurement, we marked these individuals with non-toxic paint (Acrylic paint) to avoid repeatedly sampling and released them back to each of the capture sites.

2.3 | Stage fight experiments

To examine the level of aggressiveness associated with population density, we implemented staged fight experiments in the field using pairs of size-matched residents and intruders living in each of the eight populations. We conducted the fieldwork from 9 a.m. to 4 p.m. each day. During the fieldwork, we randomly captured males in sight (i.e. intruders) and assigned each of them to a fight with a
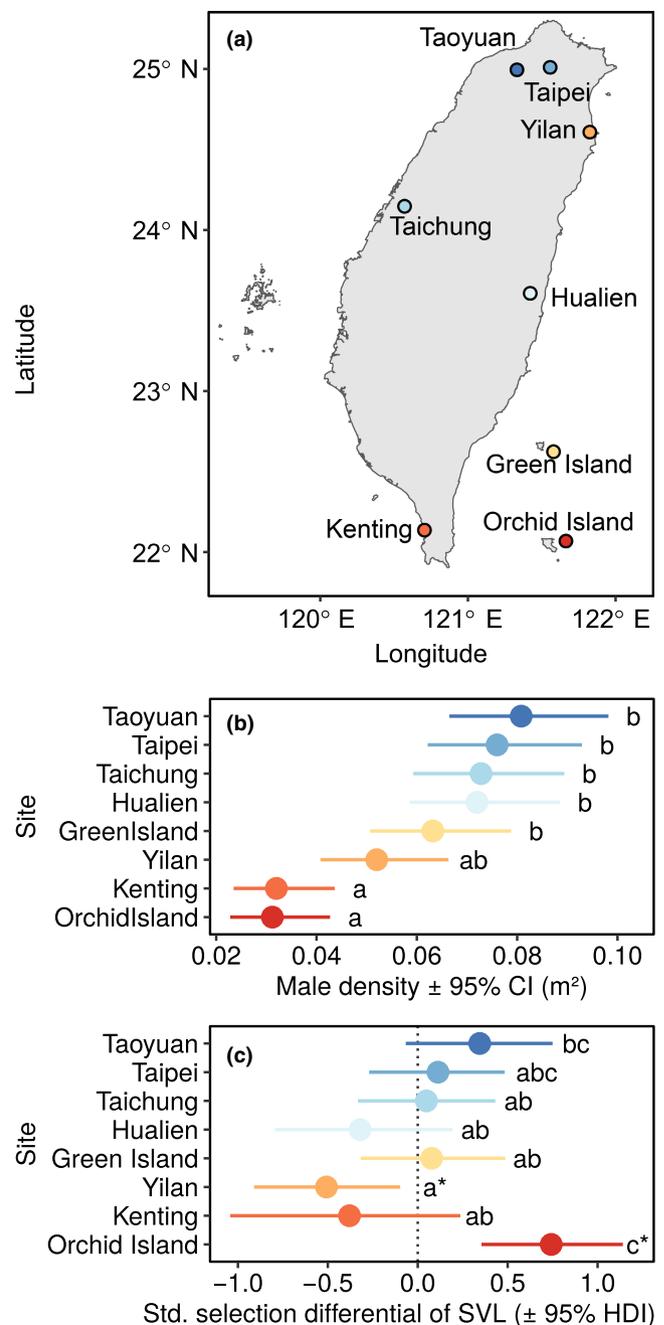

FIGURE 2 Map showing the sampling locations of the eight populations studied at low altitude tropical forests in Taiwan (a), the corresponding male densities (b), and selection differential of male snout-vent length (SVL) (c). Lowercase letters in panels (b) and (c) summarize the results of multiple comparisons; the same letter indicates insignificant difference in density or selection differentials, and vice versa. Asterisk signs in panel (c) indicate selection differentials that are significantly different from zero.

size-matched resident (estimated by the investigator). We measured the SVL and left a mark on the abdomen of each intruder for individual identification before starting a fight. To stimulate a fight without disturbing the resident male on his territory tree, one investigator carefully released an intruder to the tree (a meter below the

resident males) from the position opposite the resident male. Fights were filmed from 1.5 m away by the other investigator using a digital camera (SONY FDR-AXP55). The end of each fight was defined as when either the intruder or the resident retreated or when the fight duration was longer than 15 min (i.e. tight). We then captured the resident male and measured its SVL after the fight. Each male only participated in the test once.

The fighting performances of the residents and intruders were analysed, respectively, using the films recorded. We summarized, in total, eight aggressive behaviours that occurred in their fights (Table 1) and categorized these behaviours into three levels of fight intensity. Fights often began with non-contact displays (i.e. low intensity, such as push-ups, stand-offs and approach), followed by chase (i.e. medium intensity) and eventually physical fights (i.e. high intensity, such as pecking, crashing and biting) (Video S1). We recorded the number of behaviours displayed as well as the duration and outcome of each fight.

2.4 | Bite force measurement

We captured and measured the bite force of males in the eight populations to better interpret the variation in injury occurrence corresponding to different levels of male aggressiveness. We measured bite force indoors using a piezo high-sensitivity force transducer (Model 9203; Kistler Corporation) mounted on a holder and connected to a Kistler charge amplifier (Model 5995; Kistler) (see Kaufmann, 1983; Herrel et al. (1999) for a detailed description of the equipment set-up). We stimulated each male lizard to bite two parallel plates connected to the force transducer by gently tapping

its lower jaw. Each individual was tested five times consecutively with a 15-min interval between every two trials, and the maximum bite force was used in the further statistical analyses (Herrel et al., 2001). We released these lizards back to the capture sites after the measurement.

2.5 | Food resource survey

A food resource survey was conducted over a period of 5 days during the reproductive seasons in 2013–2014 and 2018–2020. To conduct the survey, we used sets of ground pitfall traps, each consisting of four 0.6-L plastic cups that were used as traps. Each cup contained 30 mL of 75% alcohol and was buried in the ground. The four traps were arranged in a Y-shaped pattern, with one trap in the centre and the others placed in three directions extending outward. These cups were placed under trees approximately 50 cm apart from each other, with plastic boards about 20 cm high extended between them. Insects or other animals crawling on the ground would fall into traps when moving on the plastic boards.

The food items collected were identified under a dissecting microscope at the taxonomic Order level. After identifying the invertebrates and vertebrates and excluding fragments below 3 mm, Coleoptera species, and African snails (*Achatina fulica*) greater than 3 cm from the food survey, individuals of the same Order in each group were oven-dried at 40°C for 3 days to a constant mass for dry mass measurement. We weighed the dried food on laboratory scales (±0.01 mg; Quintix® and Secura® Standard Laboratory Balances). To evaluate the average food resource diversity of a population, we calculated the mean value of the inverse Simpson index based on the

TABLE 1 A series of agonistic behaviour exhibited by *Diploderma swinhonis* during staged dyadic encounters.

Level	Behaviour	Description
Lowest	Retreat	An act of moving back or withdrawing towards the opposite direction of the opponent
Low—No involving physical contact between opponents		
	Push-up	Vertical raising and lowering of the anterior part of the body using rhythmic motion
	Approach	Movement directly towards the agonistic male can be with a fast or slow speed
	Standoff	Stand facing each other with or without an intimidating motion
Medium—Escalating the aggressive level but still not involving physical contact		
	Chase	Rapid running towards the retreated opponent
High—Physical contact and fighting; injuries may occur		
	Crash	A blow or a jolting collision
	Pecking	Pecks at another individual with its snout the mouth closed
Highest—Violent fighting; injuries usually occur		
	Bite	An act to bite the opponent, mainly in the regions of neck, body and tail base in order to expel it from the territory

abundance and dry mass of food items available to Swinhoe's lizard. We also evaluated the average food resource quantity of a population by summing the dry mass of food items.

2.6 | Stomach contents survey

Adult male lizards from eight populations ($n = 149$) were caught by lassoing for a stomach content survey. We followed a stomach-flushing procedure similar to that described by Legler and Sullivan (1979). Before stomach-flushing, we measured the SVL of collected adult male lizards with a digital calliper ($\pm 0.1\,mm$). The prey items found in the stomach were stored in 75% alcohol and identified to the level of taxonomic Order under a dissecting microscope. We analysed their stomach contents qualitatively and quantitatively, determining the number and identification of the most complete individuals and the dry mass of different Orders of prey in each stomach of a lizard (Maia et al., 2011).

We applied the same protocol as mentioned above to measure the dry mass of prey items. We evaluated the average male dietary breadth of a population by calculating the mean value of the inverse Simpson index based on the abundance and dry mass of prey items. We also evaluated the average prey quantity of a population by calculating the average of the total dry mass of prey items.

2.7 | Habitat composition

A habitat composition survey was conducted during the 2022 breeding season in the eight populations studied. We first defined the spatial boundary of each population by extending transects 10 m in each direction and dividing this area into quadrats of $10 \times 10\,m^2$. We randomly selected 30% of the quadrats to sample and measured each quadrat using a square polyvinyl chloride frame ($50 \times 50\,cm^2$) consisting of 25 grids of $10\,cm \times 10\,cm$ inside. We threw the square frame from the centre of each quadrat in four directions (N, W, S and E) and photographed each frame with a digital camera (Olympus Tough TG-5, IM005). Based on the photographs, we categorized each grid by its composition types, which included the coverage of stones, rocks, mulch, bare land, man-made structures(e.g. roads and carparcks), herbs, grass and other types if any covering covered more than 50%. We then summed the number of grids in each quadrat that fell into each composition type. In addition to composition types, we also recorded the number of wooden stems with a perimeter greater than 5 cm within a 1 m radius circle from the frame centre, as well as the diameter at breast height (DBH) of the nearest tree to the frame. The height at which DBH was measured is 1.3 m above the ground and we considered a plant individual as a tree if its DBH was greater than 5 cm. We used principle component analysis (PCA) to downscale the seven types of structures and obtained the first four principle components, which explained 90% of the variations among individuals within each population (see Table S1 for the loading vectors).

2.7.1 | Replication statement

	Analysis target	Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
(a)	Population density, sex ratio and male injury rate	Population	Sampling sites	8
(b)	Male fight aggressiveness variation among population	Population	Sampling sites	8
	Male fight aggressiveness variation within population	Individual	Individuals within each site	Taoyuan 46 Taipei 42 Taichung 40 Hualien 54 Green Island 46 Yilan 32 Kenting 48 Orchid Island 44
(c)	Bite force variation among population	Population	Sampling sites	8
	Bite force variation within population	Individual	Individuals within each site	Taoyuan 60 Taipei 62 Taichung 62 Hualien 87 Green Island 172 Yilan 58 Kenting 162 Orchid Island 166
(d)	Selection differential of snout-vent length	Population	Sampling sites	8

2.8 | Statistical analyses

2.8.1 | The association between density and aggressiveness

We compared the population densities (i.e. the sum of males and females), male densities and sex ratios (i.e. the log-transformed ratio of female over male) of the eight populations using Poisson regression models (the 'glm' function). To evaluate the selection pressure on male body size due to male–male competition, we estimated the standardized selection differentials (d) of male SVL within each population, $d = (\mu r - \mu)/\sigma$, where μr denotes the SVL of resident males in the stage fight experiment, and μ and σ denote the average and SD of male SVL in the male density survey. The absolute value of d represents the strength of selection, and the sign of d represents the direction of selection. We tested standardized selection differentials against zero within each population and also compared them among populations using a Bayesian general linear model (the 'brms' package) (Bürkner, 2017).

We extracted information on aggressiveness based on the outcome of fights and the highest aggression displayed by resident lizards, as well as the ratio of scarred lizards in each population. We tested whether residents were more likely to win fights using a mixed-effect logistic regression model (via the function

'glmer' of the package 'lmerTest') (Kuznetsova et al., 2017). We also examined whether residents tended to escalate fights using a mixed-effect ordinal logistic regression model (via the function 'clmm' of the package 'ordinal') (Christensen, 2022). The ratio of scarred males was analysed using a mixed-effect binary logistic regression model based on population survey data. We employed a two-stage variable selection procedure for the above three logistic models. In the first stage, we included individual-level covariates (i.e. resident and intruder SVLs but no individual-level covariate for fitting the scarred male ratio) and their interactions and/or quadratic terms based on the minimal Bayesian information criterion (BIC). In the second stage, we separately considered four groups of population-level covariates: intrasexual competition, habitat composition, food resources and dietary. For each group, we simultaneously included the population-level covariates belonging to the group (Table S2) in the outcome model after the first stage. We stepwise included or eliminated their quadratic terms, interactions or redundant covariates according to BIC until the minimal BIC was reached or only one population-level covariate was left in the model. We included population in the above mixed models as a random factor, which affected the intercepts of dependent variables.

2.8.2 | The association between density, morphological traits and bite performance

To better account for the association between population density and male aggressiveness, we further analysed how male density and sex ratio interactively affected fight-associated morphological traits and bite force, respectively. Given that there were multiple traits likely to determine individual fight ability (i.e. SVL, head width, head length, head height, and body weight, natural log-transformed beforehand), we first reduced the dimensions of these morphological traits by applying a PCA. We then performed a linear mixed model using the first principle scores derived. We included sex, male density, male bite force, log-transformed sex ratio (i.e. female number divided by male number), standardized selection differentials of male SVL and interactions among these factors as the fixed effects/covariates. Redundant independent variables were eliminated according to BIC.

We performed a multiple linear regression model (function 'lm') to examine the variation in bite force of lizards across populations, sexes and SVLs. We log-transformed bite force and SVL to avoid the potentially allometric growth relationship between the two traits. The log-transformed SVL, sex, male density, sex ratio and the interactions among these factors were considered fixed factors/covariates. We performed backward stepwise selection based on BIC to eliminate redundant independent variables and interactions. We tested how male density affected the adjusted bite force (i.e. the least-squares means of bite force) of the two sexes of lizards using Kendall's rank correlation tests. The bite force of each lizard individual was adjusted based on its sex, population and the average SVL

of all the lizards measured (i.e. 70.3 mm). All of the analyses were performed in R ver. 4.1.1.

2.9 | Ethics statement

Permission to collect and observe the lizards was issued by the institutional animal care and use committee of National Museum of National Science (NMNSAAAUP2019-001).

3 | RESULTS

3.1 | Male densities and selection differentials of the eight lizard populations

We caught a total of 600 male tree lizards across the eight populations (39 males in Orchid Island, 40 males in Kenting, 65 males in Yilan, 79 males in Green Island, 90 males in Hualien, 91 males in Taichung, 95 males in Taipei and 101 males in Taoyuan). The densities across the eight populations were significantly different ($\chi^2 = 61.359$, df = 7, $p < 0.001$; Figure 2b), with the highest density in Taoyuan and the lowest densities in Kenting and Orchid Island.

Overall, the selection differential for male SVLs showed a trend of gradually decreasing with lower population densities, with the selection differential in Taoyuan population (i.e. the highest density) significantly larger than that in Yilan population (i.e. lower density population, Figure 2c). The selection differential in Yilan population was less than zero (posterior median, $\delta = -0.51$ SD; 95% highest density interval [HDI], −0.91 to −0.10 SD; Figure 2c), indicating that resident males here were smaller than its population average.

Distinctively, the population on Orchid Island exhibited a significantly greater selection differential compared with most other populations despite its lowest population density (Figure 2c). The selection differential on Orchid Island was significantly larger than zero, indicating that resident males here were larger than the population average (posterior median, $\delta = 0.74$ SD; 95% HDI, 0.35–1.14).

3.2 | The association between male density and aggressiveness

We conducted a total of 176 stage fights across the eight populations. After comparing BIC of four hypothesized models with different sets of population-level covariates, we found the 'intrasexual competition model' to be the best model accounting for the probabilities of fight intensity and outcome, as well as the proportion of scarred males (Table 2). Within this model, male population density was a strong predictor negatively associated with the three indicators of fight performance (intensity: $\beta \pm SE = -31.85 \pm 12.02$ per m², $Z = -2.649$, $p = 0.008$; outcome: $\beta \pm SE = -44.10 \pm 13.00$ per m², $Z = -3.393$, $p < 0.001$; proportion of scarred males: $\beta \pm SE = -131.3 \pm 33.13$ per

TABLE 2 Coefficient and goodness-of-fit comparisons in models fitting fight intensity (a) and winning odds (b) of resident males in the contest experiment, and the proportion of scarred males (c).

	Model			
	Intrasexual competition	**Habitat composition**	**Food resource**	**Dietary**
(a) Covariate	Std. coefficient			
Individual level				
Resident SVL	0.262	0.322	0.288	0.297
Intruder SVL	0.147	0.241	0.207	0.214
Population level				
Male density	−0.560**	—	—	—
HC PC1	—	0.476	—	—
TDWP	—	—	−0.160	—
DBa	—	—	—	0.261
df	8	8	8	8
log-likelihood	−246.85	−247.95	−249.21	−248.76
BIC	534.6	536.8	539.4	538.5
ΔBIC	0	2.21	3.83	4.73
(b) Covariate	Std. coefficient			
Individual level				
Resident SVL	0.549**	0.561**	0.589**	0.622***
Intruder SVL	−0.187	−0.102	−0.098	−0.111
Population level				
Male density	−0.782***	—	—	—
HC PC1	—	0.264	—	—
$(HC\ PC1)^2$	—	0.812***	—	—
TDWP	—	—	−0.362	—
DBa	—	—	—	0.456*
DBw	—	—	—	0.613**
DBa×DBw	—	—	—	−0.571**
df	5	6	5	7
log-likelihood	−103.52	−101.55	−107.90	−100.27
BIC	232.6	236.4	241.4	236.4
ΔBIC	0	1.17	8.78	3.74
(c) Covariate	Std. coefficient			
Population level				
Male density	−0.318***	—	—	—
$(Male\ density)^2$	0.001	—	—	—
HC PC1	—	0.074	—	—
$(HC\ PC1)^2$	—	0.781***	—	—
TDWP	—	—	−0.364	—
DBa	—	—	—	0.246
DBw	—	—	—	0.456*
DBa×DBw	—	—	—	−0.394
TDWS	—	—	—	−0.339*
df	4	4	3	6
log-likelihood	−14.913	−14.913	−20.910	−14.545
BIC	38.1	38.4	48.1	41.6
ΔBIC	0	0.24	9.91	3.42

Abbreviations: DBa, dietary breadth in abundance; DBw, dietary breadth in dry mass; TDWP, total dry mass of food resource from pitfall trap; TDWS, total dry mass of dietary items from stomach.

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

mm², $Z = -3.962$, $p < 0.001$, Figure 3). Our results did not show strong support for the hypothesis of either habitat composition, food resource or dietary composition.

Overall, male residents from a population with lower male density tended to resolve territorial contests through high-intensity physical fights (e.g. Kenting: high intensity = 65%; low intensity = 8.7%), and scarred males tended to represent a smaller proportion of the population (from ~0.2 in Kenting to ~0.04 in Taoyuan). Although larger male residents had a higher probability of winning fights against intruders (odds ratio = ~1.15 per mm), this effect was dampened in high-male-density populations.

3.3 | The association between density, morphological traits and bite performance

We obtained the std. morphological index of each sampled lizard (male, $N = 398$; female, $N = 401$) by downscaling five morphological traits (log-transformed) using PCA,

$$SMI = 0.061 \text{ body weight} + 0.150 \text{ head height}$$
$$+ 0.066 \text{ head length} + 0.028 \text{ SVL}$$
$$+ 0.110 \text{ body weight} - 7.067.$$

This index explained a total of 90.7% of the variance. We found that sex, male density, sex ratio and interactions between male density and sex significantly affected fight-associated morphology (Table S3). Generally, males had significantly larger fight-associated morphology than females, and lizards from populations with lower male densities and lower sex ratios were larger (Figure 4a,b). A significant effect of the interaction between male density and sex indicated that the fight-associated morphology became significantly smaller with the increased male density in females compared with males (Figure 4a,b).

We also found that the adjusted bite force was negatively correlated with male density (male, Kendall's $\tau = -0.714$, $p = 0.014$; female, Kendall's $\tau = -0.857$, $p = 0.002$, Figure 4c,d). The bite force of males was more sensitive to changes in male density than that of females (Figure 4c,d). We also found that log-transformed SVL, sex, population, the interaction between log-transformed SVL and sex, and the interaction between sex and population had significant effects on bite force (Table S4). Larger individuals, males and lizards in the lower male-density populations had greater bite force, and such allometric trends were much more obvious in males from high-density populations (Figure S2; Table S4).

4 | DISCUSSION

By simultaneously comparing lizard phenotypic traits (Figures 3 and 4) response to perceived ecological drivers (Table 2) across eight populations, this study reveals that within Swinhoe's tree lizard populations, intrasexual competition can significantly explain variation in male territoriality. Moreover, we demonstrate
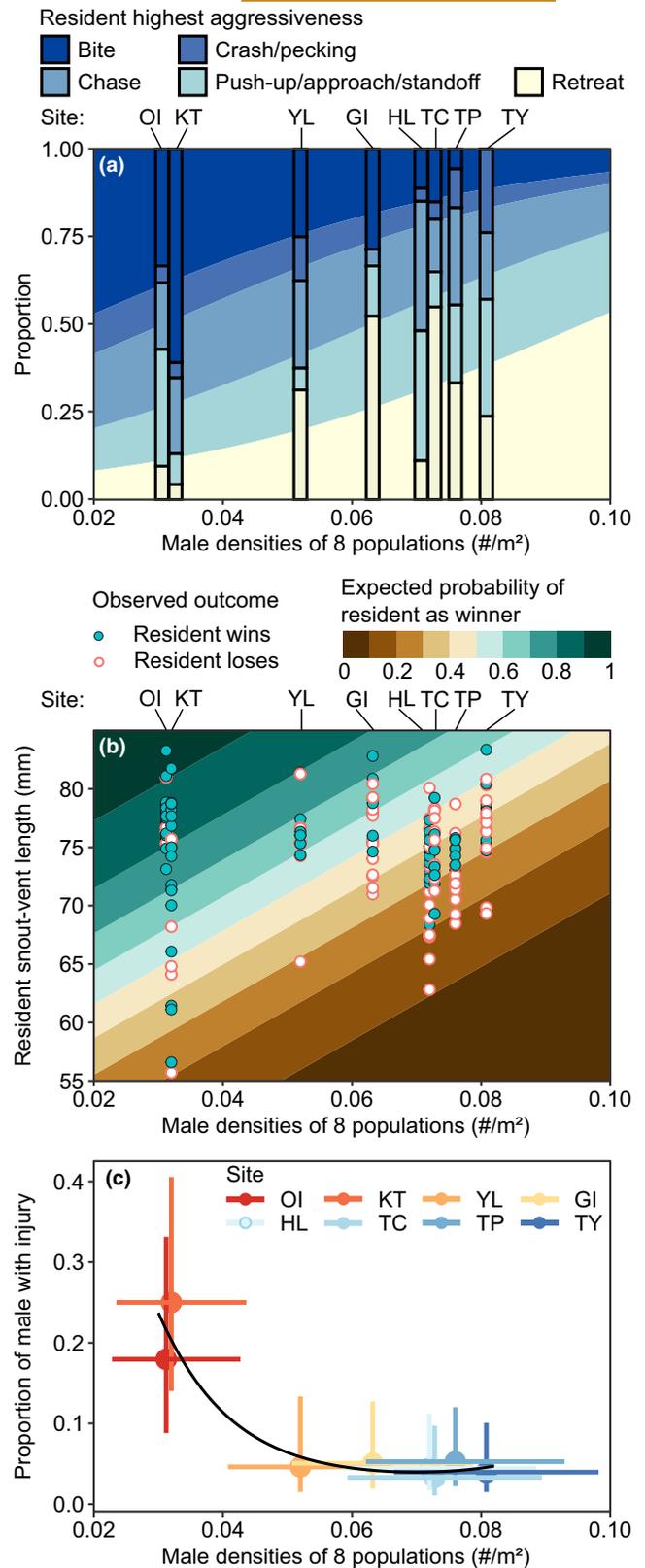

FIGURE 3 Contest results include the composition of the resident lizards' most aggressive behaviours (a), the outcome of residents as winners (b), and the proportions of residents with injuries (c) in eight populations exhibiting varying male densities. The colour patches in panels (a) and (b), as well as the regression line in panel (c), depict the expected probability/proportion based on the most BIC-favoured models.
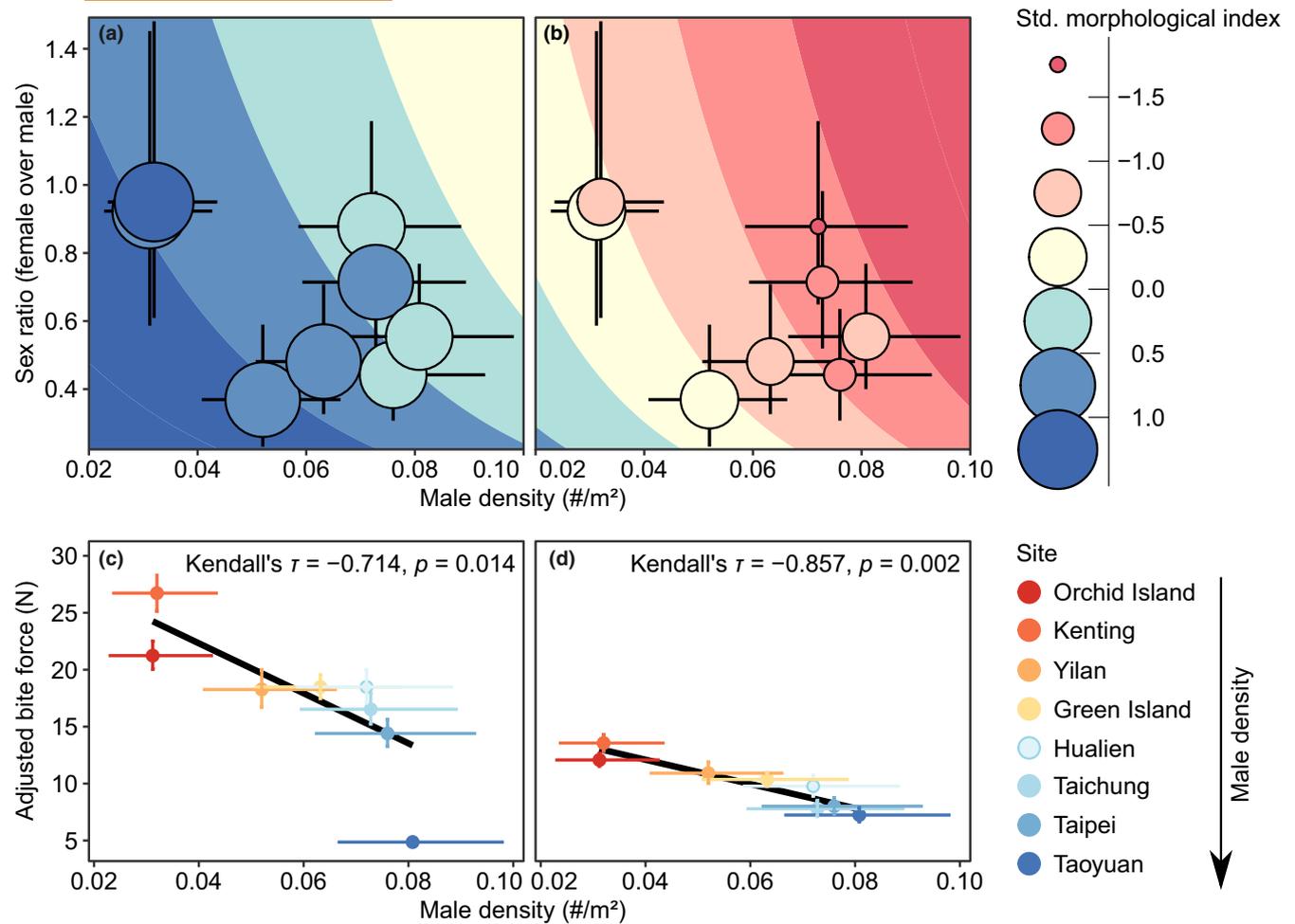
FIGURE 4 Relationship between male density and std. morphological index in males (a) and females (b) across eight populations. Std. morphological indices was the standardized scores of first principle component after downscaling snout-vent length (SVL), head width, head length, head height and body weight and explained 90.7% variance. Colour patches present the expected value according to the most BIC-favoured model. Relationships between males' (c) and females' (d) adjusted bite force (e.g. bite force when SVL constrained at 70.3 mm) and male density among eight populations. Black lines present the M-robusted regression lines. All error bars present the 95% CI.

how density-dependent intrasexual competition could drive the coevolution among fight-associated behaviour, morphology and performance. Overall, by examining the density gradient across eight populations, our findings illustrate the reaction norms of male territoriality, which are negatively associated with local male density, supporting the hypothesis that aggressiveness decreases with increasing density.

A reduction in aggressiveness due to negative density dependence may occur when the costs of maintaining territoriality outweigh its benefits. In high-density populations where individuals' home ranges may highly overlap (Schoener & Schoener, 1982; Vander Wal et al., 2014), frequent engagement in violent territorial contests may either limit males' ability to engage in other fitness-related activities or decrease their fitness due to a higher injury risk (Vervust et al., 2009). Our findings show less intense fighting tactics with lower injury rates in higher population densities, suggesting that the increased costs of territorial conflicts could be a primary evolutionary force weakening the Swinhoe's tree lizard's territoriality. Additionally, we found a weaker resident effect in higher density

populations, suggesting that increased territoriality does not necessarily provide fighting advantages to males from higher density populations, and this process may reinforce the negative density-dependent aggressiveness.

Male density could have a uniform impact on the evolution of male fight-associated behaviour, morphology and performance. We observed that males from higher density populations exhibited less pronounced fight-associated morphology and weaker bite force. This result well aligns with our findings on fight behaviour that males from higher density populations tended not to escalate fights, had a lower injury rate and possessed a weaker resident advantage in winning fights. We also found a positive allometric relationship between bite force and SVL; yet, the degree of allometry varied significantly across populations and was sexually dimorphic; males from higher density populations had far weaker bite force. An explanation is that these males may allocate more resources to other morphological weaponry than bite force due to the increased costs associated with intrasexual competition. Sexual dimorphism in bite force also suggests that its evolution

may be primarily driven by male-specific selection pressure in a context that is not associated with mate competition, given that sex ratio was not a powerful predictor to explain inter-population variation in this trait.

The evolutionary trajectory of a phenotypic trait can be shaped by multiple forces that may exert conflicting effects. In this study, we demonstrated that males from either higher density populations or populations with a less male-biased sex ratio exhibited less pronounced fight morphology. Given that these two conditions are unlikely to co-occur with increasing male density, our findings suggest that the development of fight morphology in the Swinhoe's tree lizard may have been influenced by two antagonistic forces associated with male density and sex ratio, respectively. Furthermore, although density emerged as the dominant predictor of male territorial behaviour at the population level, our results do not preclude the possibility that other ecological factors may indirectly regulate territorial behaviour or operate at different ecological scales. For example, shelter is considered to be an essential ecological resource and the availability of shelter could influence animal territorial behaviour (Chapman, 1966; Tricarico & Gherardi, 2010). We did not consider the effect of shelter availability or abundance on the variation in territorial behaviour of the Swinhoe's tree lizard in this study because, based on our field observation in the field, the lizard can use any place as a temporary refuge, such as a dense tree canopy, beneath grass or under thick leaf litter. Nonetheless, for species displaying strong habitat preferences for refuge, investigating this factor could be of significant importance.

Our findings on negative density-dependence territorial behaviour aligned with several prior studies. For example, insular lizards, birds and mammals from high-density populations have exhibited diminished aggression towards conspecifics, resulting in the establishment of smaller territories, increased overlap in territory boundaries, greater acceptance of subordinate individuals, reduced overall aggressiveness, and in certain instances, the abandonment of territorial defence (Smith et al., 1980; Stamps, 1983; Stamps & Buechner, 1985). Additionally, a meta-analysis involving 483 lizard populations has also unveiled an inverse association between lizard body size and population density (Buckley et al., 2008). Although diverse interactions between population density and aggressive behaviour have also been documented in various taxa (Jones, 1983; Knell, 2009; Mayer et al., 2020; Niemelä et al., 2012; Wegener et al., 2019), the present study on the significance of expanding the sampled population size and considering the intricate interplay among multifaceted phenotypic traits. These steps are crucial for gaining a more comprehensive understanding of the ecological mechanisms shaping animal territoriality. Moreover, to unravel the complex interplay between various ecological factors and territorial behaviour, future studies could collect individual-based environmental data and employ path analyses. This approach would provide a more comprehensive understanding of the regulatory mechanisms underlying territoriality in this species.

In conclusion, our findings provide support for the hypothesis that negative density dependence plays a significant and widespread role as a fundamental regulator driving eco-evolutionary dynamics. Our study revealed male-specific variation in fight behaviour, morphology and performance along a density gradient, suggesting the presence of different evolutionary equilibria in territoriality influenced by the costs and benefits experienced by individuals in local populations. This observed variation highlights the importance of ecological and social factors in shaping the evolution of ontogenetic growth and life-history strategies, as evidenced by the interpopulation variation in body size among the lizards (Buckley et al., 2008; Calsbeek & Smith, 2007). These insights gained from our study lay the groundwork for future investigations into the theory of pace-of-life syndromes (Wright et al., 2019). By addressing the challenge of understanding the density-dependent effects on the evolution of territoriality and the interdependence between multiple phenotypes, our study further elucidates how phenotypic adaptation may have contributed to shaping population structure.

AUTHOR CONTRIBUTIONS

Wen-San Huang conceived the idea, designed the experiment and initiated this long-term programme. Jung-Ya Hsu wrote the manuscript and conducted all laboratory experiments and the fieldwork. Chun-Chia Chou, Jhan-Wei Lin and Jung-Ya Hsu chiefly constructed and wrote the manuscript based on the draft of Jung-Ya Hsu. Chen-Pan Liao did all formal analyses, wrote the results, and revised most of the methods. Jhan-Wei Lin, Chun-Chia Chou, Jung-Ya Hsu and Wen-San Huang revised the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The authors declare that all data supporting the findings of this study are available in the article and the electronic supplementary material or upon request from the corresponding author (Hsu et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Loading vectors yielded by principle component analysis that downscaled habitat components.

Table S2: Population-level covariates used in models fitting the probabilities of fight outcome and intensity of resident males in the competition experiment.

Table S3: The results of mixed effect linear model fitting std. morphological index.

Table S4: ANOVA table presents the variation of natural-log transformed bite force partitioned with natural-log transformed snout-vent length (SVL), lizard sex, population and their interactions.

Figure S1: An adult male *Diploderma swinhonis* defended its territory on a tree trunk.

Figure S2: Relationship between snout-vent length (SVL) and bite force in males (A) and females (B) across eight populations.

Video S1: Territorial fight held by a resident and an intruder on the tree trunk