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ORIGINAL ARTICLE

Color variation and mate choice in a lacertid lizard

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Abstract

Coloration plays a crucial role in sexual selection. Stejneger's grass lizard (*Takydromus stejnegeri*) exhibits a diverse range of body colorations, including complex combinations of green or brown and striped or non-striped patterns in both sexes. In order to clarify the relationships among the variation of body coloration, body condition, and mate preference, we conducted a capture-mark-recapture survey in the wild and mate choice experiments in the laboratory. Analysis of data from 2,497 marked individuals revealed that adult females typically exhibit green patches on their sides, whereas adult males tend to display white stripes on their backs during the breeding season. These colorations did not correlate with their body condition. In the mate choice experiments, we found that green females preferred green males, while brown females preferred brown males. Conversely, males did not demonstrate a significant preference for both colors. Our findings suggest that the varied colorations of *T. stejnegeri* are influenced by life stage, sexual maturity, and potentially linked to assortative female choice.

Significance Statement

The maintenance of color variation is a fascinating subject in evolutionary biology, with sexual selection and mimicry often cited as two of the primary underlying mechanisms. However, long-term population monitoring and studies involving large sample sizes are rare, especially in reptiles—a group with elusive habits—where research is particularly scarce. This study has gathered an extensive collection of research samples to monitor seasonal variation in body color among individuals within the population and has conducted experiments on sexual selection to substantiate the maintenance of variation. We believe this research approach offers a compelling case study for investigating color variation.

Keywords Assortative mating · Capture-mark-recapture · Mate choice · Nuptial color · *Takydromus*

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Introduction

Visual signals, including actions, postures, and coloration, are crucial in animal communication. Among these signals, coloration serves as a key communicative element in animals. For example, male *Anolis* lizards exhibit various colorful dewlaps to attract potential mates during courtship and to intimidate rivals in territorial disputes (Sigmund 1983; Johnson et al. 2010). A significant challenge in evolutionary biology is to understand how these color variations evolved and their persistence within species (Roulin 2004; Gray and McKinnon 2007; Cuthill et al. 2017). Color morphs that represent a better advantage in increasing fitness are more likely to thrive in the population (Brazill-Boast et al. 2013; Galeotti et al. 2013). In the context of intraspecific communication, sexual selection can intensify the coloration, serving as a primary evolutionary force behind conspicuous color morphs (Chen et al. 2012; Pérez I de Lanuza et al. 2013). However, studies on color variations commonly use model systems showing substantially polymorphic or dimorphic between sexes (McKinnon and Pierotti 2010; Svensson 2017), while systems showing mutually colored sexes or mixed morphs remains underexplored (Nordeide et al. 2013; Stuart-Fox et al. 2021).

The color variation can present in all members of the population regardless of the sex, which is referred as color polymorphism (Huxley 1955). In this scenario, the occurrence of multiple discrete color morphs within the species results from genetic variation and is irreversible throughout ontogeny or environmental conditions (Bond 2007; Wellenreuther et al. 2014). On the other hand, sexual dimorphism in coloration describes a difference of color morphs that present in all adult members of a given sex, where one often has a more vivid and high-contrast color than the other (Pérez I de Lanuza et al. 2013; Rojas and Endler 2013). Mate choice is traditionally thought to cause this color variation between sexes (Owens and Hartley 1998; Stuart–Fox and Ord 2004; Maan and Cummings 2009). Lizards serve as ideal models for studying the evolution of coloration, owing to their diverse body colors and patterns. Equipped with color vision, they are capable of perceiving a variety of visual signals from conspecifics (Cooper and Greenberg 1992; Langkilde and Boronow 2012; Fleishman and Font 2019). In numerous sexually dichromatic systems, coloration often serves as an honest signal of body condition and reproductive status (Cuadrado 2000). Higher quality individuals tend to display more pronounced coloration, which in turn is associated with heritable traits that contribute positively to fitness (Díaz 1993; Doucet 2002; Dubuc et al. 2014; Higham and Winters 2015; Keren-Rotem et al. 2016). Consequently, wild animals with brighter or more intense colorations tend to have greater mating opportunities and reproductive success, as observed in case studies of the green anole (Sigmund 1983), the rhesus macaque (Dubuc et al. 2014, 2016), the estrildid finches (Soma and Garamszegi 2018), and a lacertid lizard (Tseng et al. 2018).

Studying Stejneger's grass lizard, *Takydromus stejnegeri* (Squamata: Lacertidae), offers a valuable opportunity to explore the role of color variation in mate choice (Fig. 1). The *Takydromus* genus, encompassing 22 species (Rodda 2020), is the only lacertid genus found in East and Southeast Asia. Various types of courtship coloration have been observed in this genus (Lue and Lin 2008; Yang and Wang 2010; Lin et al. 2020), including: 1) seasonal sex dichromatism (e.g., *T. viridipunctatus*), where males exclusively exhibit secondary sex coloration during the breeding season, while females remain dim year-round; 2) lifelong sex dichromatism (e.g., *T. sauteri*), where males and females display different colors post-sexual maturity, unaffected by seasonal changes; and 3) lifelong sex monochromatism (e.g., *T. formosanus*), where both sexes exhibit the same color throughout their lives. Species exhibiting seasonal sex dichromatism have been more thoroughly studied, as behavioral and physiological experiments are more straightforward to design for these courtship systems. For example, Tseng et al. (2018) showed that green coloration was preferred in *T. viridipunctatus* mate choice using testosterone manipulation, whereas Lin et al. (2020) utilized long-term field capture data to analyze the seasonal fluctuation of this coloration. However, the color variation in Stejneger's grass lizards, which do not conform to any of the aforementioned systems, remains largely unexplored. *Takydromus stejnegeri* features a range of colorations, including various combinations of brown and green, with or without white stripes in both sexes, making it a highly diverse species with complex color variations (Fig. 1). Furthermore, the temporal variation and significance of these colorations in the mate choice of *T. stejnegeri* are yet to be understood.

In this study, our objectives were to: 1) investigate the temporal variation in body coloration and stripes; 2) examine the relationship between body coloration, stripes, and body condition; and 3) assess the role of green coloration in the mate choice of *T. stejnegeri*. The novelty of this study resides in its examination of a system that has received limited attention in prior research, specifically focusing on the impact of various factors, such as season, sex, body condition, and sexual selection, on lizard color variation. We

Fig. 1 Color variation of adult *Takydromus stejnegeri*. (**A**) Brown lateral band with white lines; (**B**) brown lateral band without white lines; (**C**) green lateral band with white lines; (**D**) green lateral band with obscure white lines; (**E**) and (**F**) other intermediate forms. All these color combinations occur on adult males and females. Photographed by Si-Min Lin (A and B), Wan-Xin Jian (C), Jui-Chen Hsieh (D), Shih-Hsien Chan (E), and Mei-Ling Lo (F)

will compare the results of this study with those from other species within the same genus that exhibit different color patterns. Our overarching goal is to understand the evolution of these diverse color systems, ranging from lifelong to seasonal differences, within a relatively short evolutionary timeframe. Specifically, we aim to explore how natural and sexual selection influence these changes and contribute to the rapid diversification of species.

Materials and methods

Study site and sample collection

To examine the temporal variation in body coloration, we monitored a *T. stejnegeri* population at the Lotus Temple Trail, Hsinchu, Taiwan (24.87556°N, 120.96147°E). The study site (150×500 m^2) predominantly features bushes

(*Acacia confusa*, *Broussonetia papyrifera*, and *Macaranga tanarius*) and grasslands (*Miscanthus* sp., *Bidens pilosa*, and *Wedelia trilobal*) grown continuously along the two sides of walking trails. The entire area is an isolated hill, allowing us to neglect emigration and immigration rates and assume a closed population. The breeding season at this site, determined by the gravid period of females, spans from March to August, while the non-breeding season extends from September to February.

We conducted the capture-mark-recapture (CMR) survey once a month at night from October 2017 to October 2020. The grass lizards tend to sleep on the grasses during the night, and hence are easy to find by spotlighting (Lin et al. 2017, 2020; Chen et al. 2021). On the night of a sunny day in the middle of each month, five to eight experienced fieldworkers searched for lizards sleeping on the grasses or herbs along several transects for two hours. Upon spotting a lizard, we hand-captured it from its sleeping perch. The day following capture, we measured the lizard's body condition and coloration using the method described in the subsequent section. Before releasing them back into their original habitat in the evening, we marked the lizards by toe-clipping.

Body condition

We measured the snout-vent length (SVL) to an accuracy of 0.1 mm using an electronic vernier caliper (Mitutoyo 500–171-30), and the body mass to an accuracy of 0.1 g using a digital scale (Luckystone 4006878401). Following Lin et al. (2017), we recorded the autotomy index of each lizard. The sex of the lizards was determined based on the presence or absence of hemipenes. In females, the reproductive condition was recorded by counting bite marks as a parameter of courtship behavior, and assessing gravidity through abdominal palpation (Lin et al. 2020). We defined adults as individuals longer than 45 mm in SVL, which was the minimum size of a gravid lizard following the method applies in a similar species (Lin et al. 2020). Due to the absence of sexual size dimorphism in this species, this 45-mm threshold was applied to males as well. Utilizing growth records from newly hatched lizards, individuals under 30 mm were categorized as hatchlings (age less than one month), and those measuring between 30 and 45 mm were classified as juveniles. The size effect only occurs in the growing period. After the lizards are fully grown, the size effect does not persist.

To measure body condition, we calculated the scaled mass index (SMI) following Peig and Green (2009) and calculated the residual from mixed-model regression of SVL against body mass (both log-transformed), with individuals ID as the random factor (Godfrey et al. 2013). Lizards with shorter tails due to autotomy, and hence lighter body mass, were considered to be in worse body condition because there is a strong link between survival and tail regeneration (Lin et al. 2017). We identified outliers using Cook's distance and studentized residual. To investigate differences in body condition indices between color morphs, white stripes, sex, and season, we utilized a linear mixed model (LMM). We included lizard ID as a random factor in the LMM and conducted the LMMs using the lme4 package (Bates et al. 2015). The model selections were performed by comparing the AIC values. We inspected model assumptions using the check_model function in the performance package (Lüdecke et al. 2021) and weighed the unequal variances using the varIdent function in the nlme package (Pinheiro et al. 2020).

Coloration assessments

For adult lizards of both sexes, we assessed their coloration and white stripe indices. These indices were defined in reference to the long-term CMR system used for *T. viridipunctatus* (Lin et al. 2017, 2020; Tseng et al. 2018) with the following modifications:

Body color index — the coverage of green areas on the body.

[Level 1]: green color absence.
[Level 2]: green color presence only on the lateral part.
[Level 3]: green color presence and expands to neck or head.

White stripe index — the presence of lateral white stripes.

[Level 1]: white stripe absence.
[Level 2]: a pair of white stripes on dorsal or on lateral part.
[Level 3]: two pairs of white stripes on both dorsal and lateral parts.

Mate choice experiments

To evaluate mate preferences for brown or green coloration in both females and males, we conducted mate choice experiments during the breeding seasons of 2017 and 2018. In total, 171 sexually mature individuals (SVL > 45 mm) were captured, including 62 females and 56 males from Lotus Temple Trail (N24.875561°, E120.961473°) in July 2017, and 26 females and 27 males from Nangang (N25.040778°, E121.610764°) in July 2018. Each lizard was housed individually in transparent plastic containers (30 cm × 19 cm × 21 cm) using crushed walnut shells and bark mulch as the substrate, with artificial turf providing shelter. Room temperature was maintained at approximately 28 °C, with a daily photoperiod of 12 h (06:00 – 18:00 h). We fed the lizards with crickets (*Gryllus bimaculatus*), cockroaches (*Shelfordella lateralis*), or mealworms daily and provided water ad libitum. At the end of the experiments, the lizards were released back into their original habitat.

We designed the mate choice experiment as a dichotomous choice trial in an acrylic arena (40 cm length × 30 cm width × 20 cm height; Supplementary Information Fig. S1) following the successful experiment applied in its closely related species (Tseng et al. 2018). Because we could not find a material which could totally prevent from filtering the UV light, we used the thinnest available plexiglass (2 mm) to build the tank. We further used a FLAME-S-XR1-ES spectrometer (Ocean Optics) and found no UV reflection from the body of this species. The acrylic arena was separated into three compartments (Hamilton and Sullivan 2005; Tseng et al. 2018), with two small rooms and a large one separated by transparent plexiglass, but the animals in the two smaller rooms could not see each other. A cotton twine above the large room further divided it into a "preference zone" adjacent to the two smaller compartments, and a "neutral zone" at the far end of the arena. We covered the arena's bottom with a thin layer of crushed walnut shell to facilitate lizard movement.

Mate choice experiments were conducted in a bright outdoor environment during 09:00 – 12:00 (30 ± 2 °C). The lizards' coloration was assessed using the body color index described above. For the mate choice experiments, only brown (Level 1) and green (Level 3) lizards were selected as candidates. Before starting the trial, we placed an opaque board between the two small rooms and the large one. We introduced one green and one brown lizard of the same sex with similar size (SVL difference < 0.2 mm) and randomly placed them in the two small rooms. We then placed the focal lizard of the opposite sex in the large room. All lizards were allowed to acclimate for 10 min. We then removed the opaque board to allow the focal lizard to see the stimulus lizards but prevented physical contact. The focal lizard could freely move in the preference and neutral zones for one hour. We filmed the trials using a video camera (JVC GZ-E10) and recorded the time the focal lizard spent approaching the brown or green colored stimulus lizard whenever it entered the preference zone.

Statistical analysis

To analyze coloration variation among different life stages of wild lizards, we utilized a generalized linear model (GLM) with a binomial distribution. In this model, coloration (brown or green; corresponding to Level 1 and Level 2 + 3) was the response variable, and life stage (hatchling, juvenile, female, or male) was the explanatory variable. Similarly, to determine if stripe presence varied among life stages, we employed the same GLM approach with a binomial distribution, where stripes (absence or presence; corresponding to Level 1 and Level 2 + 3) was the response variable. Since hatchlings lacked stripes, we excluded them from this analysis to prevent complete separation in the model. We applied generalized linear mixed models (GLMMs) with a binomial distribution to test the effects of seasons (breeding and non-breeding), sexes, and their interactions on the coloration and stripes of lizards. To accommodate for recaptured individuals, we incorporated lizard ID as a random effect in the GLMM. We conducted GLMMs using the lme4 package (Bates et al. 2015). We performed post-hoc comparisons using the emmeans package, with p-values adjusted by Tukey's HSD test (Lenth et al. 2021).

To test mate preferences, we calculated the proportion of time that a focal lizard spent on the green stimulus lizard (T_{green}/T_{total}) or the brown stimulus lizard (T_{brown}/T_{total}). We analyzed our data using beta regression models with betareg package (Cribari-Neto and Zeileis 2010), where the proportion of time spent on brown stimulus lizard as the dependent variable. The model included the main effect of focal lizard's sex and color, as well as their interaction term. Additionally, the interaction between sex and color was included as a precision parameter. The model specification is as follows:

Proportion of time spent on brown stimulus lizard ~ sex * color | interaction (sex, color).

Then we tested whether the preference was different between green lizards and brown lizards of the same sex using emmeans package (Berge et al. 2021). We conducted all analyses using R version 4.0.3 (R Core Team 2020).

Results

Variation of body coloration across life stages

Throughout the study period (2017–2020), we captured and marked a total of 2497 lizards, comprising 1312 adults, 1011 juveniles, and 174 hatchlings (Supplementary Information Fig. S2). The recapture rate varied among the months ranging from 0.06 to 0.68 (Supplementary Information Fig. S3). The likelihood of lizards exhibiting green coloration ($x^2 = 528.31$, $p < 0.0001$) and having stripes ($x^2 = 584.65$, $p < 0.0001$) varied significantly across life stages (Fig. 2). In the younger stages, merely 1.2% of hatchlings and 11.6% of juveniles displayed green coloration. Similarly, only 10.7% of juveniles had white stripes, while these were entirely absent in hatchlings. In contrast, a significant proportion of adults exhibited green coloration, with 55.9% of females and 19.9% of males being green. Concurrently, 29.5% of females and 62.0% of males displayed white stripes in adulthood. The presence of green coloration and white stripes was not consistently correlated, indicating various combinations of green/brown and white stripes/no stripes in both females and males.

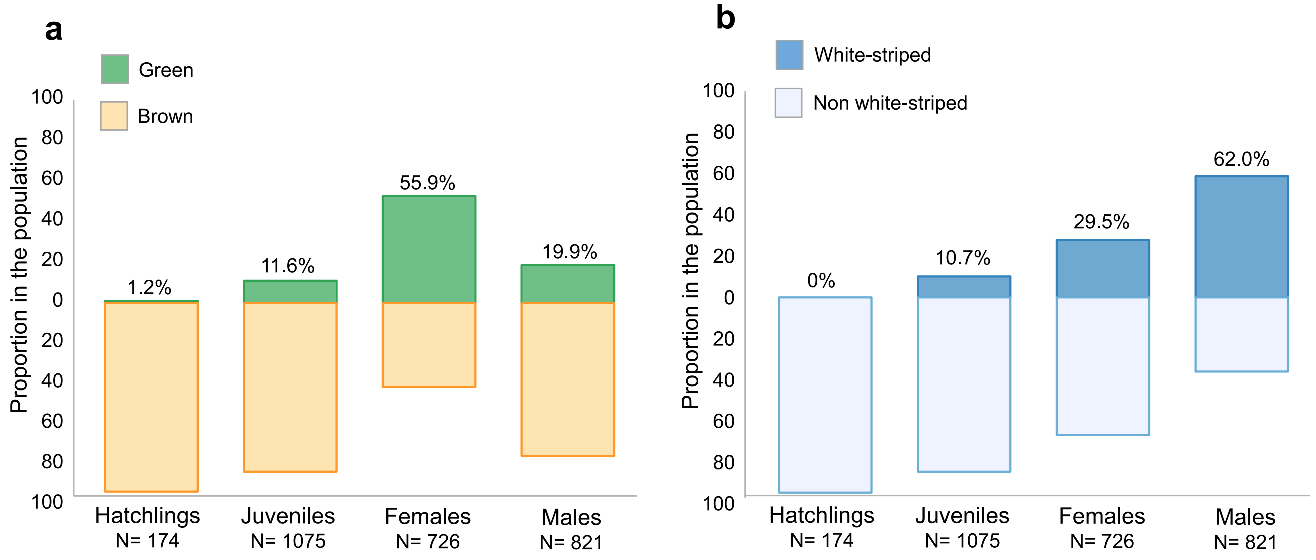

Fig. 2 Body coloration and white stripes of *Takydromus stejnegeri* across different life stages. Population proportion of both characters represented statistical significance among the groups: $p < 0.0001$ for green coloration, and $p < 0.0001$ for white stripes

Temporal variation of body coloration in adults

Beyond age-related effects, seasonal variations in body coloration were also pronounced. Green coloration in females reached its highest probability during the breeding season (|β|= 5.406, $p = 0.012$). Generally, the proportion of green females gradually increased from approximately 40% in March to a peak of around 80% in May, June, or July (Fig. 3A). In contrast, temporal variation of green color in males was not significant, with most individuals remaining brown throughout the months (Fig. 3B). Overall, the likelihood of females being green was significantly greater than that of males in both the breeding (|β|= 18.71, $p < 0.0001$) and non-breeding seasons (|β|= 13.31, $p < 0.0001$).

We observed synchronous peaks in white stripe occurrence in males, exhibiting a similar pattern annually. The probability of males with white stripes was significantly higher than that of females in both the breeding (|β|= 2.476, $p < 0.0001$) and non-breeding seasons (|β|= 0.58, $p = 0.0014$). Less than 60% of females exhibited white stripes, fluctuating irregularly (Fig. 3C). In contrast, a regular fluctuation in white stripes was found in males, who had a significantly higher probability of having stripes in the breeding season than in the non-breeding season (|β|= 2.147, $p < 0.0001$). Between May and August, more than 70% of males displayed white stripes, with all of them showing white stripes in July (Fig. 3D).

Color transition between breeding and non-breeding seasons

In analyzing the recapture data, we found that 50 females and 70 males had records across both non-breeding and breeding seasons. We used the color and stripe patterns of these individuals to compare color transitions across seasons in the same individuals. Among these lizards, 36% of females showed green coloration in the non-breeding season, increasing to 80% in the breeding season (Fig. 4). This group included 24 females (48%) that changed from brown to green and one (2%) from green to brown; 17 (34%) remained green and 8 (16%) stayed brown throughout. In contrast, males showed a moderate increase in green coloration, from 11 to 31%. Only 14 males (20%) changed from brown to green, with over 68% ($N = 48$) retaining their original brown color.

Males are characterized by the appearance of white stripes, showing a notable increase from 34% in the non-breeding season to 73% in the breeding season. More than 30 males (42.9%) switched from no stripes to having stripes, two showed the reverse (2.9%), and the rest remained unchanged. Conversely, the percentage of females with white stripes increased slightly from 18 to 26%. The majority of females (70%, N = 35) did not display stripes in either season.

Relationship between coloration and body condition

Adult males ($N = 821$) exhibited a snout-vent length range of 45.01 – 58.47 mm (mean ± SD = 50.68 ± 3.04 mm) and a body mass range of 1.28 – 3.68 g (mean ± SD = 2.35 ± 0.42), resulting in an SMI index ranging from 1.02 to 2.31 (mean ± SD = 1.66 ± 0.21). Females ($N = 725$) had a snout-vent length of 45.02 – 60.08 mm (mean ± SD = 51.46 ± 3.56 mm) and a body mass of 1.08 – 4.10 g (mean ± SD = 2.29 ± 0.47),

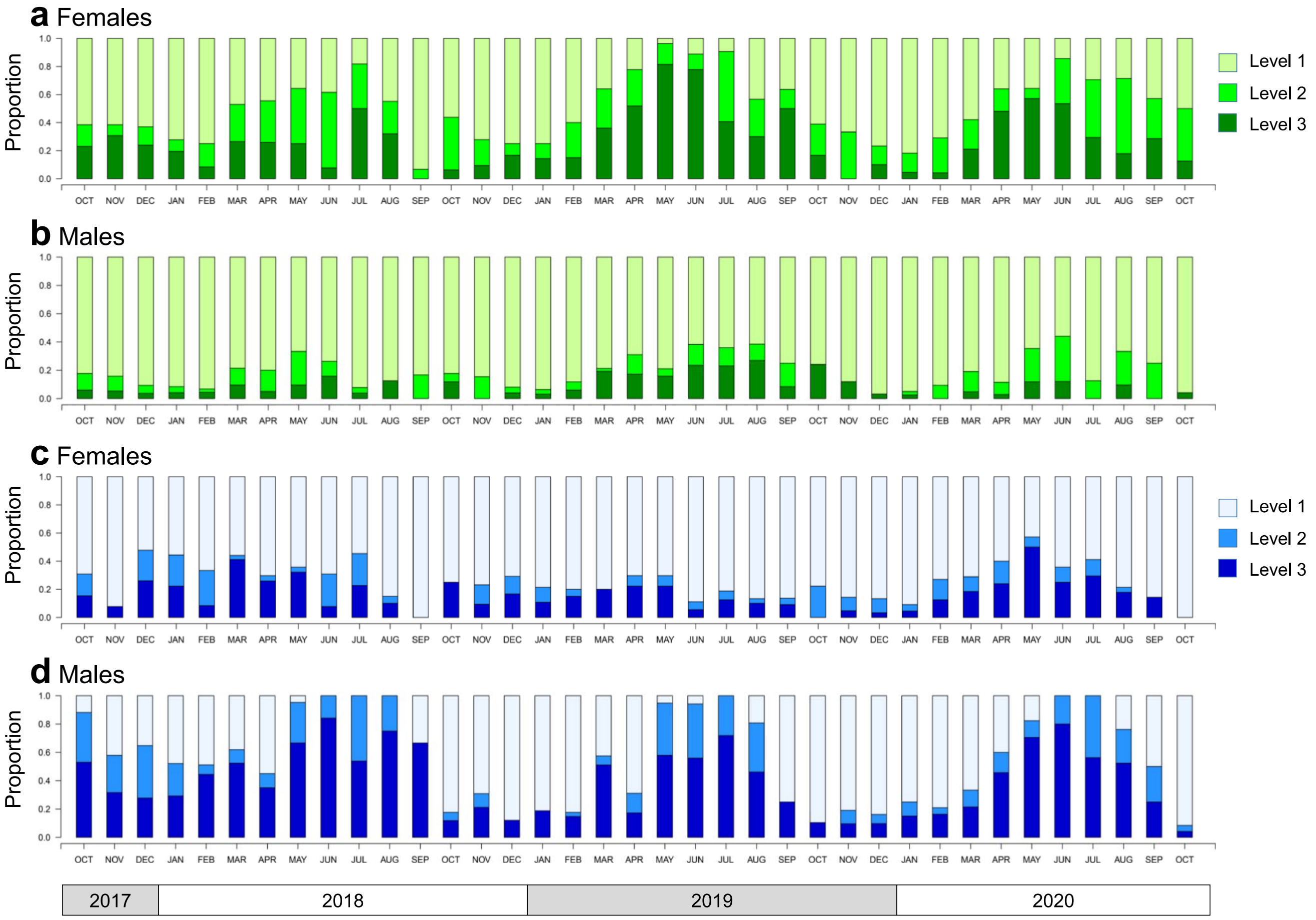

Fig. 3 Annual fluctuation of body coloration and white stripes in *Takydromus stejnegeri* from 2017–2020. Annual peaks of green color (Level 2 and Level 3) were observed in females in the breeding season (**a**), where synchronous peaks of white stripes occurred on males (**d**). In contrast, proportion of green color in males (**b**) was significantly lower than females, and that of white stripes in females (**c**) was significantly lower in males

leading to their SMI index ranging between 0.92 and 2.16 (mean ± SD = 1.54 ± 0.22). We removed the four-way interaction from the final model as it was insignificant ($|\beta| = 0.104$, S.E. = 0.120, $p = 0.385$), and the models with a higher AIC value (AIC = −492). Our analysis revealed that SMI was not significantly associated with the interaction of coloration, sex, and season ($|\beta| = 0.059$, SE = 0.051, $p = 0.246$, AIC = −529). Neither sex with green coloration exhibited a better body condition compared to those with brown coloration in either breeding or non-breeding season. Similarly, SMI was not significantly associated with the interaction of stripes, sex, and season ($|\beta| = 0.056$, S.E. = 0.047, $p = 0.237$, AIC = −547). Both sexes with stripes did not have a better body condition than those without stripes in either breeding or non-breeding season. We also found the same insignificant associations when using the residual score as the body condition index (the interaction of coloration, sex, and season $|\beta| = 0.021$, SE = 0.021, $p = 0.319$, AIC = −3318; the interaction of stripe, sex, and season $|\beta| = 0.035$, SE = 0.019, $p = 0.06$, AIC = −3339; Supplementary Information Text S1, Table S1, Table S2).

Mate choice experiment

In the trials involving female choice, green females displayed a strong preference for green males (Z = −2.451, $p = 0.014$, effect size = 0.427, Fig. 5A). When compared between different color groups of the same sex, the brown female group spent significantly more time on the brown male than the green female group (Z = 1.975, $p = 0.048$, odds ratio = 1.459). In contrast, in the trials involving male choice, green males (Z = 0.362, $p = 0.717$, effect size = 0.508) and brown males (Z = −0.267, $p = 0.789$, effect size = 0.493) showed no differences in their preference for green or brown female (Figs. 5C, D).

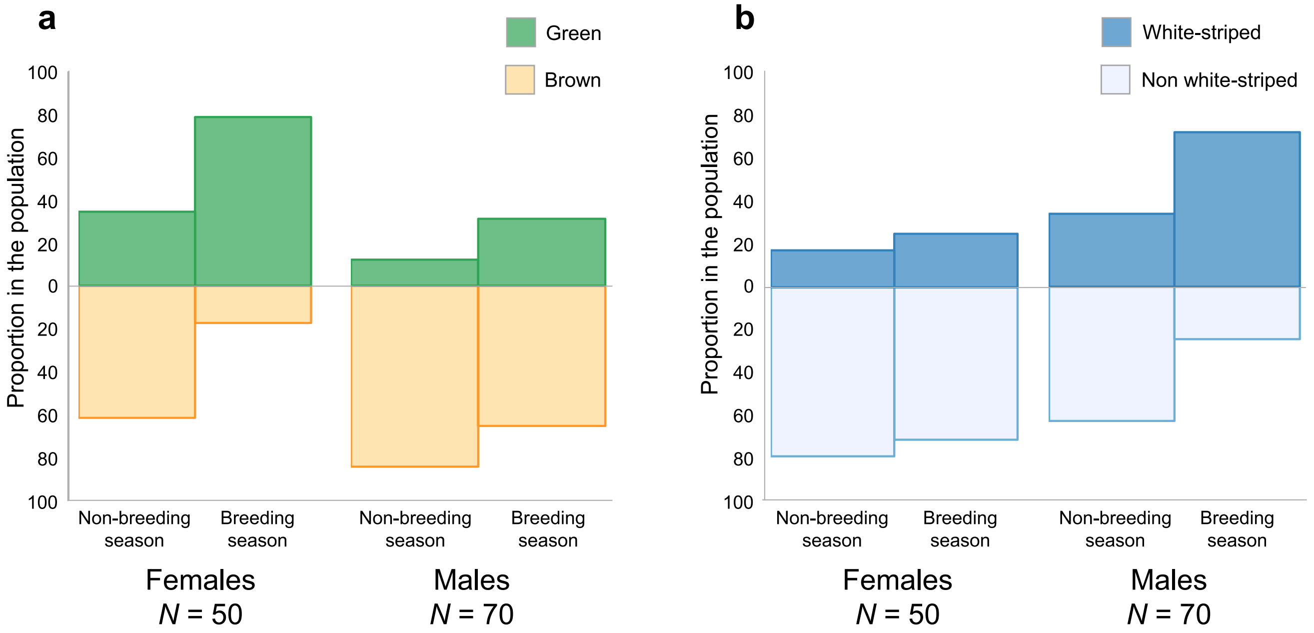

Fig. 4 Seasonal differences of body coloration and white stripes in *Takydromus stejnegeri* from recaptured individuals. Proportions of green color in females and white stripes in males increased from non-breeding season (September – February) to breeding season (March – August)

Discussion

This study highlights how body coloration in a lacertid lizard, *T. stejnegeri*, exhibits temporal variations in both sexes. Among adults, female lizards exhibit varying levels of green coloration, and a minority also show white stripes during the breeding season (March – August). Conversely, only a small proportion of male lizards display green coloration, and the majority exhibit white stripes during the breeding season. This suggests that both green coloration and white stripes in *T. stejnegeri* may serve as sexual signals during the breeding season. We suspect that this seasonal variation may be partially, but not entirely, related to hormonal changes. In the closely related lizard *T. viridipunctatus*, testosterone simply increases the coverage of green coloration in males during the breeding season (Tseng et al. 2018). However, the complex color morphs and existence/absence of white stripes in *T. stejnegeri* during the breeding season indicates a more complex signaling system compared to that of its congeners.

Based on current knowledge, *Takydromus* lizards lack the capacity for rapid chromatophore-mediated color changes; instead, their coloration transitions occur gradually over an extended period, typically requiring several weeks to manifest. Consequently, the possibility of short-term, rapid changes can be reasonably excluded. Because *Takydromus* lizards typically form crowded populations with extremely high population density, social ranking has never been observed in the wild (Lin et al. 2017, 2020; Tseng et al. 2018). Therefore, the hypothesis of coloration expressing social status is also excluded. Our study also failed support the hypothesis that green coloration and white stripes are reliable predictors of body condition, because neither morph correlated with the Scaled Mass Index (SMI), the index of body condition. These findings are very different from the phenomenon observed in *T. viridipunctatus*, which belongs to a sexually dichromatic system, and the green coloration is tightly associated to male quality (Tseng et al. 2018; Lin et al. 2020). In contrast, color variation in *T. stejnegeri* persists even among sexually mature adults, as indicated by the observed color variations at both intra- and inter-sex levels (Figs. 1 and 2).

Therefore, we infer that this variation may be linked to a polymorphic genetic background. However, this has yet to be definitively determined due to the limited understanding of the genetic basis of this non-model species. Given this situation, the origin, maintenance, and function of the color patches necessitate further discussion. In addition, we cannot rule out the possibility that coloration (and subtle variations in color characteristics such as brightness or saturation) may signal other aspects of quality or health state that were not measured in this study. These aspects may provide a foundation for future studies to explore their potential roles in our study species.

The observed mating preferences may shed light on some aspects of this question. In our study system, both green and brown females tend to choose males with the same coloration, exhibiting assortative mating (Pérez i de Lanuza et al. 2012; Roulin and Bize 2007; Sacchi et al. 2018). Given that a correlation between adult quality and this trait is likely to be weak both statistically and in our experimental

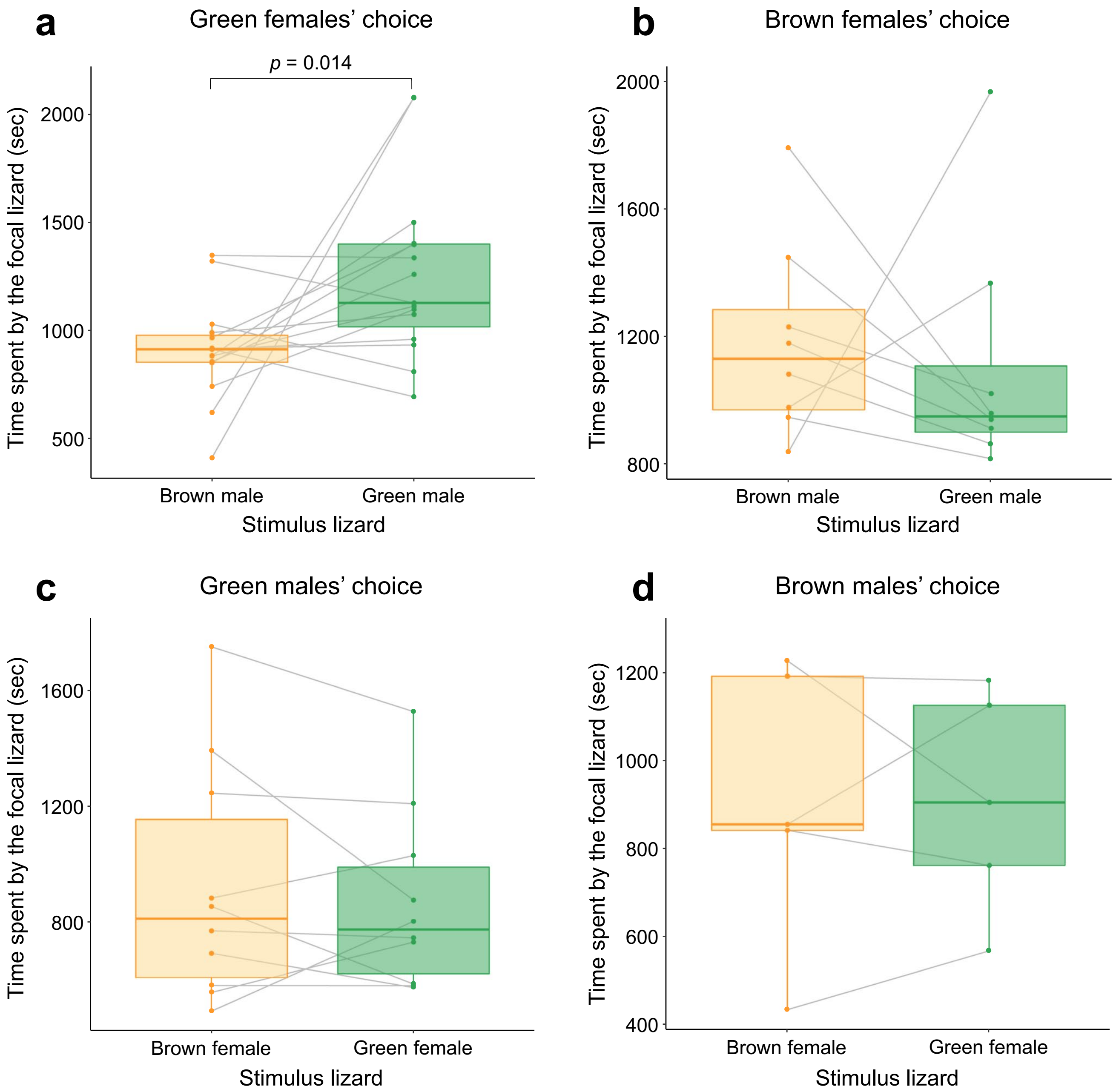

Fig. 5 Mate choice experiments in *Takydromus stejnegeri*. The data points connected by the line represent the two stimulus lizards in the same trial. The boxes show the median, interquartile range, whiskers (indicating range) and individual data points

design, this preference likely primarily stems from coloration. Meanwhile, males also exhibited no clear preference for either morphotype (although the sample size for the latter group was relatively low). Our results are consistent with classic studies indicating that overall female choice is stronger than male choice because females typically invest more in reproduction, leading them to be more selective than males (Andersson 1994). This female mating preference can be more exaggerated in our choice test design than a no-choice design (Dougherty and Shuker 2014).

In some other systems, female coloration may indicate gravid status, as seen in species like the keeled earless lizard (*Holbrookia propinqua*; Cooper and Crews 1988), the Mediterranean chameleon (*Chamaeleo chamaeleon*; Cuadrado 2000), and the striped plateau lizard (*Sceloporus virgatus*; Weiss 2002). For instance, in Mediterranean chameleons, males tend to avoid copulating with females that display gravid coloration, characterized by a black body with yellow spots. In this context, males might favor a morphotype that enhances their chances of successful mating and siring more offspring (Swierk et al. 2013; Belliure et al. 2018). We inferred that green coloration is unlikely to indicate gravid status in our study system for several reasons (Supplementary Information Fig. S4). First, this species is characterized by continuous cycles of gravidity and egg-laying throughout the breeding season. For example, a captive record showed that a single female produced four clutches of eggs over a 40-day period (Lin et al. 2004). Second, color change requires a significant amount of time, and the green coloration generally remains stable throughout the breeding season. This lack of correlation is further supported by long-term capture data, as shown in Supplementary Information Fig. S4.

The contrasting mating choices and interactions between males and females appear to sustain the frequency of different colorations in this lizard species, as suggested in previous studies on color polymorphism (Pérez i de Lanuza et al. 2012; Lancaster et al. 2014; Wellenreuther et al. 2014; Sacchi et al. 2018). Assortative mating alone is unlikely to maintain polymorphism, as it typically leads to divergence between alternative color morphs (Wellenreuther et al. 2014; Stuart-Fox et al. 2021). In our study, the lack of male mate preference could counterbalance this divergence and foster gene flow among color morphs, thereby maintaining the presence of different morphs (i.e., green and brown). However, the underlying genetic background and regulation of these color morphs in this species remains unknown. Additional research is necessary to elucidate the genetic mechanisms underlying the temporal variation in colorations of this species.

One limitation of this study lies in the difficulty of confirming the intent of the tested individuals during mate choice trials. In our experimental design, we used transparent partitions to isolate the tested individuals, ensuring that body coloration was the sole available signal. This approach effectively minimized potential confounding effects from other physical traits, such as size or behavior, which could otherwise influence mate choice. However, this also constrained our ability to interpret the behavioral intent of the individuals in the preference zone. This limitation is a challenge frequently encountered in studies utilizing similar experimental designs, but future experiments allowing direct interaction while controlling for confounding variables could provide deeper insights into how coloration and other traits influence mating decisions in this species.

The close evolutionary relationships among *T. stejnegeri* (with a polymorphic system), *T. viridipunctatus* (with a sexually dichromatic system), and other congeners with various courtship patterns suggest rapid transitions among these systems. Considering the extensive geographical distribution and the variation in color morphs within the *Takydromus* group, comparing mating systems among lineages can deepen our understanding of how colorations evolved in lizards. Moreover, recent studies have highlighted an interrelationship between coloration expression in these lizards and their avian predators, such as cattle egrets or shrikes, which lead to a high mortality rate in the lizards (Lin et al. 2017). The expression of coloration changes the lizard's contrast against the grass background, influencing survival rates under attacks from visually hunting predators (Chen et al. 2021). The evolution of coloration represents a complex balance between sexual selection and natural selection, and the interplay between these selective forces remains to be explored in greater detail.

Conclusions

Our findings indicate that *T. stejnegeri* exhibits color variation, encompassing different combinations of green and brown colors and the presence or absence of white stripes, observable in both males and females. These color variations are associated with life stage, sex, and seasonal changes. Furthermore, our results lend support to the hypothesis that sexual selection, through assortative mating, may contribute to the enhancement of color variation in Stejneger's grass lizard.

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Author contribution W-XJ, K-HL and S-ML conceived the ideas and designed methodology; W-XJ, T-WL, and FADN collected the data; W-XJ, T-CL, C-PL and K-HL analyzed the data; W-XJ, K-HL and S-ML led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability All data used in this study is available as supplementary material.

Declarations

Ethics approval All procedures conducted during this study were approved by Institutional Animal Care and Use Committee (IACUC), National Taiwan Normal University (license No. 106023) and were performed in accordance with the local law of the Wildlife Conservation Act in Taiwan. All sampling and toe clipping procedures were conducted following the protocol guidelines provided in "Taiwan Wildlife Survey—Amphibian Resources Survey Manual" (Lue et al. 1996) approved and published by Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan. In this study, toe clipping was identified as an optimal method due to its ability to yield a wealth of information from a single sampling event. This technique facilitated the collection of genetic samples, essential for longitudinal population genetics studies, and provided material for age determination via skeletochronology, all while ensuring non-lethality for the subjects. The toe-clipping procedure, proficiently conducted by S-ML and T-WL, benefited from their combined 14 years of experience in handling and researching Takydromus lizards, alongside comprehensive animal ethics training. The method of toe clipping used here, involving the partial removal of a single toe, has been scientifically proven to have no negative impact on the survival rates in a closely related congener (*T. viridipunctatus*), as supported by long-term capture-mark-recapture data (Shaner et al. 2013; Lin et al. 2017, 2020). Throughout the study, lizard handling was kept to a strict minimum (less than five minutes per individual), ensuring their prompt return to their natural habitat within the following day.

Competing interests The authors declare no competing interests.

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