



Appearance matters: multiple functional signals of body colours in a weevil

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Visual signals in animals are often shaped by sexual selection. For aposematic species, advantages may result from advertising unpalatability to decrease predation and attracting mates to increase reproductive success. On Green Island, Taiwan, most *Eupyrigops waltonianus* weevils have metallic blue spots on the body; however, some weevils have no conspicuous coloration. The role of coloration in sexual selection and any disadvantages encountered by dull individuals are unknown. To understand whether morphological traits affect mating success in *E. waltonianus*, we compared different traits between mating and nonmating individuals in the field. A manipulation experiment was also used to test whether coloration affects mating choice and behaviour. The function of colour as an aposematic signal was tested. Results showed that body size and coloration were important indicators of female mating success in field and laboratory experiments, respectively. In the field, males preferred to mate with larger, more conspicuous females, and females preferred to mate with conspicuous males; however, no such preference was shown in the laboratory. Intrasexual competition was stronger in males than in females. Predators avoided colourful weevils. Thus, conspicuous coloration in both sexes is a multifunctional trait and could be an attractive or aposematic signal for different receivers.

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Visual signals are often modified by natural and sexual selection during evolution, and different selection forces may trigger the diversification of these traits (Crothers & Cummings, 2013; Ender, 1980; Finkbeiner, Briscoe, & Reed, 2014). These diverse traits have multiple functions to increase the species' fitness, especially for survival (Nokelainen, Hegna, Reudler, Lindstedt, & Mappes, 2012) and reproduction (Lin, Zhang, Liao, Hebets, & Tso, 2015). For aposematic species, conspicuous visual signals convey an important message to predators, warning of toxins, bad smell or hardness, for example, to reduce predation risk (Finkbeiner et al., 2014; Tseng, Lin, Hsu, Pike, & Huang, 2014). However, some studies have indicated that aposematic signals also play an important role in conspecific communication (Finkbeiner et al., 2014; Jiggins, Naisbit, Coe, & Mallet, 2001; Maan & Cummings, 2008). For example, the strawberry poison frog, *Oophaga pumilio*, possesses polymorphic

aposematic patterns, and these visual signals are used not only to avoid predation (Siddiqi, Cronin, Loew, Vorobyev, &) but also to attract mates (Maan & Cummings, 2009). Therefore, conspicuous colours may function as aposematic signals for predators and as mating signals for conspecifics (Blount, Speed, Ruxton, & Stephens, 2009; Speed & Ruxton, 2007; Willink, Bolaños, & Pröhl, 2014).

Conspicuous coloration is a determining factor in sexual display in many different organisms (e.g. satin bowerbirds, *Ptilonorhynchus violaceus*, Savard, Keagy, & Borgia, 2011; budgerigars, *Melopsittacus undulatus*, Griggio, Zanollo, & Hoi, 2009; butterflies, *Hypolimnas bolina*, Kemp, 2007; strawberry poison frogs, Maan & Cummings, 2009), and individuals with these attractive traits can increase their reproductive success (Andersson, 1994; Ryan, 1997). Although cryptic individuals do not possess conspicuous coloration to attract conspecifics to mate, they may use different strategies to mate successfully. For example, the poison dart frog *Oophaga granulifera* displays two different colorations, one conspicuous (red) and the other cryptic (green), evolving from conspicuous ancestors. The cryptic frogs are less visible but increase their calling activity during courtship to increase their mating success (Willink et al., 2014).

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Behavioural mechanisms are also critical in sexual selection, and shape the evolution of mating systems (Sih, Lauer, & Krupa, 2002). There are three major behavioural mechanisms, intrasexual competition, mate choice and intersexual conflict, which decide mating success (Andersson, 1994; Sih et al., 2002). First, competition involves a struggle for resources or mating opportunities in the same sex (Andersson & Iwasa, 1996; Ospina-L et al., 2017; Sullivan-Beckers & Cocroft, 2010). Dominant or more active individuals have more opportunities to win the competition because stronger individuals usually defeat others in combat (e.g. interfere and separate copulating pairs). Furthermore, active individuals have more opportunities to encounter potential mates and increase their chances of mating and reproduction rates (Sih et al., 2002). Second, mate choice also plays an important role in mating systems. In many species, females spend more time and invest more resources in reproduction compared to males (Koivula, Koskela, Mappes, & Oksanen, 2003; Shuker, Ballantyne, & Wedell, 2006), and that may have led to the female choice and male–male competition system. Mate choice is a filter to select better genes or traits to increase the fitness of offspring (Andersson & Simmons, 2006). Third, intersexual conflict in mating behaviour arises when two sexes are in conflict over the optimal fitness of reproduction during evolution (Casalini et al., 2009; Gagnon & Turgeon, 2011). For example, if males attempt to copulate with females during an encounter, females that refuse to mate will display specific behaviours to resist male copulation, like jumping, rolling and kicking their legs to prevent males from copulating (Edvardsson & Arnqvist, 2005; Lauer, 1996; Sih et al., 2002). Females sometimes get rid of males, but sometimes males can mate successfully (Sih et al., 2002). Therefore, intersexual conflict is also an important factor that affects mating success in sexual selection.

Eupyrgops waltonianus is an endemic weevil on Green Island, off the coast of Taiwan, which has an extremely hard black exoskeleton with small metallic-coloured spots (Tseng et al., 2014). Each spot is composed of small scales, and the colour and size of each spot vary; most are bright and conspicuous. *Eupyrgops waltonianus* exhibit sexual dimorphism with females larger and heavier than males. Although the general appearance may affect the predators' perception of unpalatable prey (Lee et al., 2018), lizards can learn to avoid weevils with conspicuous coloration and the memory can last for some time (Tseng et al., 2014). Therefore, the spots on *E. waltonianus* function as warning signals to advertise unpalatability, and successfully reduce predation pressure from lizards (Tseng et al., 2014). Although conspicuous aposematic coloration can effectively protect weevils from predation, it is easy to find dull black weevils without bright markings in the field (Fig. 1a). Without coloration as protection, dull weevils may face stronger predation pressure than colourful ones. It remains unclear whether dull individuals have different strategies to survive or adapt to natural environments.

In this study, we tested whether coloration affected mating preference in the wild and in a laboratory experiment. First, we compared different morphological traits of mating and nonmating *E. waltonianus* in the field and investigated whether cryptic individuals had an advantage or disadvantage in mating opportunities. Second, we compared mating behaviours in different mating stages (male occupation, male–male competition/female–female competition, female resistance and mating success) to evaluate whether coloration affected mating success in a mate choice experiment. Third, intrasexual competition in *E. waltonianus* was investigated and, finally, an experiment was conducted to examine the effect of coloration on predation.

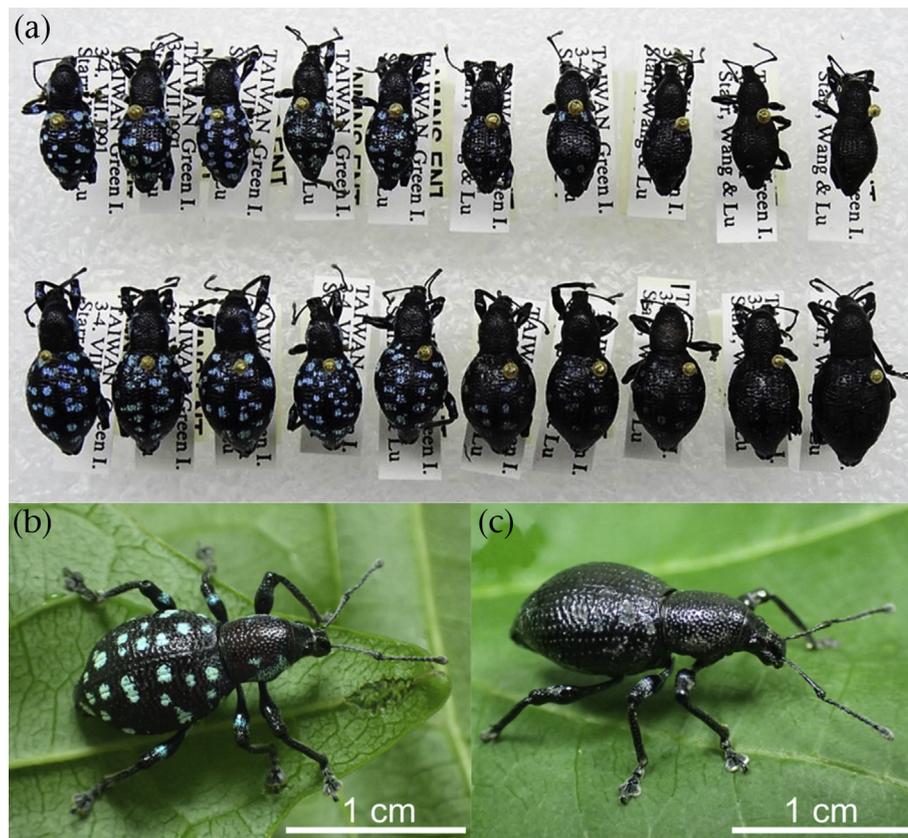


Figure 1. (a) Variation in colourful spots on specimens of *Eupyrgops waltonianus* from the National Museum of Natural Science, Taiwan. (b) Female in the spot-remaining treatment (SR+). (c) Female in the spot-removed treatment (SR–).

METHODS

Ethical Note

Eupyrgops waltonianus is a protected species in Taiwan, and all experiments were approved and conducted under animal ethics protocols of the Taiwanese Wildlife Conservation Act and were also covered by a permit (No 1061701832) from the Forestry Bureau, Council of Agriculture, Taiwan. After the experiments, we released all lizards and weevils at their exact capture location.

Field Collection

We studied *E. waltonianus* on Green Island (121°29' 5"E, 22°39'33"N), which lies ca. 30 km off the coast of southeastern Taiwan. To test the mating preference of *E. waltonianus* in the field, we collected weevils (total = 1417, male = 781, female = 677) by hand from April to November 2016 and July 2017. Mating pairs were identified if the male had inserted his genitalia into the female. Others were identified as nonmating individuals. Each pair was kept in a plastic container (3 × 3 cm and 4 cm high), and nonmating male and female individuals were placed into two plastic boxes (8 × 9 cm and 13 cm high) separately according to sex. All individuals were kept in the laboratory with a room temperature of approximately 27 °C.

Individual Measurement

In the laboratory, we put all the weevils in a 4 °C refrigerator for 30 s so that they became inactive for a short time. Then we stretched their legs and stuck them on a soft eraser to limit movement. The weevils soon recovered and were not harmed after removal from the refrigerator. Each individual was photographed (Nikon D200 with MICRO NIKKOR 60 mm lens) in this condition and the lengths of the thorax and elytra and the spot area were measured using ImageJ (version 1.52; <http://rsb.info.nih.gov/ij/>). The body weight of each individual was recorded using an electronic scale (HIRODA CT-50, 50 g/0.001 g). All nonmating weevils were kept individually in plastic containers (3 × 3 cm and 4 cm high) for the manipulation experiment.

Colour Reflectance Measurement

To confirm the differences in reflectance spectra between conspicuous and dull individuals (i.e. artificially removed spots), we chose 10 males and 10 females randomly from the field and measured the reflectance spectra of the spots and the background colour of elytra. Then we removed the spots after measuring them and recorded the reflectance again. The spots on the weevils were gently removed with a box cutter (see below). Reflectance was measured with a spectrometer (detection range, 250–800 nm; Jaz spectrometer; Ocean Optics, Dunedin, FL, U.S.A.) and a deuterium-halogen light source (DH-2000-BAL). A reflectance standard (WS-1-SL) was used for calibration in SpectraSuite (Ocean Optics).

Mate Choice Experiment

The aim of the manipulation experiment was to understand whether spots influenced mating choice in *E. waltonianus*. Nonmating individuals collected in the field were used for mating choice trials, which included male choice and female choice trials. In the male choice trials, two females with similar morphological traits and one randomly chosen male were put in an arena (5 × 5 cm and 3 cm high). All the spots on one of the females were removed and this female was regarded as the treatment individual (spot-removed treatment, SR–; Fig. 1c). We fixed the weevil by hand, and used a box cutter (180Black, OLFA, Taiwan) to scrape the scales off the elytra carefully until all the scales were removed. The weevils were not harmed during manipulation and could move naturally afterwards. The black background of the other female was wiped gently using the box cutter and she was considered as the control individual (spot-remaining treatment, SR+; Fig. 1b). The mating behaviours at different mating stages were recorded as described below. In the female choice trials, two males with similar morphological traits were chosen. The two males in the female choice trials were manipulated in the same way as the females in the male choice trials. The groups containing two males with one female were male–male–female (MMF) groups, and the groups composed of two females and one male were female–female–male (FFM) groups. During the experiment we did not supply any water or food until the trial was over. The weevils' mating behaviour was recorded by infrared digital video camera (SONY 4K FDR-AX40) for 24 h. Trials lasted for 24 h because *E. waltonianus* is also active at night.

In the mating process, we divided mating behaviours into four different stages: male occupation, male–male competition/female–female competition, female resistance and mating success. In the first stage of male occupation, occupying duration was defined as the time from when the male climbed onto the back of the female (guarding, not copulation) until it left (Klein, Trillo, Costa, & Albo, 2013; Sih et al., 2002). Total occupation duration (total time of occupation by the same male over 24 h) and occupying frequency (number of times the same male occupied the female over 24 h) were recorded.

Male–male competition and female–female competition behaviour were recorded as competitive intensity level, total competitive duration and competitive frequency. Competition only occurred during occupation or copulation when the nonmating individual interfered with the mating pair. The competition behaviour was divided into six competition intensity levels and these levels were scored from lowest 0 to highest 5 (details in Table 1). We compared the total score differences between SR+ and SR– individuals in male choice or female choice trials. The total competitive duration was defined as the total time of interference from the competitive score from the same male over 24 h. Total competitive frequency was defined as the number of males or females interfering with the others over 24 h.

Three types of female resistance were recorded: total resistance duration, resistance frequency and resistance intensity level.

Table 1

The scores and definitions of behaviours in the male choice and female choice experiments

Intensity level (score)	Definition
0	No interaction between nonmating and mating individuals
1	The nonmating individual touched the mating pair, but the mating pair did not show any reaction
2	The male raised his front legs to drive out the nonmating disturber
3	The male raised his front and middle legs to drive out the nonmating disturber
4	Only the genitalia were still connected with the female after disturbance
5	The mating pair separated after disturbance

Female resistance only occurred when a female was occupied or mated by a male; the female would raise her legs to kick the male to signal refusal of the male's occupation or mating. Female total resistance duration was the total time of the resisting behaviour displayed by the same female that had been occupied or copulated with over 24 h. Female resistance frequency was the number of times the females resisted over 24 h. Female resistance intensity was evaluated by the kicking frequency. The total number of times that the female kicked the male with one leg was counted. We only counted the kicking frequency for one leg because it was difficult to see different legs due to the limited view of the video. Then we compared the differences in female resistance between SR+ and SR− individuals.

At the stage of mating success, mating duration was defined as the time that the male copulated with the female. Total mating duration (total time of copulation of the same male over 24 h) and mating frequency (the number of copulations over 24 h) were recorded in mating success.

Prey Choice Experiment

To test the defence function of aposematic spots on *E. waltonianus*, we performed a prey choice experiment on Green Island in August 2018. We designed an arena made from cardboard (44 × 32 cm and 44 cm high), the bottom of which was covered with litter and branches. The front wall of the arena was transparent for observation during the experiment and the left wall had two entrances (12 cm × 3 cm) which were used to transport the weevils into the arena. We placed one lizard (*Diploderma swinhonis*) in the arena for 5 min before the experiment, and then simultaneously offered the lizard one SR+ and one SR− weevil in two independent 9 cm (diameter) Petri dishes with caps through the entrances. To prevent the lizard from biting the experimental prey and to avoid odours that might affect the choice of the lizard during the experiment, weevils were placed inside transparent petri dishes. If the lizard attacked one of the weevils, then the experiment terminated. The behaviour of 'attack' was defined as the lizard trying to bite the prey from outside the Petri dish. If a lizard had seen the prey and not attacked for 5 min, we removed both Petri dishes with weevils and offered one mealworm, *Tenebrio molitor*, in a Petri dish (with cap) for 5 min to check whether the lizard was hungry or not. If the lizard attacked the mealworm, the trial was terminated immediately. In contrast, if the lizard did not attack the mealworm, we considered the trial had failed, and the results were excluded from the following analysis. After each successful trial, we measured the lizard's snout–vent length (SVLs) and sex and recorded which prey it attacked (SR+ weevil, SR− weevil or mealworm).

Statistical Analyses

The mating probabilities observed in the field were separately fitted to two additive logistic models. Explanatory variables included weevils' body length, scaled mass index and spot area ratio. We applied Peig and Green's (2009) method to calculate male/female body mass indices by mathematically shifting male/female weevils' body lengths to the mean values. We allowed all explanatory variables as smoothed terms (splines with 2–4 degrees of freedom) and applied two-directional stepwise variable selections based on minimum Akaike information criterion (AIC) procedures. We also checked the relationships between body traits/conditions and spot area ratios using two linear models, which fitted spot area ratios against body length and scaled mass indices. Male and female data were analysed separately.

The morphological traits of the two females in the FFM group and two males in the MMF group were compared using two permutational multivariate analyses of variance (PERMANOVAs) with 9999 permutations. Since we conducted paired experimental designs, permutations only occurred between two competing weevils. To compare whether the behaviour of mating progress affected mating success, we used exact Wilcoxon–Pratt signed-rank paired-sample tests to compare behavioural outcomes. We adjusted *P* values using Benjamini–Hochberg procedures to reduce the false discovery rates since these behavioural outcomes were dependent on each other. FFM and MMF data were analysed separately. To identify which sex was dominant in mate competition, we performed Wilcoxon–Pratt signed-rank independent-sample tests to compare the absolute values of competition differences between MMF and FFM groups.

The outcomes of the prey choice experiment were fitted using a bias-reduced multinomial logistic regression (Kosmidis & Firth, 2011). The lizard attack target (SR+ weevil, SR− weevil or mealworm) was considered as the dependent variable, and the sex and SVL of lizards were considered as independent variables in the statistical model. The SVL of female and male lizards were independently standardized (e.g. mean = 0 and SD = 1).

RESULTS

Mating Preference in the Field

A total of 780 males and 677 females were collected in the field. The percentages of spot areas on male and female weevils were $12.43 \pm 6.23\%$ (mean \pm SD) and $10.97 \pm 5.60\%$, respectively (see Fig. A1 for the distribution of spot area ratio in males and females in the field). The result of the additive logistic models showed that male mating probability was positively associated with his spot area ratio (mating log-odds increased 3.201 ± 1.476 per spot area percentage; $Z = 2.169$, $P = 0.030$; Fig. 2a). Males with median scaled mass indices had higher mating probabilities evidenced by a significant nonparametric effect ($\chi^2_2 = 20.7$, $P < 0.001$; Fig. 2a). Male body length, as a potential predictor, was eliminated during model selection ($\Delta\text{AIC} = -1.922$; see Table A1). On the other hand, we found that females with longer body lengths had a higher mating probability in the field (mating log-odds increased 0.338 ± 0.981 per mm of body length; $Z = 3.447$, $P < 0.001$; Fig. 2b). Females with greater scaled mass indices in general also had higher mating probabilities (mating log-odds increased 0.821 ± 0.368 per 0.1 g of scaled mass index; $Z = 2.230$, $P = 0.026$; Fig. 2b). The female spot area ratio was eliminated during model selection ($\Delta\text{AIC} = 4.35$; see Table A1). To summarize, a male with median size and higher spot area ratio and a female with both greater body length and scaled mass index had a higher mating probability in the field.

According to the field data, we found that the spot area ratios of males were positively associated with male body length but negatively associated with scaled mass index (Table 2). However, the total $R^2 = 2.0\%$ showed poor predictability for both predictors (Table 2). In contrast, we found no evidence showing body length and scaled mass index were associated with female spot area ratios and the total R^2 was only 0.4% (Table 2).

Manipulation of Colour Pattern Reflectance

The spectrum of spots showed minimal reflectance of ca. 20% within UV and red light and >70% reflectance within the range 500–550 nm (Fig. 3). In contrast, black background and SR− coloration in both sexes on average only reflected ca.10% illumination (Fig. 3). These results showed that the spots on both male and female were obviously brighter than their black background

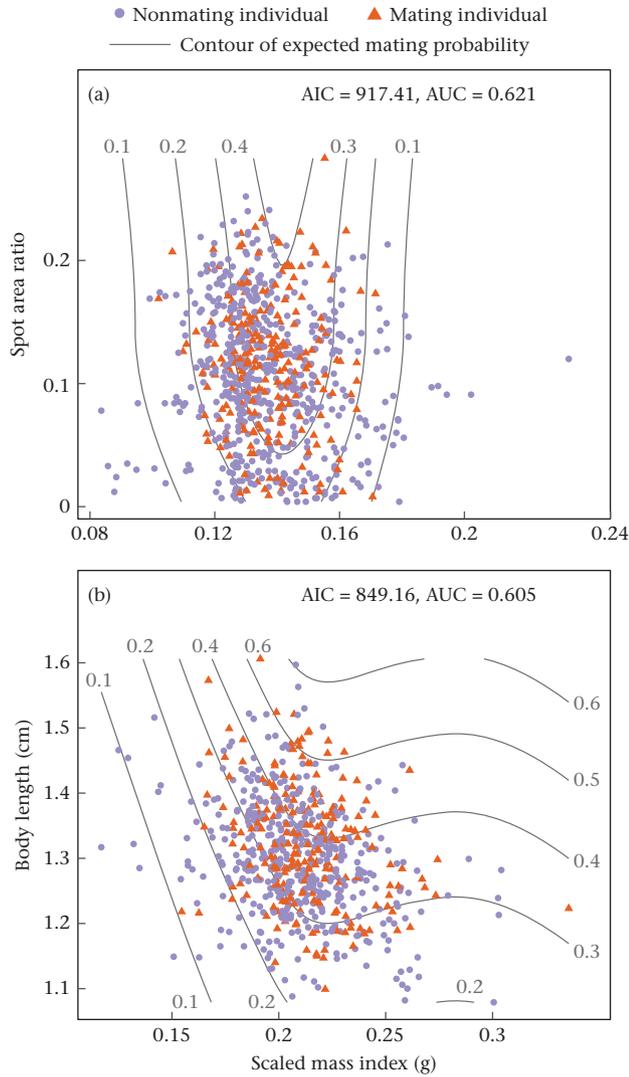


Figure 2. Contour plots of expected mating probabilities of weevils in the field. (a) Males ($N = 780$) and (b) females ($N = 677$). Scaled mass indices as body condition indicators were the estimated body mass when body length was shifted to the average value (i.e. male = 1.127 cm and female = 1.305 cm). AIC: Akaike information criterion; AUC: area under the curve.

coloration or SR– coloration, and the black background and SR– colours were optically similar to each other in both sexes.

Mate Choice Experiment

We conducted 43 trials with the MMF group and 45 trials with the FFM group. Body weights, thorax lengths, elytra lengths and scaled mass indices of the two males in each MMF group and those

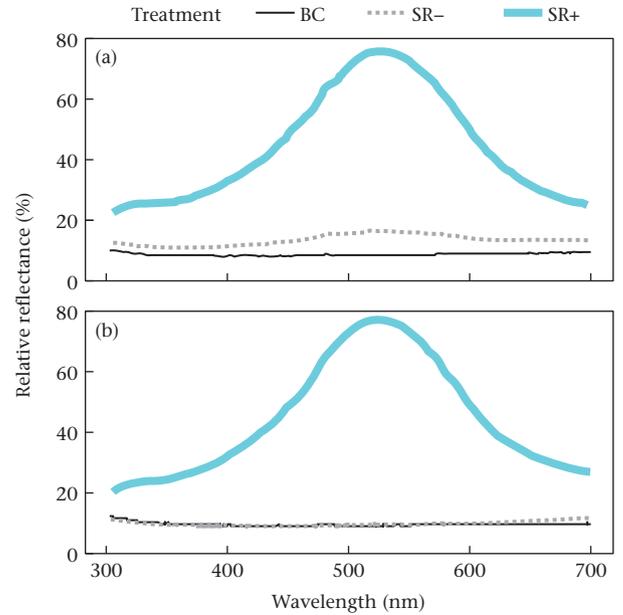


Figure 3. Average reflectance spectrum of (a) 10 male and (b) 10 female weevils in different treatments. BC: black background coloration of weevil; SR–: spot-removed coloration; SR+: spot-remaining coloration. Note that the SR– treatment is obviously different from SR+ but similar to BC in both sexes.

of the two females in each FFM group were compared by PERMANOVAs. Since we purposely chose competitors with similar body sizes, both comparisons showed no significant differences in all three traits (MMF group: $R^2 = 0.08\%$, $F_{1, 84} = 0.07$, $P = 0.955$; FFM group: $R^2 = 0.15\%$, $F_{1, 88} = 0.13$, $P = 0.899$; Fig. A2).

In the male–male competition (SR+ versus SR– males) trials, the total occupying duration ($Z = 0.428$, $P_{adj} = 0.850$; Fig. 4a), occupying frequency ($Z = -0.193$, $P_{adj} = 0.850$; Fig. 4b), competitive duration ($Z = -0.612$, $P_{adj} = 0.760$; Fig. 4c), competitive frequency ($Z = -0.386$, $P_{adj} = 0.760$; Fig. 4d) and intensity level ($Z = -0.866$, $P_{adj} = 0.760$; Fig. 4e) did not differ between SR+ and SR– males in the trials. Likewise, at the stage of female resistance and mating success, female resistance duration ($Z = 0.358$, $P_{adj} = 0.953$; Fig. 4f), frequency ($Z = -0.381$, $P_{adj} = 0.953$; Fig. 4g), intensity level ($Z = 0.063$, $P_{adj} = 0.953$, Fig. 4h), mating duration ($Z = 0.468$, $P_{adj} = 0.993$; Fig. 4i) and mating frequency ($Z = 0.013$, $P_{adj} = 0.993$; Fig. 4j) were not significant in the MMF group.

In the female–female competition (SR+ versus SR– females) trials, the total occupation duration of SR+ females was longer than that of SR– females ($Z = -2.332$, $P_{adj} = 0.038$; Fig. 4a), and the males may have preferred to mate with colourful females. However, there was no significant difference in occupying frequency between the two females ($Z = -1.705$, $P_{adj} = 0.091$; Fig. 4b). At the stage of female–female competition, SR– female showed a higher total competitive duration ($Z = 2.448$, $P_{adj} = 0.047$; Fig. 4c) and intensity

Table 2
Linear regression fit for spot area ratios of males and females observed in the field

Variable	Coefficient (95% confidence interval)	<i>t</i> (<i>df</i>)	<i>P</i>	Partial R^2 (%)
Male				
Intercept	0.0513 (–0.0338, 0.136)	1.184 (777)	0.237	–
Scaled mass index (g)	–0.249 (–0.504, 0.00704)	–1.909 (777)	0.057	0.798
Body length (cm)	0.0820 (0.0229, 0.141)	2.725 (777)	0.007	1.275
Female				
Intercept	0.117 (0.0242, 0.209)	2.479 (674)	0.013	–
Scaled mass index (g)	–0.120 (–0.314, 0.0744)	–1.211 (674)	0.226	0.282
Body length (cm)	0.0252 (–0.0298, 0.0802)	0.901 (674)	0.368	0.186

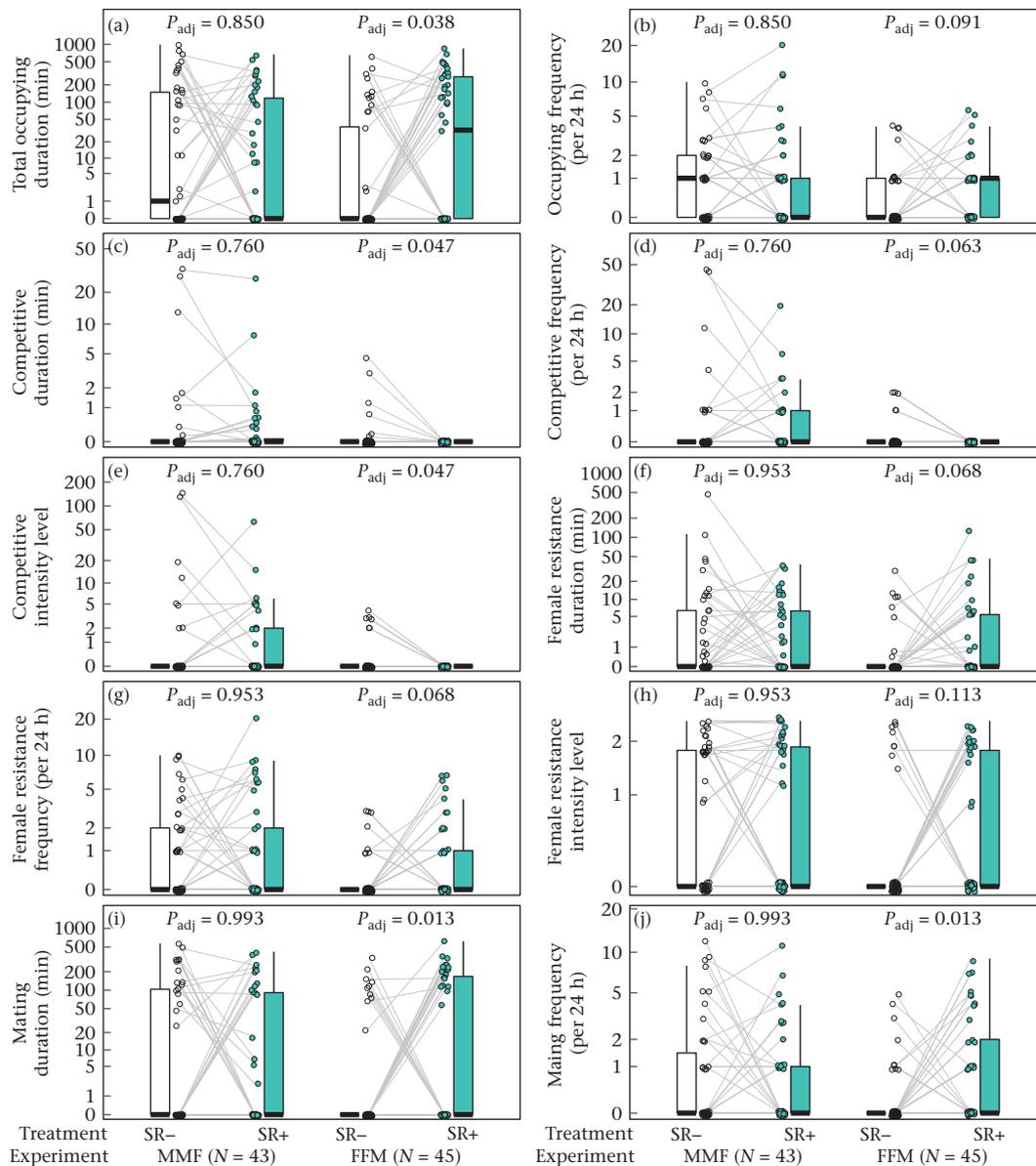


Figure 4. Comparison of the differences in mating behaviours between weevils in the spot-remaining (SR+) and spot-removed (SR-) treatments. (a) Total occupying duration, (b) occupying frequency, (c) competitive duration, (d) competitive frequency, (e) competitive intensity level, (f) female resistance duration, (g) female resistance frequency, (h) female resistance intensity level, (i) mating duration and (j) mating frequency. Points linked to each other indicate the outcomes in the same trial. Thick lines, boxes and whiskers indicate the medians, 25%/75% percentiles and $1.5\times$ interquartile ranges, respectively.

level ($Z = 2.448$, $P_{\text{adj}} = 0.047$; Fig. 4e), but SR+ and SR- females did not differ significantly in competitive frequency ($Z = 2.235$, $P_{\text{adj}} = 0.063$; Fig. 4d). In terms of female resistance, female resistance duration ($Z = 2.048$, $P_{\text{adj}} = 0.068$; Fig. 4f), resistance frequency ($Z = -1.999$, $P_{\text{adj}} = 0.068$; Fig. 4g) and intensity level ($Z = -1.592$, $P_{\text{adj}} = 0.113$; Fig. 4h) were not significantly different between SR+ and SR- females. Finally, at the mating success stage, the total mating duration ($Z = -2.499$, $P_{\text{adj}} = 0.013$; Fig. 4i) and mating frequency ($Z = -2.473$, $P_{\text{adj}} = 0.013$; Fig. 4j) of SR+ females were significantly higher than those of SR- females.

Comparing the competition stage of MMF and FFM groups showed that the competitive duration difference ($Z = 6.160$, $P_{\text{adj}} < 0.001$; Fig. 5a), frequency ($Z = 6.076$, $P_{\text{adj}} < 0.001$; Fig. 5b) and intensity level ($Z = 3.842$, $P_{\text{adj}} < 0.001$; Fig. 5c) were significantly higher in male–male competition than in female–female

competition. Therefore, we considered that the potential intra-sexual competition pressure was higher for males than for females.

Prey Choice Experiment

To test whether male and female lizards respond differently to prey choice (Lee et al., 2018), we compared the prey preference of males and females separately. In total, we collected 82 female and 132 male lizards for the prey choice experiment. Since 37 female lizards and 52 male lizards did not attack weevils or mealworms, these data were excluded from analyses. According to the data in which the lizard attacked either weevils or mealworms, we found that both female and male lizards significantly preferred to attack cryptic weevils compared with conspicuous weevils (female lizards: 37.8% versus 4.44%, odds ratio = 8.910, $N = 45$, $Z = 2.898$,

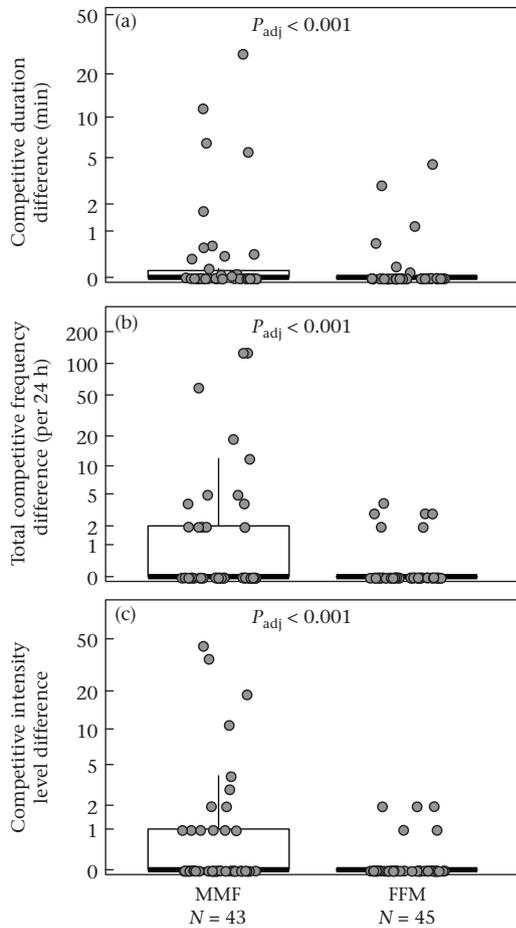


Figure 5. Comparisons of (a) competitive duration differences, (b) competitive frequency differences and (c) total competitive intensity level differences between male–male–female (MMF) groups and female–female–male (FFM) groups. Thick lines, boxes and whiskers indicate the medians, 25%/75% percentiles and 1.5× interquartile ranges, respectively.

$P = 0.004$; male lizards: 21.3% versus 8.75%, odds ratio = 2.519, $N = 80$, $Z = 2.009$, $P = 0.045$; Fig. 6). Although female lizards preferred to attack weevils more than males (42.2% versus 30.0%), we found no evidence that lizard attack preferences differed significantly between the sexes (likelihood ratio test: $\chi^2_2 = 4.273$, $P = 0.118$). We also found that lizard SVLs had little effect on attack preferences (marginal likelihood ratio test: $\chi^2_2 = 0.726$, $P = 0.696$).

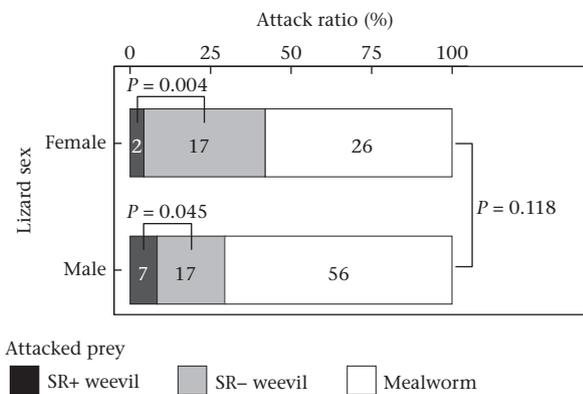


Figure 6. The results of the prey choice experiment of with female and male lizards. Arabic numbers shown inside the columns indicate the empirical number of lizards. SR+: spot-remaining treatment; SR-: spot-removed treatment.

DISCUSSION

Our study demonstrated that the spots on *E. waltonianus* appeared to play an important role in survival and reproduction, and other traits, such as body mass and body length, also affected mate choice depending on sex. Duller individuals were more frequently attacked by lizard predators and had a lower chance of mating due to the absence of conspicuous coloration. Weevils become less conspicuous with age and dull weevils seem to face negative selection pressure through predation. It is therefore expected that predation pressure would eliminate dull individuals from the wild; nevertheless some dull individuals can be observed in the field. A possible explanation may be that predators integrate different cues to recognize prey, such as walking behaviour and shape (Lee et al., 2018), and as a result, duller weevils may be able to persist in wild populations.

Conspicuousness Represents Reproductive Fitness

Conspicuous coloration of females not only functions as an attraction signal, but is also correlated with individuals’ reproductive success (clutch size and fledgling success in birds; Amundsen, Forsgren, & Hansen, 1997; Doutrelant et al., 2008; Gladbach, Gladbach, Kempnaers, & Quillfeldt, 2010). In *E. waltonianus*, colourful males were preferred in the field experiment. Compared to SR- individuals, SR+ females were more attractive to conspecifics and more successful in courtship in the laboratory experiment (Fig. 4a, i, j). In the laboratory experiment competitors did not differ in morphological traits (body weight, thorax length, elytra length and spot area), except coloration. Therefore, conspicuous spots on *E. waltonianus* was the only characteristic that differed systematically between treatments. More conspicuous individuals with larger spot areas are likely to be younger than weevils with dull coloration, because the scales are easily abraded over time. Based on captive-breeding experiments with *Pachyrrhynchus sarcitis*, newly emerging weevils are soft with bright coloration (Huang et al., 2018). Therefore, younger *E. waltonianus* are more conspicuous and older individuals are duller and body coloration may reflect the age of weevils.

Age can influence the reproductive ability of both sexes in various species (Arnqvist & Nilsson, 2000; Burley & Moran, 1979; Gibbons & McCarthy, 1986). In the hide beetle, *Dermestes maculatus*, females that mated with intermediate-age males laid more eggs and had higher fertilization success than with younger or older males (Jones & Elgar, 2004). It has also been confirmed in the Mexican fruit fly, *Anastrepha ludens*, that older or sexually experienced males are more competitive and may maintain higher-quality ejaculate than younger males (Pérez-Staples et al., 2010). Xu and Wang (2009) showed that male *Ephestia kuehiella* preferred younger females, and the fecundity and fertility of younger females were significantly higher than those of older females. In our study, *E. waltonianus* may distinguish the age of mates through the size of the spot area, and mate with younger individuals to increase fitness.

Besides the age effect, conspicuous coloration may be correlated with other body qualities (Lim & Li, 2007; Peters, Delhey, Goymann, & Kempnaers, 2006; Siefferman, Hill, & Dobson, 2005). For example, the conspicuousness of aposematic signals on strawberry poison frogs is positively correlated with toxicity, and more colourful individuals are more toxic (Crothers & Cummings, 2013). In some other species (e.g. marine opisthobranchs, Cortesi & Cheney, 2010; ladybirds, Arenas, Walter, & Stevens, 2015), the aposematic signals not only reflect higher toxicity, but also effectively defend against predators and increase the survival rate. The scales on moths can display colours that not only protect the moths from sticking to spider webs (Eisner, Alsop, & Ettershank, 1964), but also

absorb the ultrasonic echolocation calls emitted by bats (Zeng et al., 2011). In *E. waltonianus*, the colourful markings functioned as aposematic signals and lizards spat out these weevils after attacking them (Tseng et al., 2014). The hardness of weevils plays a secondary defence role against predators (Wang, Huang, Tang, Huang, & Lin, 2018). Therefore, conspicuous coloration represented the predator defence capability of individual weevils.

Effect of Size on Mate Choice in the Field

Females used body mass and coloration as indicators to choose males, and male mate choice depended on the body mass and length of females. Although coloration is usually an important trait in sexual selection, this trait may change in different environments. For instance, the environment may affect the conspicuousness of individuals. The spots on weevils are made of scales, like the scales on butterflies but attached on the elytra more tightly. Therefore, the scales can be rubbed off the elytra. If weevils inhabit dense forest, their body may frequently rub on leaves and twigs and their spots will abrade easily and become cryptic. Compared to colourful scales which may easily change, body length and weight may honestly reflect the individual's quality in the field, such as potential reproductive abilities (Wickman & Karlsson, 1989). Xu and Wang (2009) demonstrated that heavier female *E. kuehiella* had higher reproductive ability and higher fecundity (mean number of eggs) and fertility (mean number of fertile eggs) than lighter females. Besides reproductive potential, larger or heavier individuals are often dominant during competition and mate choice or have higher mating success (Jaffe, Mirás, & Cabrera, 2007; Kraak & Bakker, 1998; McElligott et al., 2001). Our field study showed that male *E. waltonianus* preferred heavier and longer females, which may indicate that heavier females have better potential reproductive abilities.

Comparing the experiments in the field and in controlled laboratory conditions, it seems that both males and females used different traits to evaluate their potential mates under the different conditions. In the wild, *E. waltonianus* encountered each other easily because they aggregated on host plants. However, in the manipulation experiment, there was a choice of only two weevils, one with or one without markings. The differences in the results between the two experiments may be because of density-dependent mate choice. Many studies have shown that mate choice plasticity is affected by conspecific density (Atwell & Wagner Jr, 2014; Fowler-Finn & Rodríguez, 2012; Lehmann, 2007; Rhainds, Gries, & Mat Min, 1999), and females become more choosy in a low male density environment (Atwell & Wagner Jr, 2014; Lehmann, 2007), even changing their preferred male traits (Fowler-Finn & Rodríguez, 2012). In addition to density-dependent mate choice, other factors, such as the previous mating experience of weevils in the wild, may also affect the mating preference of *E. waltonianus*.

Sexual Competition and Interference in Mating

Sexual interference is a common strategy in reproduction. Many species show that intrasexual interference could effectively increase mating opportunities for unmated individuals (Brown, Chimenti, & Siebert, 2007; Wey, Chang, Fogarty, & Sih, 2014). In our study, SR—females significantly interfered with mating pairs in competitive duration (Fig. 4c) and competitive intensity level (Fig. 4e). Whether the competition level of female *E. waltonianus* was affected by the quality of the rival (i.e. younger females may lay more eggs and consume more resources) still needs to be confirmed by further study.

Both the field surveys and manipulation experiment in a controlled environment showed that the body mass, body length and colourful markings of *E. waltonianus* increased mating success. These traits might reflect better body quality and younger age, so weevils mating with conspicuous mates can increase reproductive fitness. In *E. waltonianus*, it seems that males and females rely on different morphological traits to choose their mates under different conditions. The conspicuous coloration of *E. waltonianus* provided multiple functional signals for deterring predators and attracting mates. Moreover, our study also demonstrated that *E. waltonianus* displayed higher male–male competition than female–female competition in the mating system.

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Appendix

Table A1

The Akaike information criterion (AIC) values and their differences of candidate models fitting mating probability of male and female weevils in the field

	Sequence	Independent variable set	AIC	Step Δ AIC
Male	1	BL + SMI + SAR	941.52	–
	2	BL + spline(SMI, DF = 2) + SAR	923.36	–18.16
	3	BL + spline(SMI, DF = 3) + SAR	920.27	–3.09
	4	spline(SMI, DF = 3) + SAR	918.35	–1.92
	5	spline(SMI, DF = 3) + spline(SAR, DF = 2)	917.41	–0.94
Female	1	BL + SMI + SAR	855.46	–
	2	BL + spline(SMI, DF = 2) + SAR	851.11	–4.35
	3	BL + spline(SMI, DF = 2)	849.58	–1.53
	4	BL + spline(SMI, DF = 3)	849.16	–0.42

Sequences indicate the order of stepwise variable entering/eliminating. BL: body length; SMI: scaled mass index; SAR: spot area ratio.

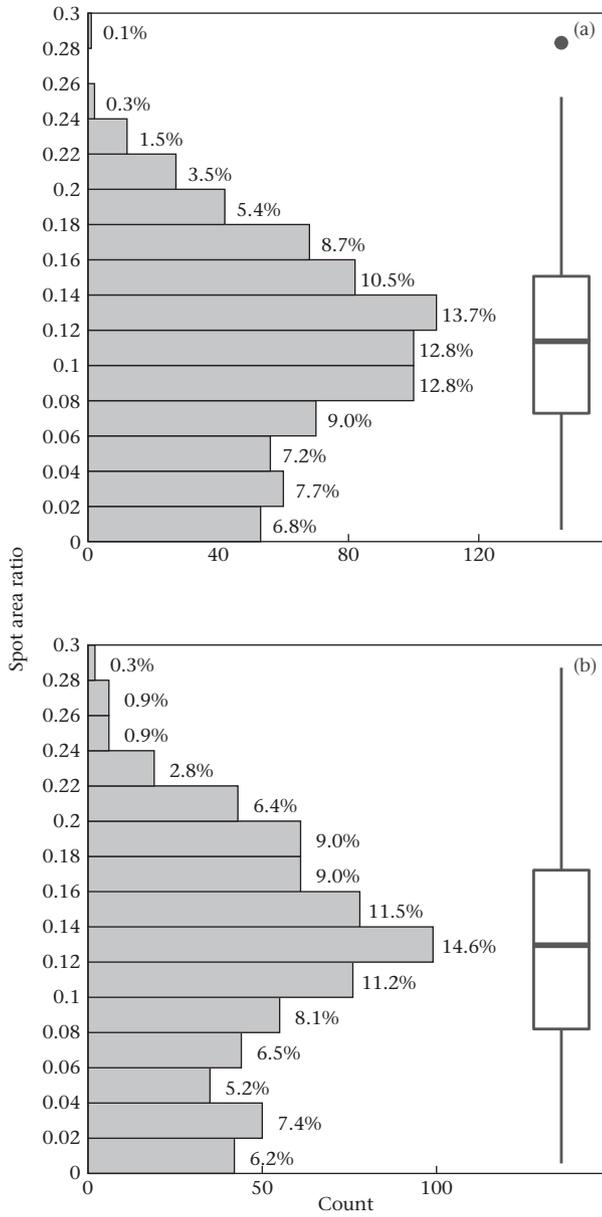


Figure A1. The empirical distribution of spot area ratio of (a) males and (b) females in the field. Thick lines, boxes and whiskers indicate the medians, 25%/75% percentiles and 1.5× interquartile ranges, respectively.

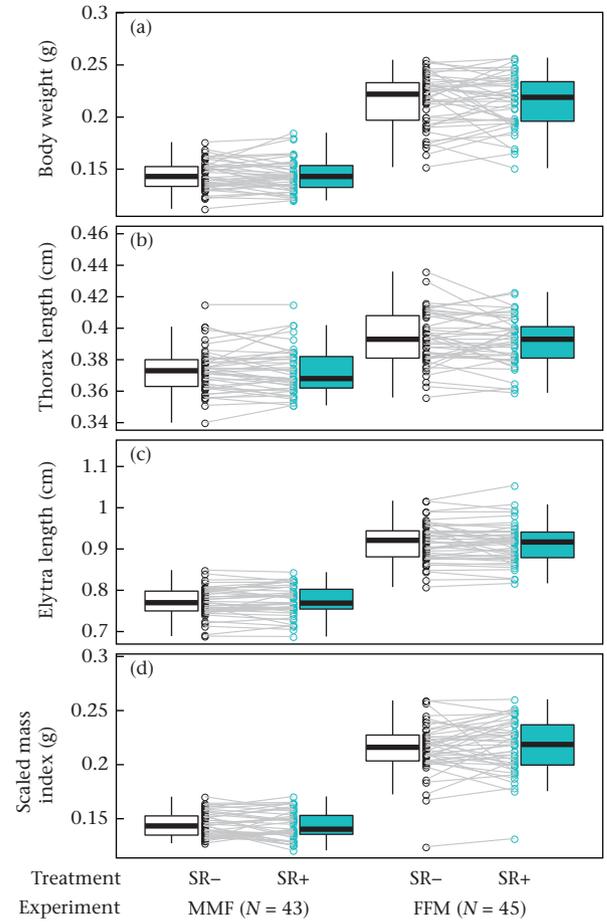


Figure A2. Comparisons of (a) body weights, (b) thorax lengths, (c) elytra lengths and (d) scaled mass indices. Linked points indicate the outcomes in the same trial. Thick lines, boxes and whiskers indicate the medians, 25%/75% percentiles and 1.5× interquartile ranges, respectively. Scaled mass indices as body condition indicators were the estimated body mass when body length was shifted to the average value (i.e. male = 1.145 cm and female = 1.308 cm). SR+: spot-remaining treatment; SR-: spot-removed treatment. MMF: male – male–female group; FFM: female – female–male group.