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It takes two to tango: functional roles, sexual selection and allometry of multiple male weapons in the flower beetle *Dicronocephalus wallichii bourgoini*

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Males of many insect species exhibit multiple sexually selected weapons, but the functional roles and evolution of them are poorly understood. The males of the flower beetle, *Dicronocephalus wallichii bourgoini* are equipped with exaggerated horns and elongated forelegs. To explore the selective forces acting on these two weapons, we investigated their functions by examining male–male competition and allometric relationships between the weapons and body size. We found that competition between mate-guarding (i.e. owners) and unpaired males (i.e. intruders) frequently occurred in the field. In the early phase of the contests, males mainly used their forelegs, likely, to assess the body size of their opponents. If the foreleg length of the owner was shorter than that of the intruder, the intruder approached to take over the owner's mate. Escalated contests occurred, in which both opponents tried to drag each other away from the females or the substrate using their horns. This suggests that the multiple weapons in this species are specialized for specific phases of contests. The males with larger bodies and weapons were more successful in defending their ownership of mates or taking over guarded females. The allometric slope of horns was positive in small males, but it decreased in large males. In contrast, male forelegs exhibited isometry without a switch point, and the slope was significantly steeper than that of female forelegs. Our findings suggest that sexual selection acts on both male weapon traits in *D. w. bourgoini* but that antagonistic natural selection constrains the further exaggeration of these traits in different ways.

ADDITIONAL KEYWORDS: alternative reproductive tactics – combat – intrasexual selection – rose chafer – Scarabaeidae – sneaking copulation.

INTRODUCTION

Males of several animal species engage in fights with conspecific males for access to females or limited resources that attract females. This intrasexual competition favours the exaggeration of sexually dimorphic weapon traits such as the antlers of deer and horns of rhinoceros beetles (Darwin, 1871; Andersson, 1994). The fighting can incur costs for contestants in terms of expenditures of time and energy and risks of injuries. During fighting, males gather information about the costs and benefits of persisting in the contest to make strategic decisions about whether to withdraw from the contests (Maynard Smith & Parker, 1976). If contestants can assess the resource-holding potential (RHP) of their own and the opponents (mutual assessment), they will adjust their behaviour based on the estimated differences in RHP (Enquist & Leimar, 1987). Alternatively, if they can only evaluate their RHP (self-assessment), they will persist in contests according to their own RHP (Taylor & Elwood, 2003). In mutual-assessment process, the assessment of opponents is based on traits correlated with RHP. Male weapon traits often function as tactile or visual signals of RHP (Hyatt & Salmon, 1978; Hongo, 2003). With greater discrepancy of weapon size between contestants, costly physical fighting is less likely to occur (Enquist & Leimar, 1987; Snell-Rood & Moczek, 2013). On the other hand, when the size difference is minimal between opponents, and the rival males increasingly show high-intensity behaviours, the contests are likely to escalate towards more energetic behaviours,

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*Corresponding author. E-mail: koj.wataru@gmail.com & treehopper@ntnu.edu.tw and then the male sexual traits will function as armaments (Enquist & Leimar, 1987, 1990; Snell-Rood & Moczek, 2013).

The scaling relationships between weapon traits and body size have been of major interest because they provide great insight into the evolutionary pressure acting upon these traits (Eberhard & Gutierrez, 1991; Bonduriansky & Day, 2003; Tomkins, Kotiaho & LeBas, 2005; Kodric-Brown, Sibly & Brown, 2006; Knell, 2009). Secondary sexual traits, including animal weapons, typically show positive allometry, whereby larger males have disproportionally larger weapon traits than smaller males (Kodric-Brown, Sibly & Brown, 2006). Larger males are thought to gain a greater fitness return by investing resources into the traits than smaller males (Kodric-Brown, Sibly & Brown, 2006; Bonduriansky, 2007). On the other hand, the theoretical model by Bonduriansky & Day (2003) suggests that sexually selected traits can exhibit isometry or negative allometry depending on the precise nature of the net selection on body size and trait size. Some sexually selected traits, including the forceps of earwigs (van Lieshout & Elgar, 2009) and the forelegs of fruit flies (Eberhard, 2002), do not show positive allometry. These traits may be candidates for antagonistic natural selection against higher allometric values (Eberhard, 2002; van Lieshout & Elgar, 2009).

Furthermore, the allometric relationship between body size and weapon size deviates from simple linearity in some holometabolous insects (Nijhout & Wheeler, 1996; Knell, 2009). The nonlinear patterns include curvilinear, sigmoid and discontinuous allometries (Knell, 2009). For example, in some stag beetles, male mandibles show a smoothly curvilinear allometry in which individuals with relatively larger mandibles have slopes that show greater declines with increasing body size (Huxley, 1931; Knell, Pomfret & Tomkins, 2004). This pattern is mainly explained by the depletion of resources available for the rapid development of the weapon within the closed pupal environment (Nijhout & Wheeler, 1996; Knell, Pomfret & Tomkins, 2004; Pomfret & Knell, 2006). Because the largest traits are most likely to be affected by resource depletion, the slope for weapon/body size allometry is thought to decrease as body size increases. Another example is sigmoid allometry in horns of dung beetles *Onthophagus* spp. (Tomkins, Kotiaho & LeBas, 2005; Emlen *et al.*, 2005; Emlen, Lavine & Ewen-Campen, 2007). This is likely an adaptation for size-dependent reproductive strategies (Emlen, 1997). Obviously, the large males are specialized for fighting, whereas small males are specialized for sneak copulation (Emlen, 1997; Moczek & Emlen, 2000).

The weapons of beetles take a variety of forms, such as horns, mandibles, the rostra and elongated forelegs (reviewed in Emlen, 2008). Among them, the morphology and function of the horns of scarab beetles and the mandibles of stag beetles have attracted considerable attention (e.g. Huxley, 1931; Siva-Jothy, 1987; Tatsuta, Mizota & Akimoto, 2001; Knell, Pomfret & Tomkins, 2004; Hongo, 2007; Sugiura, Yamaura & Makihara, 2007; Emlen, 2008; McCullough, Tobalske & Emlen, 2014; McCullough *et al.*, 2015; Romiti *et al.*, 2015), but little is known about the functional significance of other types of weapon traits (Eberhard, 1977; Zeh, Zeh & Tavakilian, 1992; Painting & Holwell, 2013, 2014). Elongated male forelegs have evolved repeatedly in various beetle lineages, such as stag beetles (e.g. *Chiasognathus* spp., *Casignetus* spp.), long-armed scarabs (Euchirinae), flower beetles (e.g. Goliathini, *Dicronocephalus* spp., *Phaedimus* spp., *Theodosia* spp.), weevils (e.g. *Gasterocercus* spp., *Cyrtotrachelus* spp.), longhorn beetles (e.g. *Acrocinus* spp., *Batocera* spp.) and rhinoceros beetles (e.g. *Chalcosoma* spp., *Golofa* spp., *Megasoma* spp.). Furthermore, many of them are already equipped with enlarged weapons on their heads for fighting, such as mandibles and horns (e.g. *Chiasognathus* spp., *Casignetus* spp., Goliathini, *Dicronocephalus* spp., *Chalcosoma* spp., *Golofa* spp., *Megasoma* spp.). The co-occurrence of these male traits raises the intriguing questions of what specific roles these male traits play in intraspecific competition and whether the forelegs, mandibles and horns are functionally related and thus represent correlated evolution. Nevertheless, few studies have examined in detail how these multiple weapons are used in male–male contests under natural conditions (e.g. Eberhard, 1977). Given that the diversity in weaponry reflects specific fighting styles (McCullough, Tobalske & Emlen, 2014), it is important to clarify the behavioural and ecological contexts in which these male traits are used to understand the evolution of multiple weapons.

The present study investigated the allometry and sexual selection of elongated forelegs and horns in the Taiwanese flower beetle, *Dicronocephalus wallichii bourgoini* Pouillaude 1914 (Coleoptera: Scarabaeidae: Cetoniinae). This species is distributed in the lowland forests of Taiwan. Adult individuals of *D. w. bourgoini* aggregate at sap sites or around the fruits of host trees during the daytime. This species is univoltine, and adults appear from April to June (Šípek, Král & Jahn, 2008). The males of this species have a pair of horns on their heads, and their forelegs are longer than those of females. Laboratory observation by Šípek, Král & Jahn (2008) suggests that these two traits are used in different phases of male–male contests, but the morphology and detailed function of these male traits in their habitats have not been examined. In this study, we first explored the functional role of the two secondary sexual traits and the patterns of fighting escalation under natural conditions. Second, we examined the effect of weapon and body size on mating success by testing three specific predictions from the hypothesis of sexual selection for exaggerated weapons: (i) the body size of males paired with females is larger than that of unpaired males; (ii) male body size is positively correlated with mate-guarding duration; (iii) larger males are more likely to win fights than smaller males. Finally, we examined whether forelegs and horns exhibit positive or negative allometry to assess the strength of sexual selection on these traits.

MATERIAL AND METHODS

STUDY SITE

The field study was conducted in a lowland forest in the district of Tamsui, Taipei, Taiwan (25°11′N, 121°29′E), from 1 May to 4 June 2016. At this site, *D. w. bourgoini* adults (Fig. 1A) feed on the fruits of various plant species, including cherry, hoe and arrowwood, during the daytime. They aggregate on bamboo shoots in a bamboo forest of approximately 500 m^2 at the study site, where they mate and feed on the sap by biting the shoots using their mandibles. All field and laboratory methods were conducted following the animal ethics protocols of the current Taiwanese Wildlife Conservation Act, and a research permit was issued and supported by the New Taipei City Government Animal Protection and Health Inspection Office (No. 1053063951).

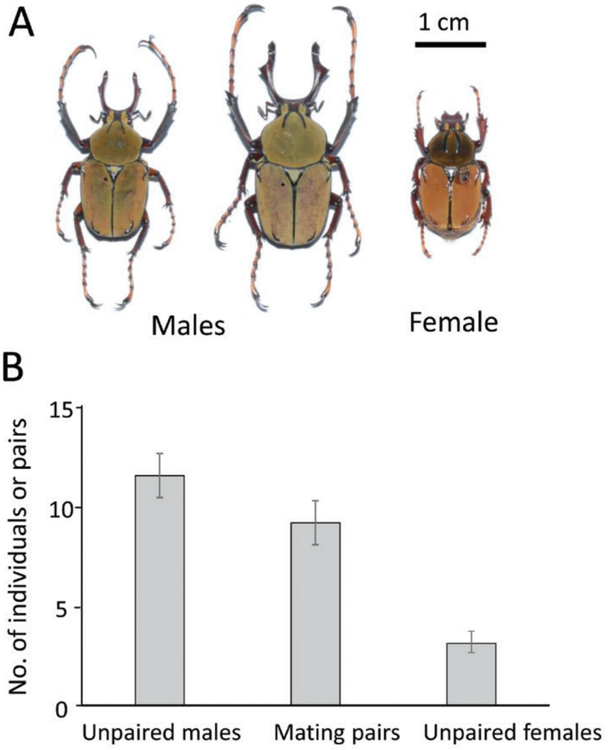

Figure 1. (A) A female, a small male and a large male of *Dicronocephalus wallichii bourgoini*. (B) The number of unpaired males, paired males and unpaired females (*M* ± SE) of *D. w. bourgoini* over 5 days and 33 counting events.

FIELD OBSERVATIONS

The behaviour of the beetles on bamboo shoots was observed using 8 × 42 binoculars (Nikon Monarch, Nikon Corp., Tokyo, Japan). The distance between the observer and the beetles was approximately 5 m. The observation was conducted on sunny days every 1–3 days between 1 May and 7 June 2016 (16 days in total). We observed the beetles between 7:30 and 16:30 if the weather conditions permitted; however, the observations were terminated around noon on most days due to heavy rains.

Mating behaviour

We predicted that a male-biased sex ratio at the feeding and mating site would drive intense male–male competition in this species. To test this prediction, we visually counted the number of all mating pairs, unpaired males and unpaired females of *D. w. bourgoini* on focal bamboo shoots every hour after 7:30. Flying individuals were excluded. The counting was conducted over 5 days between 9th and 21st of May, and the number of counting events was 33 in total throughout these days.

Males of this species guard females after copulation, but we categorized both mate-guarding and copulating pairs as 'mating pairs' in the field for the following reasons. First, we could not always watch the beetles close enough to discriminate between the two postures. Second, the males were sometimes observed to insert genitalia during mate guarding. In addition, genital insertion was frequently interrupted by disturbance from other males. Thus, it was not practical to clearly define their mate-guarding and copulating behaviour in the field.

Sequence of contests and functions of male weapons

To determine the functions of the male weapons, we examined the behavioural sequence of contests. We first recorded videos of 72 male–male contests using a digital video camera (HDR-SR7, Sony, Japan) on the initial days of the field observation. Based on the videos, we identified distinctive behavioural elements. These elements were mutually exclusive and classified into state or point events (Table 1). The interactions where contestants used forelegs and horns were classified into 'low-intensity' and 'high-intensity' behaviour, respectively. Since the second day of the field observation, the transition between these behavioural

Table 1. Behavioural elements of male–male fighting in *Dicronocephalus wallichii bourgoini*

Behavioural element	Description	Intensity level	Behaviour type
Sense orientation	The owner perceives an approaching intruder and turns his body toward him	Low	Point
Tapping	The owner stretches and taps the opponent's body with his forelegs	Low	State
Push	The owner pushes the intruder with his horns and forelegs from behind	High	State
Intruder escape	The intruder escapes from the owner	–	Point
Pry	The owner tries to pry the intruder away from substrate or his mate using his horns and forelegs	High	State
Flip	The owner flips away the intruder	High	Point
Fall with intruder	The owner falls onto the ground with an intruder during combat	–	Point
Owner flipped	The owner is flipped by the intruder	High	Point
Fall with female	The owner falls onto the ground with his mate while struggling with the intruder	–	Point
One escapes*	One opponent escapes	–	Point
One flips*	A male flips away an opponent	High	Point
Both fall together*	Two males fall together during the fight	–	Point

*Behavioural elements for interactions between two unpaired males.

elements has been recorded from encountering of contestants to the end of the contests. All interactions occurring on the focal bamboo shoot were recorded if possible; however, if multiple interactions occurred simultaneously, we recorded a randomly chosen interaction. We also set up the video camera attached to a tripod to record the interactions that occurred on another bamboo shoot when multiple bamboo shoots were preferred. In total, we analysed 330 cases of owner–intruder interactions and 138 cases of interactions of unpaired males.

SIZE ADVANTAGES IN COMPETITION

To explore the advantages of having a large body with large weapons, we conducted three experiments in both field and laboratory.

(i) Comparison of body / weapon size between paired and unpaired males

We compared the body/horn/foreleg size between paired and unpaired males collected in the field. We randomly collected unpaired (i.e. solitary) and paired (i.e. mate-guarding or copulating) males on the bamboo shoots between 12:00 and 14:00 once every observing day, except for the 5 days when we conducted beetle counting. The number of males collected each day was less than ten (<30%) to minimize the impact of decreased insect density on subsequent behavioural observations at the site. We collected 42 unpaired males and 35 paired males in total, and kept them in a freezer for morphological measurement.

The pronotum width and horn length from the base to tip were measured using a digital calliper to the nearest 0.01 mm. The pronotum width is the most commonly used index of body size of scarab beetles (e.g. Eberhard & Gutierrez, 1991; Emlen, 1997; Tomkins, Kotiaho & LeBas, 2005). For the measurement of the length of the forelegs, midlegs and hindlegs, each right leg was dissected and fixed onto a sheet of white paper with cellophane tape. The legs were photographed, and the length from the femur to tarsus was measured using ImageJ software (http://imagej.nih.gov/ij) (Fig. S1).

(ii) Body size and mating duration

We examined the relationship between male body, foreleg and horn size and the mating duration (i.e. sum of mate-guarding and copulating duration) in the field. To measure the mating duration of each male, we observed the initiation of copulation, and continued the observation of the pair until they separated. After separation, we immediately caught the male of the pair. Its pronotum width, horn length and foreleg length were measured using a digital calliper to the nearest 0.01 mm. The measured foreleg length was the sum of the lengths of the tibia and tarsus of the forelegs instead of total length (i.e. femur to tarsus) (Fig. S1) because we needed to measure the morphology of living insects (see following paragraph) and it was impossible to measure the total length of legs without dissecting the beetles. We recorded mating duration of 14 pairs and measured morphological traits of seven males. Additionally, we observed 16 artificially staged pairs on the bamboo shoots to increase the sample size of paired beetles.

For this experiment, we collected males and females at the study site and measured the three morphological traits by a digital calliper in the laboratory before the staged trial. We marked each individual (male and female) on the pronotum and elytra with three or four small dots of ink in different colour combinations to identify the beetles in the field. Each beetle was placed in a 60 mL plastic cup and fed with insect jellies for 1–3 days. They were returned to the bamboo shoots at the study site, and one male and one female were placed together in a cage on the site at 9:00–11:00. They usually began copulation soon upon release from the cage. The copulating pairs were immediately moved to 1.5–2.5 m high on the bamboo shoots, where many beetles aggregated.

(iii) Size difference and outcome of contests

We examined the relationship between the difference in trait size between owner and intruder and the outcome of contests under laboratory conditions. A male (i.e. owner) marked with permanent marker and a female collected from an arrowwood fruit at the study site were introduced to a plastic cage (27 × 20 × 20 cm height). In the cage, a wood stick (*c.* 20 cm in length and 3 cm in diameter) was set up vertically, and the bottom of the cage was covered with tissue papers. A small piece of insect jelly was coated onto the middle of the stick as a feeding site. After mating occurred (usually at the feeding site), another male (i.e. intruder) marked with a different colour was introduced to the bottom of the same cage. We staged 28 pairs to record the behavioural sequence of the contests between the owner and intruder until the end of the contests. If there were no interactions within 1 h after the introduction of an intruder, these males were excluded from the analyses; however, such cases were rare.

We tested whether relatively larger owners were more likely to deter the intruders using foreleg tapping alone (i.e. ritualized behaviour in the early phase of contests; see also Table 1 and Results). We also tested whether the mates of relatively smaller owners were more likely to be taken over by the intruder.

Allometry analyses

We collected females (N = 85) and males (N = 245) of *D. w. bourgoini* that aggregated on the fruits of arrowwood at the study site. The trees were about 100 m away from the bamboo forest where we conducted the behavioural observations. Thus, the collection of beetles was less likely to directly affect the density and behaviour of the beetles in the bamboo forest. The beetles were kept in a freezer after collection. The pronotum width and the lengths of the right horn (in the case of males) and the three right legs from the femur to the tarsus were measured following the previously mentioned procedure (*comparison of body / weapon size between paired and unpaired males*).

To test the linearity of the allometric relationship between body size and trait size, we adopted the following model (Eberhard & Gutierrez, 1991):

$$\ln Y = \alpha_0 + \alpha_1 \ln X + \alpha_2 (\ln X)^2 + \varepsilon \left(\text{Model 1}\right)$$

where ln Y is the log-transformed trait size (i.e. horn length or leg length), ln X is the log-transformed pronotum width, α_1 is the regression coefficient, and ε is the residual error. If the coefficient α_2 differed significantly from zero, further analysis was conducted using another model (Eberhard & Gutierrez, 1991), as the regression was considered to be nonlinear.

$$\ln Y = \beta_0 + \beta_1 \ln X + \beta_2 \left(\ln X - \ln X_0\right) D + \beta_3 D + \varepsilon \left(\text{Model 2}\right)$$

where ln Y is the log-transformed trait size (i.e. horn length or leg length), ln X is the log-transformed pronotum width, ln X_0 is the proposed switch point value, $D = 0$ if $\ln X < \ln X_0$ or otherwise $D = 1$ and β_i is the regression coefficient. The optimal value of ln X_0 was determined to give the maximum adjusted R^2 value calculated following the method of Sugiura, Yamaura & Makihara (2007), and this value was fitted to Model 2 to give the regression coefficients. If β_3 was significantly different from zero, then the allometry was discontinuous at ln X_0. If the value of β_2 was significantly different from zero, the slope changed at the point.

We also examined the allometric slope between a trait and the body size using the equation $\ln Y = \alpha \ln X + \ln a$ (Model 3), a linearized form of $Y = aX^{\alpha}$, in which Y is the trait size (i.e. horn length or leg length) and X is the pronotum width (Huxley, 1931). When $\alpha > 1$, large individuals had disproportionally larger traits relative to their body size (positive allometry). When $\alpha = 1$, the trait was proportionally constant regardless of the body size of the individual (isometry). When $0 < \alpha < 1$, the traits of large individuals were comparatively smaller in relation to their body size (negative allometry).

Statistical analyses

All statistical analyses were conducted using R ver. 3.1.1 (R Development Core Team, 2013). For the transition analysis of contests, chi-square statistics were used to test non-randomness in the behavioural patterns. Freeman–Tukey deviates were calculated for each transition to identify the transitions occurring more frequently than expected by chance (Goodman, 1968). The pronotum width, foreleg length and horn length between unpaired and mating males were compared using *t* tests. These measurements were subjected to Shapiro–Wilk tests and *F* tests to check for normality and homoscedascity, respectively. The relationship between the male body/horn/foreleg size and mating duration was tested using the Cox proportional hazards model (Cox, 1972). We also tested whether small males were more likely to have their mates taken over using simple logistic regression (owner loss = 1, win = 0). The relationships between the difference in the body/horn/foreleg size (owner–intruder trait size) and the intruder's response to the owner were examined using simple logistic regression. If the intruder retreated in response to tapping by the owner, the response of the intruder was defined as 1. If the intruder ignored the tapping and approached the owner, his response was defined as 0. To test whether the mates of relatively smaller owners were more likely to be taken over by the intruder, the relationships between the difference in the body/horn/foreleg size (owner–intruder trait size) and the incidence of takeover were examined using simple logistic regression (owner loss = 1, win = 0).

The significant difference between the allometric coefficients for traits and the slope value of 1 of the isometry was tested (Warton *et al.*, 2012). Sexual difference in mean length of forelegs, midlegs and hindlegs was tested using Welch *t*-test after checking for normality and homoscedascity. To test the sexual difference in allometric slopes of forelegs, midlegs and hindlegs, we first obtained 1000 bootstrapped slopes for males and females. Secondly, sexual differences of the slopes were obtained for each bootstrap iteration. Finally, we tested if zero was included within the 95% confidence interval of the bootstrap distribution.

RESULTS

BEETLES OBSERVED ON BAMBOO SHOOTS

Dicronocephalus wallichii bourgoini aggregated on bamboo shoots at a height of *c.* 0.5–4 m. They preferred the shoots prior to the leafing stages. When the bamboo shoots began to extend foliage, the number of *D. w. bourgoini* on the shoots decreased. The preferred trees changed over a short time span (5–7 days) according to the growth of bamboo. There was at least one preferred bamboo shoot throughout the study period.

The number of unpaired females, males and mating pairs on the bamboo shoots changed throughout the day, but the pattern of transition was different among days. The total number of beetles gradually increased from morning to noon (Fig. S2A). The number of unpaired males and mating pairs was similar (*M* ± SE, 11.5 ± 1.1 and 9.14 ± 1.1 per counting event, respectively) but much larger than that of unpaired females (3.11 ± 0.6) (Fig. 2B). The sex ratio and the number of individuals also varied among days, but did not show any specific pattern over the five observing days (Fig. S2B).

CONTESTS

Interactions between mate-guarding males and unpaired males

We observed 330 interactions between mate-guarding males (i.e. owner) and unpaired males (i.e. intruder) in the field. We identified 12 behavioural elements and classified them into 'high-intensity' or 'low-intensity' behaviour (Table 1). In 38 cases, additional unpaired males joined the fighting, and details are described in the subsequent section 'Sneaking behaviour'. The sequence of the behaviour of owners in the remaining 292 interactions is described in Fig. 2F.

The owner abruptly turned to an approaching intruder ('Sense orientation'), even when the intruder came from behind. The owner stretched and touched the intruder's body or forelegs and subsequently intensely moved his forelegs ('Tapping', Fig. 2A, F). The intruder also showed tapping with the forelegs in response to the owner's behaviour. After a few seconds of tapping, the intruder walked away from the owner in many cases ('Intruder escapes', Fig. 2F). If the intruder continued to approach and/or tried to mount the female, the owner pushed him away with his horns ('Push') or pried him away from the female's back or substrate with his horns ('Pry', Fig. 2B, F and Movies 1, 2). The forelegs of the owner moved intensely and touched the opponent's body during 'Push' and 'Pry'. The contests usually began with 'Tapping', but this step was occasionally skipped, especially when the intruders directly flew to the guarding males or dashed into them (Fig. 2F).

During 'Pry', the intruder resisted the owner's movements, and the two males tussled for a few seconds to one minute, holding each other's horns tightly (Fig. 2B, F). The intruder escaped ('Intruder escape', Movie 2) or was flipped away ('Flip', Movie 3) in the end. While the owner lifted the intruder's body to flip him, the intruder struggled to cling onto the owner's body. The owner rapidly moved his forelegs and midlegs to disengage the intruder's body from himself (Fig. 2C).

The owner was found to drive the intruder *c.* 10–50 cm away from his mate using the horns and forelegs during 'Push' or 'Pry' (Movie 3). In such cases, the owner returned to his mate immediately after winning the fight. Females frequently (49 out of 140 cases that escalated into 'Pry' or 'Push') tried to escape from the owner during the prolonged fighting (Table 2).

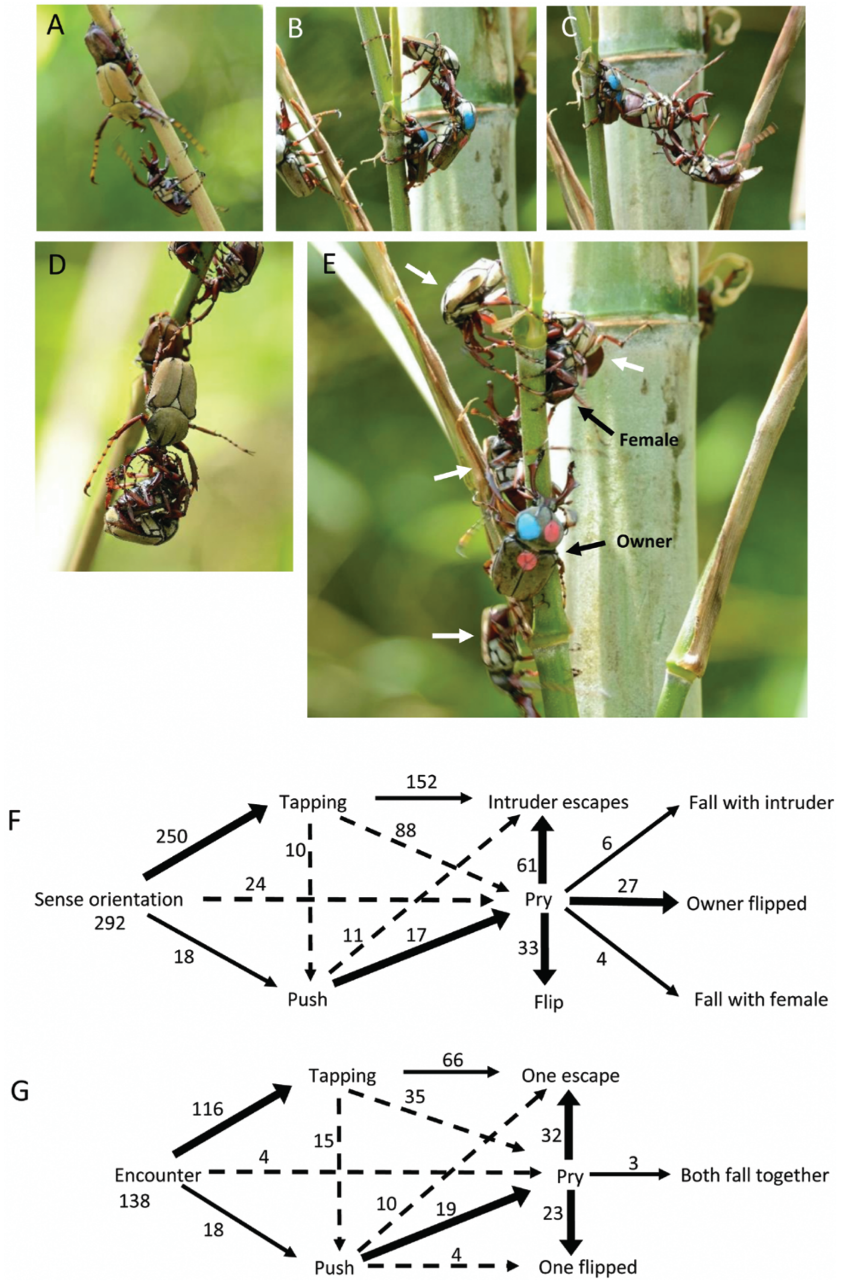

Figure 2. (A) A mate-guarding male (upper) extended his forelegs and tapped an intruder (lower) of *Dicronocephalus wallichii bourgoini* (Tapping; Table 1). The intruder also exhibited Tapping. (B) A mate-guarding male (lower) and an intruder (upper) engaged in a fight using their horns (Pry). The owner and his mate were marked on their backs with coloured ink for identification. (C) A mate-guarding male flipped away an intruder (Flip). (D) A mate-guarding male (upper) tried to flip another pair (lower). (E) Four sneakers/intruders (indicated by a white arrow) and a pair (the owner and his mate, indicated by a black arrow). His mate is also indicated by an arrow and was mounted by a sneaker/intruder. (F) The sequence of behavioural transitions in fights between owners and intruders. (G) The sequence of behavioural transitions in fights among unpaired males. The number of an observed behavioural element is indicated. Transitions that occurred more frequently than expected are indicated by arrows. Thick arrow: $P < 0.005$; thin arrow: $0.005 < P < 0.05$; dashed arrow: $P > 0.05$.

Females were usually re-captured by the owner, but we found that seven females successfully escaped before her owner returned (Table 2).

The owner was flipped away by the intruder, and takeover occurred ('Owner flipped') after 'Pry' (Fig. 2F). In some cases, the owner and intruder were

Table 2. Behaviour of females when their mates engaged in escalated fighting in *Dicronocephalus wallichii bourgoini*

Behaviour of females	Percentage of incidence
Keep staying	65 (91/140)
Try to escape but captured by the owner	30 (42/140)
Success in escape	5 (7/140)

Table 3. Outcome of sneaking males in *Dicronocephalus wallichii bourgoini*

Outcome of sneaking	Percentage of incidence
Fail	87 (33/38)
Success because the sneaker flips the owner	8 (3/38)
Success because the owner accidentally falls	5 (2/38)

found to fall together during escalated tussling ('Fall with intruder') (Fig. 2F). The owner accidentally fell together with his mate during the contests in other cases ('Fall with female') (Fig. 2F). The males were never obviously injured during fighting.

Interactions between two unpaired males

Unpaired males often walked around on the bamboo shoots or fed on the sap of bamboo and did not establish obvious territories. Nevertheless, a contest began when two unpaired males encountered one another. We observed 138 contests between two unpaired males. The sequence was highly similar to that of the interactions between unpaired males and guarding males (Fig. 2G). Briefly, 'Tapping' usually occurred at the initial stage, and one opponent often escaped after 'Tapping'. When the contests were escalated to the stage of 'Pry', one opponent was sometimes flipped away.

Other interactions

When an unpaired male encountered an unpaired female, the male mounted the female, and copulation began immediately without courtship. Females ($N = 23$) never showed refusing behaviour. In nine cases (33%), females were attacked by males with horns and forelegs, in which three females were flipped off.

We recorded four cases of female–female competition. The two females pushed each other with their heads until one opponent retreated, and flipping was not observed.

We also found that the owners of females tried to pry and flip away approaching unpaired conspecific females, conspecific mating pairs (Fig. 2D) and other flower beetles (*Anthracophora eddai* and *Protaetia* spp.).

Sneaking behaviour

We observed 38 cases of sneaking behaviour in males (Table 3 and Movies 3, 4). While an owner fought with an intruder, additional males (i.e. sneakers) flew or rapidly walked toward the female. The sneakers often fought against the males encountered (owner, intruder and/or other sneakers). The sneakers tried to copulate with the female when the owner left her to fight against the intruder or other sneakers (Fig. 2E). The mounting sneakers were usually dragged away by the former owner immediately after the former owner returned (Table 3). However, some of the mounting sneakers flipped the former owner and won the female (Table 3). We caught one of the successful sneakers and found that his pronotum width was 10.4 mm, which was much larger than the mean pronotum size for males (9.61 mm). Moreover, some sneakers successfully copulated with a female because the former owner male accidentally fell off with an intruder (or another sneaker) while fighting (Table 3).

SIZE ADVANTAGES IN COMPETITION

(i) Comparison of body / weapon size between paired and unpaired males

We compared the body/horn/foreleg size between paired and unpaired males. The pronotum width of paired males was significantly larger than that of unpaired males (Fig. 3; *t* test: $t = 5.62$, d.f. = 80, $P < 0.001$, Table S2 for raw data). The horn length and foreleg length were also significantly different between unpaired males and paired males ($t = 5.44$, d.f. = 80, $P < 0.001$ and $t = 5.47$, d.f. = 80, $P < 0.001$, respectively).

(ii) Body size and mating duration

The duration of male mate guarding/copulation was highly variable in nature, ranging from 2 to 215 min [Fig. 4A; 48 ± 10 min (M ± SE), $N = 30$, Table S1 for raw data], with a majority of these events lasting for less than 1 h (73%, 22/30). There was no significant difference in the mating duration between naturally occurring (37 ± 16 min, $N = 14$ pairs) and artificially staged pairs (58 ± 12 min, $N = 16$ pairs; Cox proportional hazards model, $\chi^2_1 = 1.16$, $P = 0.28$), and no obvious handling effect occurred in the staged pairs. There was no significant relationship between the mating duration and male body size (Cox proportional hazards model: $\chi^2_1 = 1.42$, $P = 0.23$), foreleg length ($\chi^2_1 = 3.33$, $P = 0.068$) or horn length ($\chi^2_1 = 1.18$, $P = 0.28$).

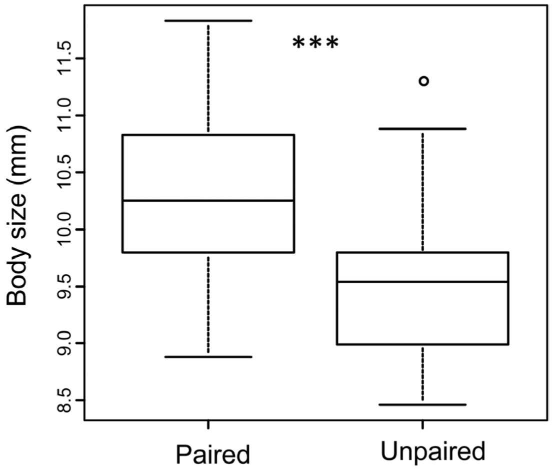

Figure 3. The body size (M ± SE pronotum width) of the paired and unpaired males of *Dicronocephalus wallichii bourgoini* collected randomly once a day. $^{***}P < 0.001$.

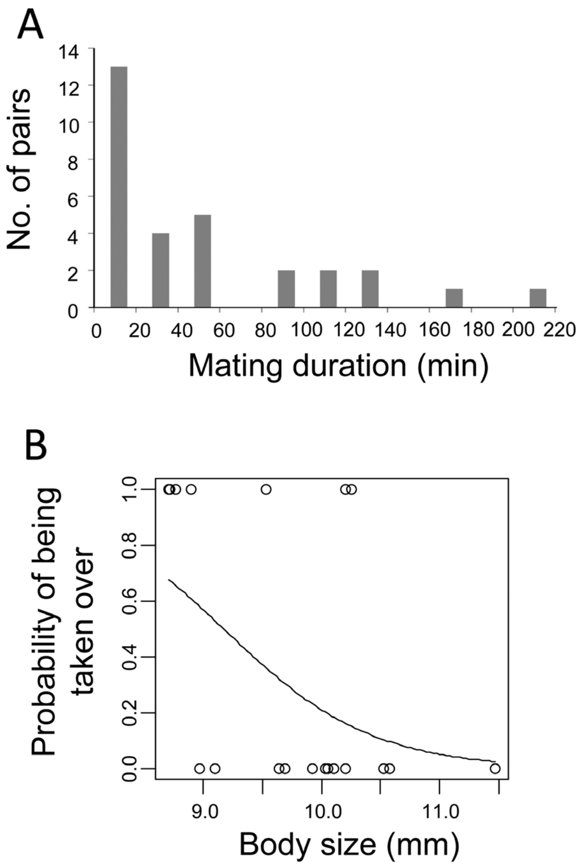

Figure 4. The analyses of mating behaviour of *Dicronocephalus wallichii bourgoini*. (A) A histogram of mating duration. (B) The relationship between male body size (pronotum width) and probability that the owner's mates were taken over by an intruder.

The factors that terminated male mate guarding/copulation are summarized in Table 4. The most frequent cause was female escape (11/30 cases). Most escapes occurred when the male owner was fighting with one or multiple intruder(s) (8/11 cases, 72%). The males searched for their mates after fighting but failed to capture them. There was no significant relationship between the incidence of female escape and male body size (logistic regression: $\chi^2_1 = 1.73$, $P = 0.19$). The second most frequent cause was the female being taken over by an intruder. There was a significant relationship between the incidence of being taken over and the owner's body size (Fig. 4B; logistic regression: $\chi^2_1 = 4.40$, $P = 0.036$), suggesting that smaller males were more likely to be overtaken during mate guarding/copulation. There were also significant and marginally significant relationships between the incidence of being taken over and foreleg length ($\chi^2_1 = 13.3$, $P < 0.001$) and horn length ($\chi^2_1 = 3.73$, $P = 0.053$), respectively. Some females slowly walked away from the owner when he was feeding or mounting, and males did not chase her, which is described as 'Pair naturally separates' in Table 4. In the remaining cases, pair separation was caused by accidental factors.

(iii) Size differences and outcome of contests

Three out of 28 cases where no interactions occurred were excluded from the analysis. Some intruders retreated in response to only tapping by the owners (Table 5). This type of response was more likely to occur when the foreleg length or body size of the owner was larger than that of the intruder (Fig. 5A; logistic regression: $\chi^2_1 = 5.29$, $P = 0.022$ for foreleg and $\chi^2_1 = 3.80$, $P = 0.048$ for body size, Table S3 for raw data).

Some intruders took over the owner's female after fighting (Table 5). Relatively larger intruders were more likely to be successful in taking over the females from the owners (Fig. 5B; logistic regression: $\chi^2_1 = 10.2$,

Table 4. The causes of separation of mating pairs and their frequency in *Dicronocephalus wallichii bourgoini*

Cause of separation	Percentage of incidence
Female escapes	37 (11/30)
Owner is thrown away by a rival male	30 (9/30)
Pair naturally separates	13 (4/30)
Pair is thrown away by *Rhomborrhina splendida*	6.7 (2/30)
Pair falls without disturbance	6.7 (2/30)
Pair is thrown away by a male	3.3 (1/30)
Owner falls with a male during combat	3.3 (1/30)

Table 5. Outcome of contests in the laboratory experiment

Outcome	Percentage of incidence
Intruder retreats by owner's intense behaviour	44 (11/25)
Intruder wins	32 (8/25)
Intruder retreats in response to tapping	24 (6/25)

$P = 0.001$). If we used foreleg length or horn length instead of body size in the analysis, significant relationships were also detected ($\chi^2_1 = 12.2$, $P < 0.001$ for foreleg and $\chi^2_1 = 16.0$, $P < 0.001$ for horn).

ALLOMETRY

For the relationships between body size and the length of forelegs, midlegs and hindlegs in both sexes of *D. w. bourgoini* (245 males and 85 females), the α_2 coefficients in Model 1 were not significantly different from zero ($P > 0.07$), suggesting that the relationships were not significantly different from linearity. On the other hand, the relationship between male body size and horn length was not linear (Table 6). Thus, further analyses were conducted using Model 2. The switch point ($\ln X_0$) value that gave the maximum adjusted R^2 value was 1.01 (10.23 mm). In Model 2, the β_2 coefficient was significantly different from zero, but β_3 was not (Table 6). This indicates that the relationship between body size and horn length is not discontinuous but rather that the linear slope changes significantly on either side of the switch point (Fig. 6A).

Male horns showed significant positive allometry (Fig. 6A, Table 7; $\alpha = 2.43$) at small body sizes (<10.23 mm), but the allometric slope was less steep ($\alpha = 0.68$) at large body sizes (≥10.23 mm). Allometric slope for male forelegs was 0.95 (Fig. 6B) and not significantly different from isometry, whereas female forelegs showed significant negative allometry (Table 7). The midlegs of males showed significant negative allometry, and those of females showed nearly significant negative allometry (Table 7). The hindlegs of males also showed significant negative allometry, and those of females showed nearly significant negative allometry (Table 7). Bootstrapping tests showed that there was a significant sexual difference in allometric slope of forelegs ($P < 0.05$) but not in that of midlegs and hindlegs ($P > 0.05$).

All morphological variables were normally distributed (Shapiro–Wilk test: $P > 0.42$). Variances were significantly different between sexes in the length of three legs (F test: $P < 0.01$). There were significant differences between sexes in foreleg length (Welch t test: $t = -59.5$, d.f. = 314, $P < 0.001$), midleg length ($t = -35.3$, d.f. = 269, $P < 0.001$) and hindleg length ($t = -29.4$, d.f. = 252, $P < 0.001$).

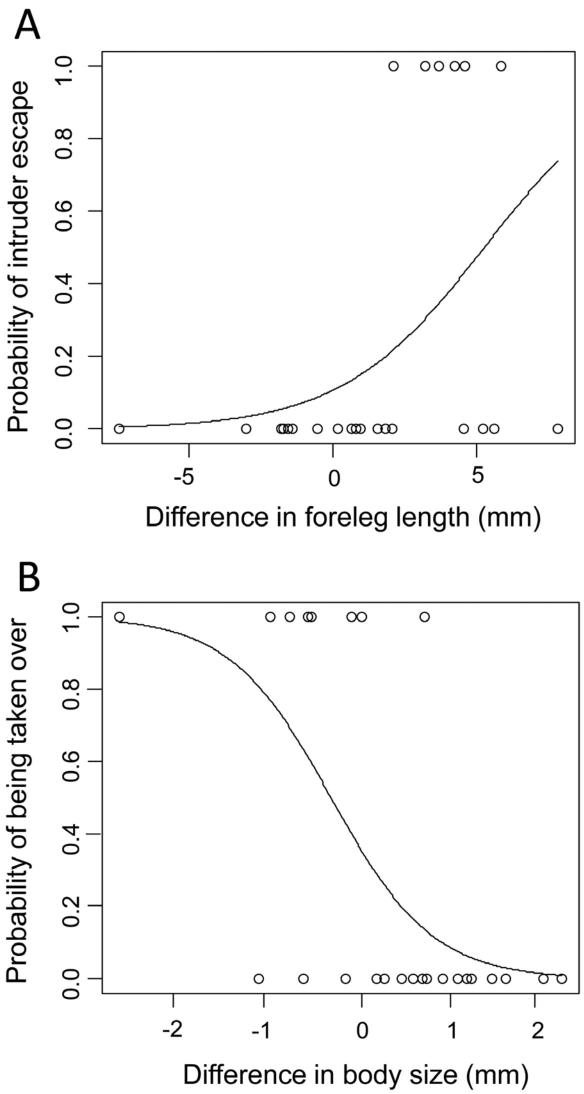

Figure 5. The analyses of fighting behaviour of *Dicronocephalus wallichii bourgoini.* (A) The relationship between the difference in foreleg length (owner – intruder) and the probability of intruder escape in response to tapping by owners. (B) The relationship between the difference in body size (pronotum width, owner – intruder) and the probability that the owner's mates were taken over by an intruder.

DISCUSSION

The results of the behavioural observations indicate that the elongated forelegs and horns of male *D. w. bourgoini* function as weapons in intrasexual

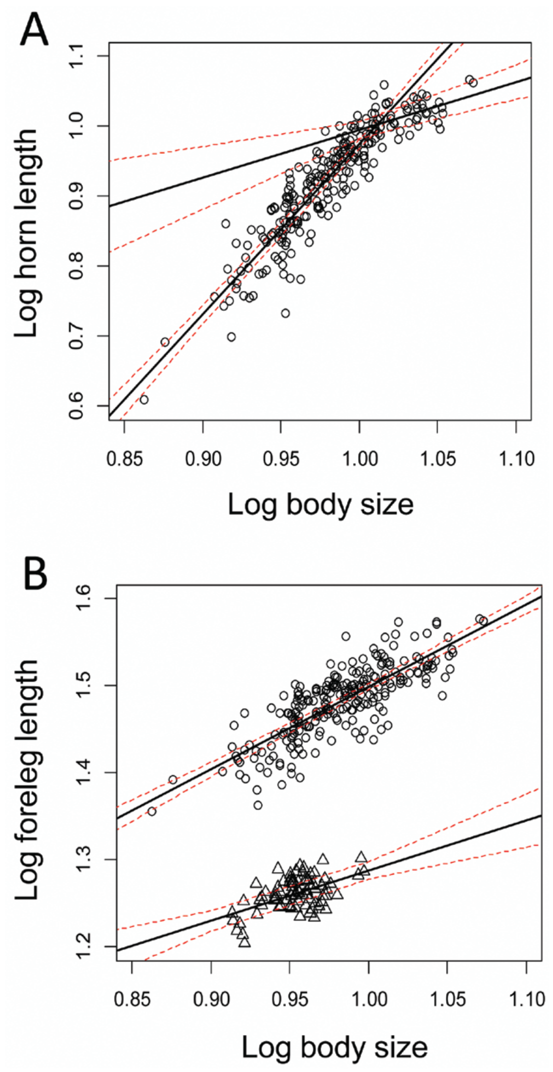

Figure 6. The allometric relationships of *Dicronocephalus wallichii bourgoini* between log-transformed body size (i.e. pronotum width) and horn length (A) and foreleg length (B). Legends for males: circle, for females: triangle. The 95% confidence intervals are shown as red dash lines.

competition, especially in defending the ownership of their mates and taking over females from other males. Allometric analyses also suggest that sexual selection acts on these male traits. Males of this species guard females from rival males after copulation. Given that the sex ratio was male-biased at the mating and feeding sites (males: females = 2.5: 1) and that the number of females guarded by males was three times that of unpaired females, unpaired males should have more difficulty in finding unpaired females than guarded females. In such ecological contexts, sexual selection has probably favoured the evolution of exaggerated male weapons and takeover tactics in this species.

Our field observations showed that the elongated forelegs and horns of *D. w. bourgoini* were used at different stages of a contest, which is consistent with a previous report based on laboratory observations by Šípek, Král & Jahn (2008). In the beginning of a contest, males extended and intensively vibrated their forelegs. If the intruder had shorter forelegs (or a smaller body) than the guarding male, the intruder was more likely to retreat at this stage. Indeed, about a half of all contests were terminated before escalating to direct combat in the field. In addition, the *D. w. bourgoini* males were never injured in fighting. These observations are consistent with mutual-assessment mechanism, in which contestants assess each other's body size, physical condition and fighting potential using their forelegs. This ritualized assessment would confer benefits to both the intruder and the owner. Ritualized behaviour in weapon use is widespread among animals (Enquist & Leimar, 1990). For example, males of the rhinoceros beetle *Trypoxylus dichotomus* face and shove each other with their horns in the early phase of a contest to assess one another (Hongo, 2003). In this study, both owners and intruders of *D. w. bourgoini* likely avoid spending unnecessary time and energy in escalated combat. The owners may, furthermore, avoid the risk of losing ownership of their mates due to sneaking copulation or female escape because these

Table 6. Parameters of Model 1 and 2 for allometry analyses of male horns in *Dicronocephalus wallichii bourgoini*

	Coefficients	Estimate ± SE	95% lower limit	95% upper limit	*t*	*P*
Model 1	α_0	−7.50 ± 1.08	−9.65	−5.38	−6.94	<0.001
	α_1	15.1 ± 2.27	10.8	19.5	6.85	<0.001
	α_2	−6.60 ± 1.13	−8.87	−4.43	−5.91	<0.001
Model 2	β_0	−1.46 ± 0.080	−1.61	−1.30	−18.2	<0.001
	β_1	2.43 ± 0.083	2.27	2.59	29.5	<0.001
	β_2	−1.75 ± 0.29	−2.32	−1.18	−6.09	<0.001
	β_3	0.0028 ± 0.0083	−0.013	0.019	0.341	0.733

Table 7. Parameters of Model 3 and *P*-values for isometry test in *Dicronocephalus wallichii bourgoini*

Sex	Traits	Coefficients	Estimate ± SE	95% lower limit	95% upper limit	*P* for isometry test
Male	Horns in large males	*A*	0.68 ± 0.17	0.34	1.02	0.066
		ln*a*	0.31 ± 0.17	−0.037	0.66	–
	Horns in small males	*A*	2.43 ± 0.089	2.26	2.61	<0.001
		ln*a*	−1.46 ± 0.086	−1.63	0.086	–
	Forelegs	*A*	0.95 ± 0.046	0.86	1.03	0.24
		ln*a*	0.55 ± 0.045	0.46	0.64	–
	Midlegs	*A*	0.82 ± 0.039	0.75	0.90	<0.001
		ln*a*	0.55 ± 0.039	0.47	0.62	–
	Hindlegs	*A*	0.73 ± 0.041	0.65	0.81	<0.001
		ln*a*	0.66 ± 0.040	0.58	0.74	–
Female	Forelegs	*A*	0.58 ± 0.10	0.37	0.78	<0.001
		ln*a*	0.71 ± 0.10	0.51	0.91	–
	Midlegs	*A*	0.78 ± 0.11	0.56	1.00	0.053
		ln*a*	0.50 ± 0.11	0.29	0.71	–
	Hindlegs	*A*	0.81 ± 0.098	0.62	1.01	0.060
		ln*a*	0.51 ± 0.094	0.33	0.70	–

two conditions typically occur when the owners leave their mates to physically fight intruders.

The enlarged horns and forelegs of male *D. w. bourgoini* were mainly used to pry and flip the opponents away from the females or bamboo shoots in the escalated phase of contests. The use of different weapons depending on the escalation level of contests has also been reported in the horned weevil, *Parisoschoenus expositus*. Males of *P. expositus* use their elongated rostrum to push and shove opponents in the early stages of contests. If the contests are not resolved, the males use their horns to trap the opponent and raise him off the substrate (Eberhard & Garcia-C, 2000). These two examples in *D. w. bourgoini* and *P. expositus* provide insights into the evolution of multiple weapons in many insects that could be specialized for specific contest phases.

We showed that *D. w. bourgoini* males employ sneaker-like behaviour to gain access to females. The sneakers were attracted to the owner and intruder in combat and tried to copulate with the owner's mate while the owner was busy fighting against other males. This type of tactic has been reported in the rhinoceros beetle, *T. dichotomus* (Hongo, 2007). In general, sneaking behaviour occurs more frequently in smaller males than in larger males in many species (Shuster & Wade, 1991; Rasmussen, 1994; Emlen, 1997; Moczek & Emlen, 2000; Leary *et al.*, 2005; Hongo, 2007; Neff & Svensson, 2013; Painting & Holwell, 2014). For example, small males of the dung beetle, *Onthophagus acuminatus*, excavate side tunnels and sneak into breeding tunnels guarded by large males in order to copulate with females without fighting. The extremely small horns of the small males in *O. acuminatus* are suggested to be an adaptation for rapid and undetected entry into guarded tunnels (Emlen, 1997). Although we do not have enough data on the body size of sneakers in *D. w. bourgoini*, in contrast to *O. acuminatus* and other species, we speculate that small *D. w. bourgoini* males are unlikely to be more successful in sneaking copulation than large males because the sneakers must win in fights against the owner that comes back to his mate even if the sneakers are able to successfully mount the female. Further observations on sneaking behaviour are required to clarify if males use both sneaking tactics and takeover tactics and how they switch these tactics.

The guarding males of *D. w. bourgoini* were larger than the unpaired males. This pattern is probably explained by the advantages of larger males in contests. The laboratory experiment and field observations both indicated that larger males were more successful in guarding their mates and in taking over females from other males. In addition, larger males may win fights and occupy the sites where females frequently visit to feed. Our predictions (i) and (iii) (see Introduction) were verified. However, we found no evidence to suggest that large males successfully guard females for a longer duration than smaller males in contrast to our prediction (ii). This is probably because mate guarding is frequently disrupted by female escape or other accidental factors that occur independently of male body size. Males of some insects, including Coleoptera, Hemiptera and Odonata, are known to adjust their mate-guarding duration depending on the sex ratio, population density, their own body size and female body size (Alcock, 1994; Schöfl & Taborsky, 2002; Saeki, Kruse & Switzer, 2005). By contrast, males of

D. w. bourgoini were rarely able to control the duration of mate guarding themselves; thus, decision making by males may have a negligible effect on mate-guarding durations under natural conditions.

The guarding males of this species attacked not only conspecific males but also conspecific females and other scarab beetles that were attracted to the bamboo sap. The aggressiveness of owners towards females and other beetles might be attributed to error in recognition. The intense mate-guarding behaviour in this species is probably beneficial for paternity assurance given the strong tendency towards multiple matings in females. We found that some females remated with another male within a few minutes after the end of a previous mating under both laboratory and natural conditions (W. Kojima, Personal observation). Additionally, females never refused copulation. Females of insects are generally thought to gain fitness benefits, such as enhanced offspring viability, a reduced cost of inbreeding and enhanced access to nutrients, from multiple matings (Hosken & Stockley, 2003), but the type of benefits that females may gain is unclear in this species or related flower beetles. Considering the high motivation of females to escape from owners after copulation, females may incur fitness costs from an owner's prolonged mate guarding, including the loss of opportunities to feed and mate with multiple males.

It is a challenge to identify which phenotypic traits of males influence mating success and the outcome of fighting because the morphological measurements of males (i.e. body size, horn length and foreleg length) are highly correlated with one another. To partition the effects of each trait on male fighting success, experimental approaches such as staged fights between size-matched males are needed (Emlen, 1997; Moczek & Emlen, 2000). However, our behavioural observations of *D. w. bourgoini* suggest that both forelegs and horns play important functions in contests. Moreover, as known in many beetles, including species with exaggerated weapons (Lawrence, 1986; Otronen, 1988; Hongo, 2003; Lailvaux *et al.*, 2005; Lee *et al.*, 2014), having a large body is probably important in winning physical fights in *D. w. bourgoini*. In addition, the combination of long horns, enlarged forelegs and a large body in males has evolved several times in Cetoniinae (flower beetles) (e.g. *Theodosia* spp., *Dicronocephalus* spp. and Goliathini). Therefore, in *D. w. bourgoini*, sexual selection might have favoured an integrated male morphology that is advantageous in fighting rather than one particular phenotypic trait.

Our allometric analyses provide evidence of sexual selection acting on the forelegs and horns of *D. w. bourgoini* males, in which the horn length exhibited positive allometry typical of sexually selected traits (Kodric-Brown, Sibly & Brown, 2006). The allometry slope of horns in large males was decreased. This pattern is commonly found in weapons of stag beetles (Knell, Pomfret & Tomkins, 2004) and rhinoceros beetles (Siva-Jothy, 1987; Hongo, 2007; McCullough *et al.*, 2015), and generally explained by the depletion of resources for pupal development (Nijhout & Wheeler, 1996; Knell, Pomfret & Tomkins, 2004). However, there are alternative hypotheses for the decline of slope for large males. For example, larger males may not gain net benefits from greater investment in horns when horn size becomes a less important predictor of victory as body size increased (Pomfret & Knell, 2006). If there is a resource competition between horns and other body parts including wings, eyes and testes (Emlen, 2001; Simmons & Emlen, 2006), selection for flight ability, visual precision and sperm competition may constrain the further exaggeration of the horns. Moreover, males with large horns could have an increased locomotory cost (Goyens, Dirckx & Aerts, 2015) and risk of predation (Kojima *et al.*, 2014; Romiti *et al.*, 2015).

In contrast to horns, the male foreleg length showed isometry ($\alpha = 0.95$), but the slope was much steeper than that for female forelegs ($\alpha = 0.58$). The sexual difference in allometric slope was only found in forelegs. Therefore, male forelegs are obviously also a target of sexual selection. A resource allocation model shows that isometric or negative allometry occurs in sexual traits that are the targets of antagonistic natural selection against higher allometric values (Bonduriansky & Day, 2003). All legs of both sexes, except male forelegs, showed significant negative allometry or nearly significant negative allometry ($\alpha = 0.58$–0.82), suggesting that natural selection favours proportionally shorter midlegs and hindlegs in *D. w. bourgoini*. Thus, the naturally selected function of male forelegs (i.e. walking and grasping) may constrain their further exaggeration and result in the observed isometric pattern. The midlegs and hindlegs of males were significantly longer than those of females, but the allometric slope was similar between sexes. It is possible that the sexual dimorphism in midlegs and hindlegs is a product of natural selection rather than sexual selection. Sexually selected weapons are often coupled with correlated modifications in other traits that mitigate the cost of the exaggerated weapons (Husak & Swallow, 2011; Husak *et al.*, 2011) or enhance their functions in fighting (Okada *et al.*, 2012). The long midlegs and hindlegs in male *D. w. bourgoini* may be an adaptation for balanced and stable walking, resisting attacks from opponents, or lifting up the body on an opponent during contests. Alternatively, male long midlegs and hindlegs may enable grasping substrates during mounting on female's back.

In summary, our results suggest that both forelegs and horns in *D. w. bourgoini* males are sexually selected traits that are specialized for different phases of intrasexual contests. The decreased allometric slope for horn length of large males and isometric pattern for male foreleg length show constraints of further exaggeration for these traits. Future studies on costs of the weapons and the relationship between weapon size and mating tactics will help us to better understand the evolution of the multiple weapons in this species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Measured morphologies in male *Dicronocephalus wallichii bourgoini*.
Figure S2. The transition of the total number and sex ratio of *Dicronocephalus wallichii bourgoini* on bamboo shoots within daytime and season.
Movie 1. An owner deterring an approaching intruder by tapping with forelegs. The intruder rapidly retreated.
Movie 2. An owner tapped an intruder, but the intruder tries to mount the owner's mate. The owner pried the intruder using his horns.
Movie 3. While an owner was fighting with an intruder, a sneaker approached from above and tried to mount his mate. The sneaker was thrown away by the owner after the owner flipped the intruder.
Movie 4. As soon as the owner began to fight against an intruder, a sneaker approached from below and tried to copulate with the owner's mate. The sneaker eventually retreated as a result of being attacked by the horns of the owner.
Table S1. Mating duration and male traits.
Table S2. Measurement of traits.
Table S3. Outcome of contests and difference in male trait size between owners and intruders.