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Stag beetle battle behavior and its associated anatomical adaptations

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Stag beetle battle behavior and its associated anatomical adaptations

Male stag beetles battle for females with their impressive, oversized mandibles. We describe their fighting behavior, which is essential to understand the evolution and morphology of their weaponry. Our behavioral analysis reveals several anatomical structures that are important for fighting, and our morphological investigations show how these may be adapted for their functions. Stag beetle fights are much more variable than other armed beetles' battles. They spend considerable time and effort in dislodging their opponent, that clings to the substrate with its tarsal claws. These tarsal claws are also indispensable to maintain balance in the most spectacular battles, when they lift a rival high in the air. The male claws are highly curved and have an increased height for this purpose. The prothoracic muscles are hypertrophied to support the lifting movement. The largest beetle wins in 85% of the fights and the smaller the difference in mandible length is between the rivals, the longer the battles can last. The long mandibles enable males to reach the opponent's legs in order to dislodge it. For this purpose, they bite with all parts of their mandibles, even though the distal part is more vulnerable for failure and transfers less bite force. Blindfolded experiments prove that visual information is not a requisite for a successful battle.

Various weapons, evolved by sexual selection and used in male-male combats, are found in a very wide range of taxa: trilobite spines; crustacean chelipeds; stag beetle mandibles; scarab horns; humps, saws and spines of fishes; horns, spines and tusks of frogs; crests, frills, plates and spines of dinosaurs; antlers of deer, elks and moose; horns of bovids and rhinoceros (for a review, see (Emlen 2008)). Knowledge of the nature of fighting tactics and styles is primordial to understand the evolution and morphology of these armaments (Eberhard 1977; Caro et al 2003; Emlen et al 2005; Emlen 2008; Gotoh et al 2012). However, the fighting behavior, as such, has received surprisingly little scientific attention (Emlen 2008). Only for ungulates has the relationship between fighting style, mating system and antler morphology been intensively studied (Caro et al 2003; Hoem et al 2006; Emlen 2008).

We aim to fill this gap for stag beetles (Lucanidae), by investigating their fighting behavior in relation to their morphology. Stag beetles are one of the paradigms for sexual selected weaponry, because the males are equipped with spectacularly large mandibles to bite and fight fiercely for females. In some species, these mandibles can become as long as the body (Kawano 2006; Goyens et al 2015a), and also the variation in shapes and ornamentations within the Lucanidae family is impressive (e.g. (Kawano 2000; Emlen 2008)). This gave rise to numerous investigations on the male mandibles, but like most other animal weapons, its usage remained largely unexplored. Several studies have been performed on the sexual dimorphism itself and its allometry (Kawano 2000; Shiokawa and Iwahashi 2000b; Tatsuta et al 2001; Tatsuta et al 2004; Knell et al 2004; Kawano 2006). Also the impact of mandible size on locomotion (running and flying; (Goyens et al 2015a; Goyens et al 2015c)) has been described, as well as the influence of mandible size on fighting and mating success (Shiokawa and Iwahashi 2000a; Lagarde et al 2005; Okada and Hasegawa 2005; Harvey and Gange 2006; Okada and Miyatake 2006). In a few stag beetle species, males with longer mandibles are known to have a higher winning chance, but the underlying function remains unknown. We hypothesize that long mandibles are used as a visual cue of the individual’s qualities, to impress rivals with smaller mandibles. Alternatively (but not mutually exclusively), we will investigate whether long mandibles have functional benefits in the fights, either directly or indirectly (through a correlation with muscle size and strength).

Most stag beetle studies have been mainly focused on the mandible apparatus itself. However, it has been argued that not only the mandibles, but the entire male head should be seen as part of the weaponry (Shiokawa and Iwahashi 2000b; Goyens et al 2014a): the enlarged male head houses massive, hypertrophied mandible adductor muscles to generate the high bite forces that are needed in the battles (Goyens et al 2014a). We hypothesize that also the prothoracic muscles are enlarged, as an adaptation to raise their head during lifting motions. Preliminary, qualitative, observations show that male stag beetles lift opponents, whose body mass can equal or even exceed their own mass, high into the air (Shiokawa and Iwahashi 2000b; Goyens et al 2014a). We further hypothesize that the beetles are statically instable in such lifting motions. Even during simple running, male stag beetles suffer severe instability because of their heavy armature (Goyens et al 2015a), and during lifting they have to additionally bear the weight of the rival. If this indeed causes instability while lifting opponents, we will investigate whether the male tarsal claws are adapted to pull, in order to counterbalance the heavy weight at the rostral part of their body.

As a first step in our search for the function of morphological structures in battles, we will describe and analyze the fighting behavior of male *Cyclommatus metallifer* stag beetles. We chose this species because of its oversized mandibles (Kawano 2006; Goyens et al 2015a) and its eagerness to engage in male-male battles. Next, we will measure several morphological parameters (body and mandible size; prothoracic muscle size; tarsal claw morphology) and relate these to our behavioral observations. We will compare the morphological characteristics with those of conspecific females. After normalization, the female morphology can be used as a proxy for that of a hypothetical non-dimorph male, as males and females can hardly be discerned from each other in stag beetle species without the sexually differentiated male mandibles (cf. (Goyens et al 2014a; Goyens et al 2015a)).

MATERIAL AND METHODS

Fight Experiments

Ten adult male and ten adult female *Cyclommatus metallifer* stag beetles were obtained from a commercial dealer (Kingdom of Beetle, Taiwan). We housed the animals individually in plastic containers (length 39 x width 28 x height 14 cm), at a temperature of between 20 and 25 °C and with a natural light-dark cycle of about 16u/8u. Newsprint and moist moss provided shelter, cage enrichment and humidity. The beetles were fed beetle jelly and water *ad libitum*. All experiments were approved by the ethical committee of the University of Antwerp (approval nr 2011-63) and were conducted conform to the legal requirements.

In order to observe their fight behavior, we mimicked a natural environment in the lab using leaves, twigs, branches and tree logs under natural day light. For the fight experiments, two males were placed 10 cm apart on a horizontal tree log. The tree log provided a natural fight location, as males normally fight over nest sites (decaying wood used by females to deposit eggs) or food sites (sap runs from trees) (Araya 1993; Knell et al 2004; Rink and Sinsch 2007; Emlen 2008; Inoue and Hasegawa 2012). We did not provide a feeding site (e.g. a cup of beetle jelly) to exclude biasing effects (e.g. prior residence effects) and because the males were already very motivated without the presence of an actual feeding site. Also other fight experiments on stag beetles and horned beetles were successfully preformed without feeding site (Eberhard 1977; Moczek and Emlen 2000; Lagarde et al 2005; Kuan 2011). The log provided grip for the beetles and made it easy for us to recognize the end of a contest when the loser was tossed off. 84 fights were recorded with a digital camera (3.3 megapixel, 25 frames second^{-1}, JVC GZ-V515, JVC Kenwood Corporation, Kanagawa, Japan). The movies were analyzed for the following parameters (also see Table 1):

Battle duration was timed from the first contact until one of the beetles won. We noted which beetle won, and whether the losing beetle fell off the tree log or ran away. If there was no clear winner, this was also noted.

Actions during the battle were scored. We made a note of the attempts to haul and dislodge the opponent, and whether one of the beetles lifted its opponent off the ground. We also observed whether, and when, the beetles adopted their typical aggressive, upright, posture with opened mandibles.

Mandible regions used to bite were registered. For this purpose we divided the mandible into 5 regions (see Fig. 5).

Behavior after settling the battle of both the winner and the loser were observed (aggressive posture, pumping, running away, falling off tree log).

Stability While Lifting Opponents

When a male stag beetle lifts an opponent, it has to balance their combined body center of mass (bCOM). As long as this combined bCOM stays above its leg base, it is statically stable. We quantified this static stability with high speed video recordings (Redlake HR1000, Redlake digital Imaging Systems, IDT vision, Tallahassee, FL, USA; top view; 125 frames second^{-1}; spatial resolution: 1280x1024 pixels). Video recordings in high speed was necessary to capture the fast movements during lifting. However, it was difficult to capture both entire beetle bodies during the complete lifting movement because of the trade-off between the field of view, sufficient detail and a fixed camera position. Yet, despite the practical limitations we were able to obtain high speed movies of 5 complete lifting movements (out of 51 recordings), featuring 5 different individuals. For this specific purpose, the floor of the battle area was covered with smooth cork in order to have a flat substrate and to provide grip. With the video analysis tool *DLTdv5* by T. Hedrick [Open source software: (Hedrick 2008); Matlab application; Matlab R2013b, 64 bit version, Natick, MA, USA;], we digitized the 2D location of all points of support (the distal ends of the tibia of the individual on the ground) from the onset of the lifting event until the opponent was released. In the same way, we digitized the 2D location of the anterior and posterior ends of the body (without mandibles) of both beetles. These were used to locate the bCOM of each individual separately, as we know that this is positioned at 58% along the body axis (from posterior to anterior; (Goyens et al 2015a)). This location is hardly influenced by leg positions (Goyens et al 2015a). The combined bCOM was subsequently determined as the average of both, using their body mass as a weighting factor. Finally, we established whether or not the combined bCOM fell within the support polygon for every frame (with the Matlab function 'inpolygon').

Importance of Visual Cues

To test the importance of vision to engage in and to successfully finish a fight, we set up 15 additional fights between blindfolded males on the tree log. For this purpose, their eyes were covered temporarily with acryl paint. After the experiments, the paint could easily be peeled off.

Morphological Measurements

We measured the male body length (abdomen – head; without mandibles) and mandible length (hinge – tip) on high resolution digital photographs with GIMP (GNU Image Manipulation Program, fee software, www.gimp.org: average precision: 30 µm $pixel^{-1}$). The males' bodies were 3.56 ± 0.75 cm long, with mandibles of 3.75 ± 0.25 cm (averages ± standard deviation), and with a strong positive correlation between both (R^2=0.81, see Fig. 1). Therefore, we used mandible length in the analyses of the fights.

Stag beetles have two tarsal claws at the distal end of each leg. To examine the morphology of the claws of a front, middle and hind leg, we made high resolution digital photographs of the tarsi of deceased stag beetles of the same species (*Cyclommatus metallifer*) with a stereo microscope (Leica M165C, Leica Camera AG, Solms, Germany; average precision: 2.5 µm $pixel^{-1}$; see Fig. 2). Some beetles lost one or more tarsi during their lives, but we were still able to measure a total of 20 male (of 8 individuals) and 24 female tarsi (of 10 female individuals). We analyzed the claw morphology with parameters comparable to those of (Zani 2000; Dai et al 2002) (see Fig. 2):

- Claw length (a), normalized for body size by dividing it through the length of the posterior body part (meta- & mesothorax and abdomen) (Goyens et al 2014a). We did not use the total body length for this purpose, as the head is also a part of the sexual dimorphism.
- Claw curvature (r, radius of curvature of the best fitting circle inside the claw), normalized in the same way as the claw length.
- Claw height (b), normalized in the same way as the claw length.
- Claw sharpness (ratio of the major axis to the minor axis of the best fitting ellipse on the claw tip).

We determined the physiological cross-sectional area (PCSA) of the prothoracic muscles on micro CT scans of a male (resolution: 8.2 µm, for details see (Goyens et al 2014b)) and female specimen (resolution: 13.4 µm, for details see (Goyens et al 2014a)). The reconstructed slice images transversely section the prothoracic muscles. In GIMP, we measured the maximal cross-sectional area of the prothoracic muscles (see Fig. 3) and normalized it to body size (by dividing the PCSA by posterior body mass$^{2/3}$, see (Goyens et al 2014a)).

RESULTS

Fight Behavior

All reported frequencies and statistical tests are summarized in Table 2.

Male stag beetles typically adopt an aggressive posture when initiating a battle. The beetle raises its opened mandibles high, and often places its front legs forward to lift the anterior body part (see Fig. 4). They also display this posture when they are disturbed by the researcher. In 32% of the recorded battles, the beetles adopts this position at the first physical contact; in 43 % of the cases they already adopted the position beforehand.

The course of the subsequent battle is very variable (for examples, see Online Resource 1). In 26% of the battles, there is only very brief contact between both males (most often a tap or a snap of the mandibles). Yet, this is sufficient to determine a distinct winner in 82% of the cases, and it is the male with the longest mandibles that wins in 89% of these fights (16 out of 18 battles; one-tailed paired *t* test: t_{17}=3.2, p=0.0024). In the battles with more than only minimal contact, the fight consists of hauling, pushing and biting in each other's body and mandibles. We do not observe a ranked order in these conducts, neither does an individual always use the same strategy. Instead, they seem to adapt their actions to their motivation and opportunities that occur.

Hauling and wrestling is vitally important to destabilize and dislodge the rival, and this can take several minutes (see Online Resource 2). Males often immediately target the competitor's legs, and we observe a distinct dislodging phase in 29% of the fights.

Biting The stag beetles conveniently use their mandibles to grab and hold their rivals. We analyzed 163 biting events in the 42 battles in which they occurred (see Fig. 5) and found that they use every mandible region to bite. They bite least frequently with the very tip of their mandibles (10%, region A), and most often with the concave, distal region (29%, region C).

Lifting In the most spectacular fights (33% of the battles), one of the males finally lifts its opponent off the ground (see Fig. 6 and Online Resource 2,4). This is normally achieved by grabbing the opponent's body between its mandibles and pinching it while lifting. But sometimes it is the opponent who bites, or the mandibles of both are interlocked so that no bite force is required (e.g. see Fig. 11). To initiate the lifting movement, the stag beetle on the ground lifts its head relative to the posterior body parts, and pushes its head further upwards with its front legs. Occasionally, the male repositions its front legs forward for this purpose (see also section 3.3). Typically, the male on the ground releases the loser high in the air. The loser then falls on its back and/or falls off the tree log. Sometimes the beetle that is lifting, is not able to release its opponent and has to put it back on the ground, for example when the opponent bites in its body. Occasionally, the male walks around with the opponent still lifted in the air (see Online Resource 2).

In 86% of the battles (with or without lifting) there is a distinct winner and loser. In 63% of these fights, the loser decides to run away. In 29% of the cases it falls off the tree log. In the remaining 8%, there is a clear supremacy of the winner and the loser stayed within close proximity to the winning stag beetle. The beetle with the longest mandibles wins in 85% of the contests with a distinct outcome (one-tailed paired t-test: t_{71}=6.5, p<0.001). Also body length is positively correlated with winning chance (one-tailed paired *t* test: t_{71}=4.9, p<0.001). In 21% of the battles, the winner exhibits 'pumping' behavior following the battle. He repeatedly lifts its body by a few millimeters with its legs (see Online Resource 3). We saw a loser make these movements only once.

The duration of the battle is related to the difference in mandible length between both aggressors: short fights are always possible, but the contests only last a long time when their sizes are matched. This results in a monotone negative correlation between battle duration and difference in mandible length (Spearman's rank correlation, see Table 3 and Fig. 7). The correlation coefficient is slightly lower, but still highly significant, when the length difference is expressed relatively to the average mandible length of both opponents. When we look to both opponents separately, we find that battles last significantly longer when the loser is larger (Spearman's rank correlation, see Table 3 and Fig. 8). This correlation is absent for the mandible length of the winner. There is no clear correlation between a male's mandible length and the frequency of lifting either (see Fig. 9). We never saw males in the smallest length class lift their opponent (see Fig. 9). Fighting males lift each other more frequently when they are more size-matched (see Fig. 10A). But concomitantly, such contestants are often a match for each other and do consequently often not succeed in dislodging each other. Sometimes, this impasse lasts for several minutes and therefore these long fights often lack lifting and they often end without a clear winner (see Fig. 10B-D). Yet, the engagement is obviously intense:

their exoskeletons creak and bend while they nervously search for grip with their legs (see e.g. Online Resource 2).

Stability While Lifting Opponents

Fig. 11 shows a representative example of the static stability analysis during lifting. The combined bCOM is located in front of the beetle on the ground, but moves closer to the support base as the opponent is lifted higher (from blue to red in Fig. 11). Because the bCOM never falls above this leg base, the beetles are statically instable during the entire lifting phase. This is the case in 4 out of 5 digitizations. In the fifth situation, the beetles are statically stable for (only) 21% of the time. Yet, the beetles are always able to finish the lifting operation successfully, without falling. We occasionally saw the beetle on the ground reposition its front legs forwards while lifting its opponent, which may be an attempt to move the frontal border of the support base closer to the bCOM (see Online Resource 4).

Probably, the tarsal claws (especially those of the hind limbs) are responsible for maintaining balance during lifting, by pulling on the substrate. In order to determine the role of these claws, we staged 4 fights on a smooth surface (a standard lab table top). The beetles are able to run slowly on this substrate, and succeed in grabbing their opponent. Yet, they fail completely in the lifting phase. Raising the opponent in the air is impossible, and after flailing about with their legs for a while, they usually land on their side or back. Finally, they often fall into aimlessly turning around together (see Online Resource 5).

Importance of Visual Cues

We find that the stag beetles still fight fiercely when blindfolded, and that they even still succeed in lifting their opponents in 22% of the fights (4 out of 15 blindfolded battles, see Table 2). In 7 out of 15 blindfolded battles, the male with the longest mandibles wins (47% of the blindfolded battles, see

Table 2). Hence, the advantage of long mandibles is lower in the blindfolded condition than in battles with vision (when 85% of the battles is won by the largest antagonist).

Morphological Measurements

By comparing linear models that took the factor 'individual' into account, we find that the normalized claw length is not statistically different between sexes (ANOVA: p=0.43) or between legs (p=0.28). Also the interaction between leg and sex is insignificant (p=0.053).

For normalized claw curvature, the same statistical approach shows a significant interaction between leg and sex (p=0.047). For males, the claws become more curved from the anterior to the posterior (front: 0.0346 ± 0.0023; middle: 0.0329 ± 0.0016; hind: 0.0323 ± 0.0023). For females, the difference between legs is a lot smaller and not ascending from anterior to posterior (front: 0.0295 ± 0.0019; middle: 0.0291 ± 0.0010; hind: 0.00294 ± 0.0015).

Using the same approach for claw height (normalized for body size), we find that claw height increases from anterior to posterior (p=0.036) and that male claws are higher than those of females (p=0.021). On average (± SD), male claws are 0.417 ± 0.033 mm high (normalized: 0.0214 ± 0.0016), female claws 0.295 ± 0.016 mm (normalized: 0.0201 ± 0.0013). There is no significant interaction between leg and sex (p=0.48)

For claw sharpness, we find no interaction between leg and sex (p=0.068), and no significant difference between legs (p=0.30) or sexes (p=0.35). For both males and females, we see that the claw ends are worn down.

The male subject has a prothoracic muscle PCSA of 8.4 mm², the female of 2.4 mm². When normalized to body size, we find that the male has a 2.2 times larger prothoracic muscle PCSA than the female (respectively 958 and 442).

DISCUSSION

Numerous studies have been performed on the impressive stag beetle weaponry. Yet, the present study is the first to search for morphological adaptations for stag beetle battles and their specific function.

The stag beetle battles do not follow a strictly established course. Instead, the employed fighting tactics and styles are very variable (see Fig. S1). This is also true for *Cyclommatus mniszechi* stag beetles, for which 11 distinct behaviors were distinguished (Kuan 2011). All these conducts were also present in our *Cyclommatus metallifer* observations, supplemented with the 'pumping' behavior that was not reported for *Cyclommatus mniszechi.* We saw this peculiar shaking behavior ('pumping') of the winning beetle after 21% of the battles. To our knowledge, this has not yet been described in literature, and we can only guess at its function. We suggest that it may perhaps be a method to show its dominance and to impress the loser. Winning rhinoceros beetles perform a similar movement (but they move sideward and combine it with stridulation), which was also interpreted as an intimidation behavior (Eberhard 1977). For *Golofa porteri* and *Trypoxylus dichotimus* rhinoceros beetles, it was possible to determine a standard chain of escalating events (Eberhard 1977; Siva-Jothy 1987; Hongo 2003; McCullough and Zinna 2013). Stag beetle battle behavior is more variable: for *Cyclommatus mniszechi, Lucanus maculifemoratus* and *Prosopocoilus inclinatus* stag beetles, respectively 3, 10 and 13 different sequence patterns could be distinguished (Kuan 2011; Hongo and Okamoto 2013). Also in our experiments, the sequence of conducts was very variable, and seemed to depend on the beetle's motivation and opportunities that occur during the scuffle. Some battles start hesitatingly and intensify gradually, however, other fights are pugnacious from the beginning, without any explorative phase. Lifting occurs even in the shortest of contests. The battle is settled quickly when there is a large size difference between both opponents because the largest beetle wins with ease. In contrast, when the size difference between the rivals is small, the battles can be very lengthy. Even though lifting is harder in this situation, it happens more frequently. This higher intensity of long fights, together with the longer battles when the opponents are size-matched and when the loser has larger mandibles, point to an **assessment strategy**. However, because of the absence of a correlation between the mandible length of the winner and the battle duration, we cannot distinguish whether or not they follow the pure self-assessment model, the cumulative assessment model or the mutual assessment model (Arnott and Elwood 2009). Similar results (negative correlation between battle duration and difference in mandible length; positive correlation between battle duration and size of the loser (see Figs. 7,8)) were observed for *Cyclommatus mniszechi* (Kuan 2011). Even though battles between size-matched males can last very long, we observed that they can also end quickly, for example when one of them loses grip to the substrate. This results in a gradual enlargement of the variation in battle duration for smaller mandible length differences (see Fig. 7), which was also found for orb-weaving spiders, but not found for *Cyclommatus mniszechi* stag beetles (Bridge et al 2000; Kuan 2011). In our experiments, all males (small and large) engage in violent fights. For *Trypoxylus dichotimus,* a rhinoceros beetle species with two male morphs (minor and major males), dissimilar observations were made: Iguchi found that both morphs fight, while Siva-Jothy observed that minor males avoid battles (Siva-Jothy 1987; Iguchi 2001). Iguchi also describes that major *Trypoxylus dichotimus* rhinoceros beetles fight more violently than minors (Iguchi 2001). This agrees with the absence in our observations of lifting by males in the smallest size-class, but we did not find an overall correlation between male size and lifting frequency (see Fig. 9).

Our high speed recordings of fighting stag beetles prove that the beetles are almost always statically instable throughout **lifting**. Yet, they can complete this spectacular action successfully and without falling. The only apparent method they may be using to remain upright, is pulling on their **tarsal claws**. This is confirmed by our fight experiments on a smooth substrate without grip for the tarsal claws: males cannot lift their rivals anymore, and they fall over while trying to do so. It is clear that the tarsal claws are indispensable to brace themselves when grabbing the opponent and to maintain balance while lifting. Males have a larger claw height than females, a feature that has been related to clinging performance on rough substrates and a climbing, arboreal life style in many animal taxa (Zani 2000; Tulli et al 2009; Tulli et al 2011; Crandell et al 2014). As our *Cyclommatus metallifer* stag beetles were very eager to climb in the lab (as opposed to other stag beetle species such as *Dorcus titanus*), their locomotion habits probably played a role in the evolution of their claw morphology as well. In climbing reptiles and mammals, interlocking with the substrate is also facilitated by a high claw curvature, while gently curved claws are typical for ground-dwelling species (Tulli et al 2009; Tulli et al 2011). Compared to these animals, male as well as female stag beetle claws are highly curved. Moreover, the hind leg claws, which are probably most important while lifting, are the highest curved claws in males. However, male claws are less curved than female claws (respectively 121° and 109°), so male claws do not seem to be especially adapted for superior grip in this regard. Finally, claw sharpness has also been correlated to a climbing life style and it determines grip in relation to surface roughness (Dai et al 2002; Tulli et al 2011), but this parameter is not significantly different between male and female stag beetle claws.

Preliminary measurements on male *Trypoxylus dichotomus* rhinoceros beetles show that they have enlarged **prothoracic muscles** to generate the torque to dislodge rivals of trunks and branches (McCullough and Tobalske 2013). As dislodging is equally essential in stag beetle fights, we expected that they may have hypertrophied prothoracic musculature as well. Moreover, male stag beetles actively lift their opponent: they pick up rivals whose weight may exceed their own body weight, and they do so at an ungainly long distance from their own body center of mass. Our measurements confirm that their prothoracic muscles are strongly proliferated for this purpose: taking account of their larger body size, males have 2.2 times larger prothoracic muscles than females. This is very comparable to the sexually selected enlargement of the massive mandible closer muscles to enable the high male bite force (almost 2.5 times larger than normalized female muscles; (Goyens et al 2014a)). Additionally, also the male leg length may be adapted for the lifting movement: the legs become sequentially longer from posterior to anterior, probably as an adaptation to push the body further upwards (Goyens et al 2015a).

The beetle with the **longest mandibles** wins in a large majority of the battles. This confirms findings for other stag beetle species (*Lucanus cervus*, *Prosopocoilus inclinatus* (Lagarde et al 2005; Inoue and Hasegawa 2012)) and rhinoceros beetles (*Onthophagus Taurus*, *Trypoxylus dichotomus*, (Moczek and Emlen 2000; Hongo 2003)). Hongo and Okamoto found an interesting exception on this general rule of size-advantage: *Prosopocoilus inclinatus* stag beetles overcome their smaller body size compared to *Lucanus maculifemoratus* stag beetles due to a different aggressive behavior (mandible nipping behavior) in interspecific fights (Hongo and Okamoto 2013). It was suggested that in rhinoceros beetles, a longer horn is advantageous because it is more effective to pry rivals or to block tunnel corridors (Eberhard 1977; Emlen et al 2005). However, literature remains vague on the practical advantage of longer mandibles in stag beetle battles (Tatsuta et al 2001; Lagarde et al 2005; Inoue and Hasegawa 2012). We found that long stag beetle mandibles have a functional benefit: they enable the males to reach forward to their opponent's legs in order to detach them. This finding is reinforced by our observation that mandible length is more important than body length to win battles. As *Cyclommatus metallifer* males battle on thin branches (Suzuki 1996), they cannot simply walk around their opponent to reach its legs but they have to attack their rival frontally. Hence, the contestant with the longest mandibles has the best reach for its opponent's legs. This finding also shows that tarsal claws are not only crucial for a stag beetle to lift an opponent, they are also indispensable to avoid being dislodged and lifted. As a consequence, an important part of the battles consists of grasping at each other's legs and hauling and wrestling to dislodge each other. Therefore, an extended reach towards the rival's legs was probably a driving force towards the elongation of the male mandibles through sexual selection. Moreover, males with long mandibles are able to **bite** more forcefully (paired *t* test: t_8=16, p<0.001; calculated with data from (Goyens et al 2014a)), even though they face a mechanical disadvantage (a long output lever). This correlation is probably the indirect effect of larger males having both longer mandibles and bigger muscles. Male stag beetles are known to have an adapted mandible and muscle morphology to bite very forcefully (Goyens et al 2014a). The result is visible in our battle observations: during the dislodging phase we regularly saw their exoskeleton (e.g. hard wing covers) bend, which was often associated with a chilling harsh sound. Although the stag beetles are obviously pinching powerfully to tear their rival loose, they do not confine themselves to the base of their mandibles, which would be mechanically advantageous and would, therefore, produce the highest bite forces. Instead, most frequently they use a more distal region which confirms the utility of the long mandibles to reach to and to grab their opponents' legs.

The observations above (further reach + higher bite force) explain why males with long mandibles are the favorite in scuffles. Mandible length may, therefore, be used as a **visual cue** of the rival's qualities. Yet, our blindfolded fight experiments prove that vision is not compulsory to engage in a fight. The stag beetles fight fiercely and they still lift opponents in 22% of the battles. As they are active in the twilight (Rink and Sinsch 2007; Inoue and Hasegawa 2012), they probably rely on the strength and pugnacity of their opponent, which is facilitated by the mechanosensors in their mandibles (Goyens et al 2015b). We observed that the stag beetles react strongly to the substrate vibrations (e.g. when we gently scratch the tree log), which are likely detected with the long hears between their tarsal claws (see Fig. 2). Further, they may also exchange information via pheromones, just like male *Lucanus cervus* stag beetles use chemical cues to find females (Rink and Sinsch 2007). Yet, our experiments do not exclude that stag beetles use visual cues whenever possible. This explains the advantage of large males in minimal contact battles and scuffles with vision. In 43% of the fights, there is a phase of aggressive posture (without any physical contact) before the first physical contact, which may be intended for visual display. Such visual display ('mandible opening') before the physical attack is even more frequent in *Prosopocoilus inclinatus* and *Lucanus maculifemoratus* stag beetles, (respectively 89.2% and 89.9%) (Hongo and Okamoto 2013). In these species, the size differences between contestants in escalated battles are significantly smaller than in non-escalated battles, which indicates that they indeed visually assess their opponent during the mandible opening phase (Hongo and Okamoto 2013). However, when we compare the minimal contact fights with more intense fights, we find no difference in mandible length (t-test; absolute mandible length difference: t_{36}=0.52, p=0.61; relative mandible length difference: t_{36}=0.13 , p=0.90).

The main purpose of the battles is to push, throw or chase away the assailant, not to **hurt** it. The tough exoskeleton largely defends the male body from being hurt, but once, we saw a mandible grabbing under the hard wing cover of its opponent during a fight. In such a situation, the opponent definitely risked tearing its wings. Also, our study suggests that males risk breaking off their own valuable mandibles when biting. They avoid this by modulating their muscle force for tip biting (Goyens et al 2014b). Finally, the legs are at risk during the fights, too: in a preliminary fighting experiment, one of the beetles lost a leg while being dislodged by its opponent. We also saw females attack each other by snapping at each other's legs with their scissor-like mandibles. As we observed our male stag beetles cut through the wire mesh in the cover of their boxes with their mandibles, it seems probable that they are also able to bite legs off.

CONCLUSION

We described for the first time the conducts and strategies in stag beetle battles. The course of the fights is very variable, with dislodging as an important and recurring phase. In the most spectacular fights, one of the beetles lifts its rival above its own head. We confirmed our hypotheses regarding morphological adaptations for the contests:

- The morphology of the stag beetle's tarsal claws is adapted to pull on the legs in order to prevent being dislodged and to balance the static instability during lifting.
- Prothoracic muscles are hypertrophied to support the lifting moment of the body.
- The largest male wins in a majority of the battles. The long mandibles have a functional benefit: they are crucial to grab the rival's legs in order to dislodge it. Males with longer mandibles are also able to bite more forcefully. When possible, males probably evaluate the

opponent's mandible length as a visual cue of its qualities to determine their engagement in the battle.

- Previous investigations showed adaptations of the leg length (to push the body upwards for the lifting movement) and mandible closer muscle size (to enable high bite forces).

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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FIGURE CAPTIONS

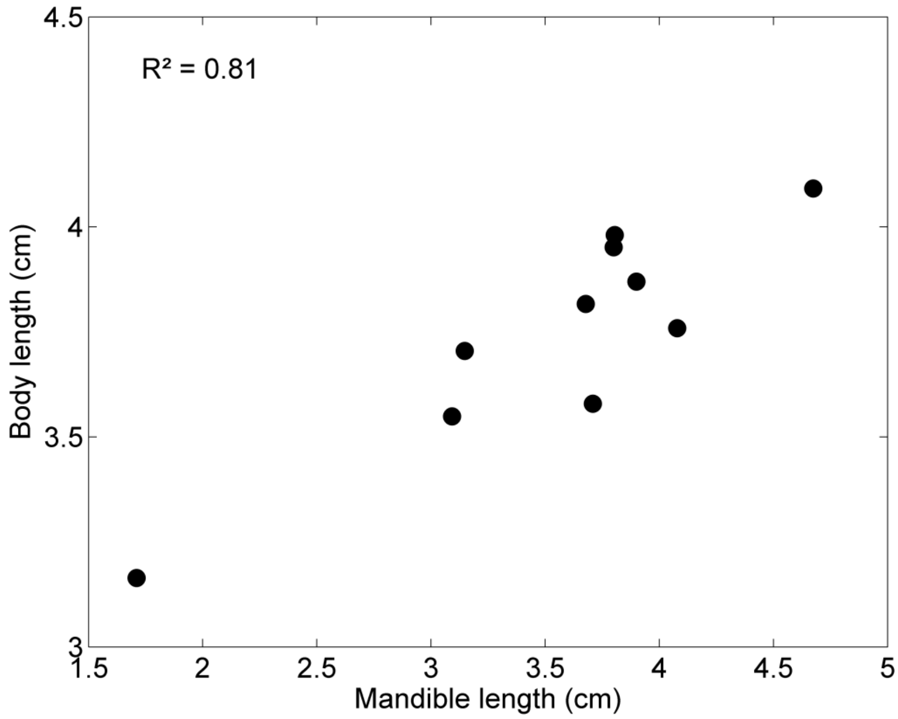

Fig. 1 Correlation between body length (without mandibles) and mandible length and the coefficient of determination (R^2)

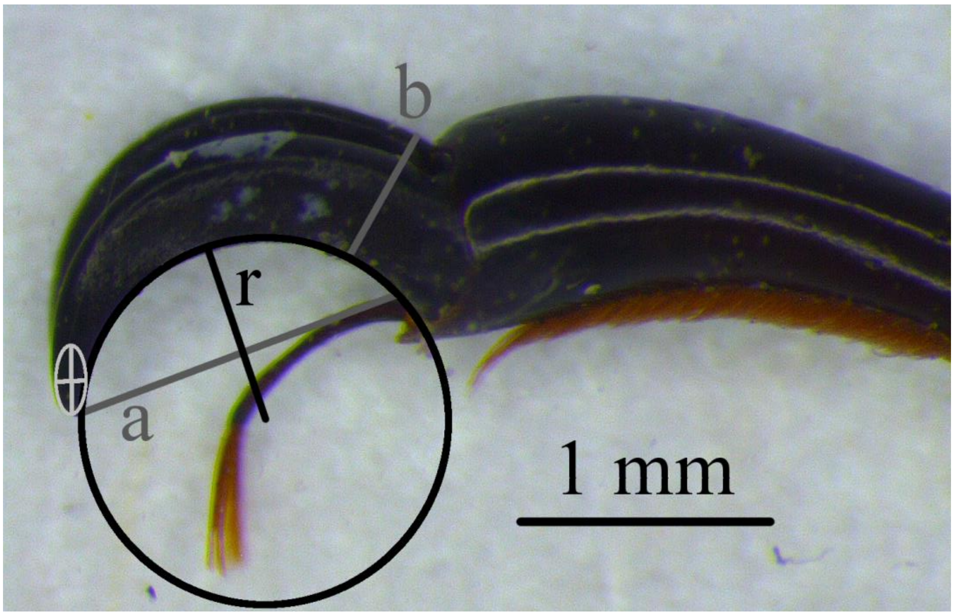

Fig. 2 Tarsal claw of a male stag beetle. a is the claw length, b is the claw height and r is the radius of curvature. The ellipse on the claw tip is drawn to measure the sharpness of the claw

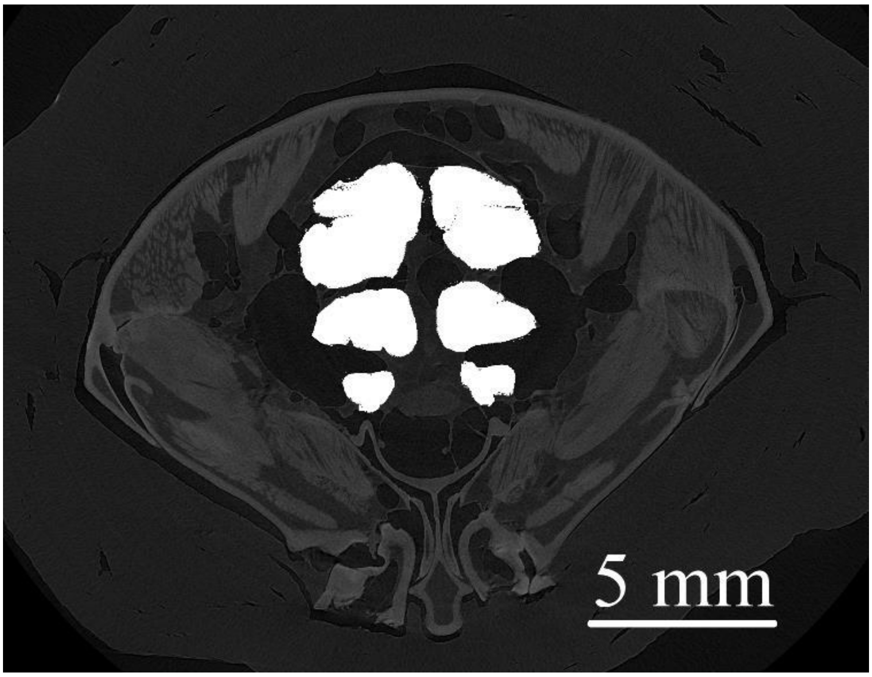

Fig. 3 Transverse micro CT slice image through the prothorax of a male stag beetle specimen. The measured prothoracic muscles are indicated in white

Fig. 4 Male stag beetle in the aggressive posture

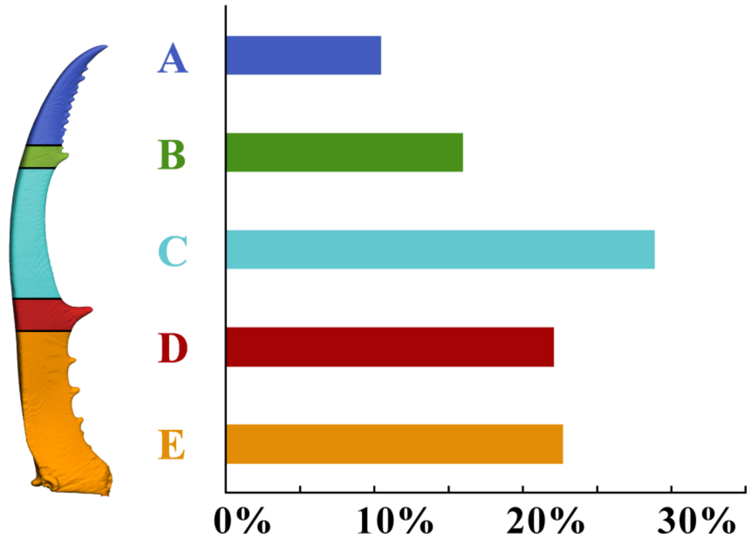

Fig. 5 Proportional use of mandible regions. The colored zones on the mandible (A-E, left) correspond to the bars with the same color (right)

Fig. 6 Representative screenshots from the lifting behavior in stag beetle fights. The complete sequence lasted 0.56 s

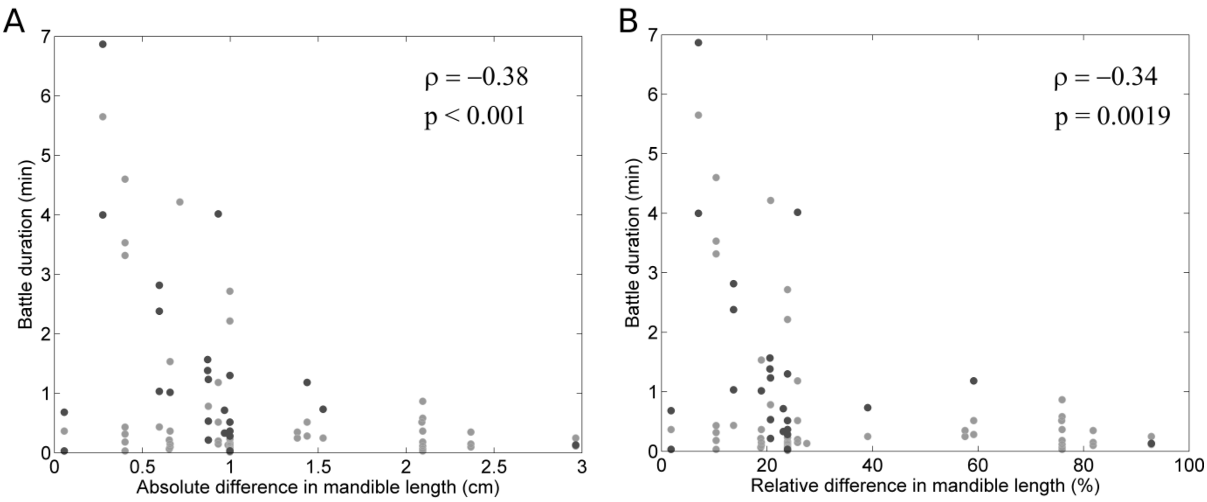

Fig. 7 Duration of the battles in relation to the absolute (A) and relative (B) difference in mandible length between both grapplers, Spearman's rank correlation coefficient ρ and the associated p value. Dark markers indicate fights with a lifting event

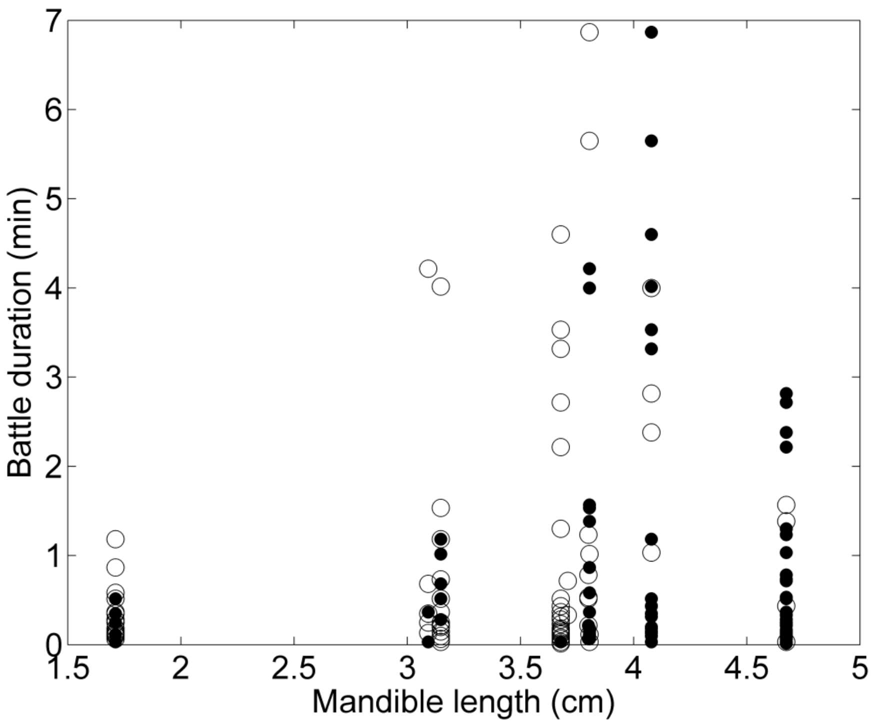

Fig. 8 Duration of the battles in relation to the mandible length of the winner (filled circles) and the loser (open circles)

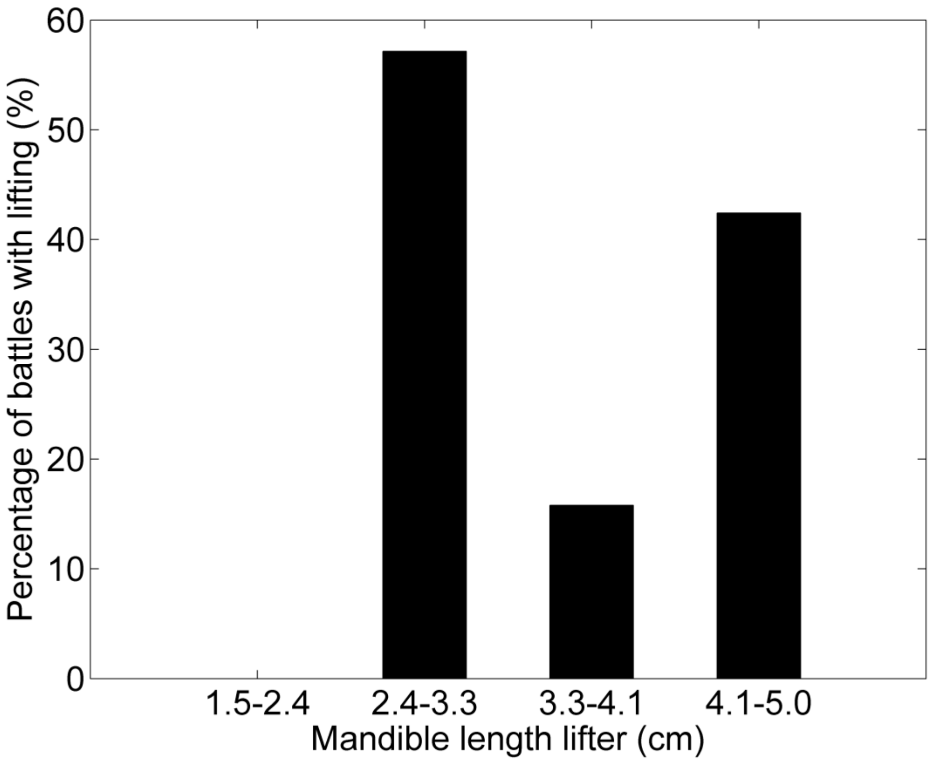

Fig. 9 Percentage of battles with lifting in relation to the mandible length of the male that lifts its opponent

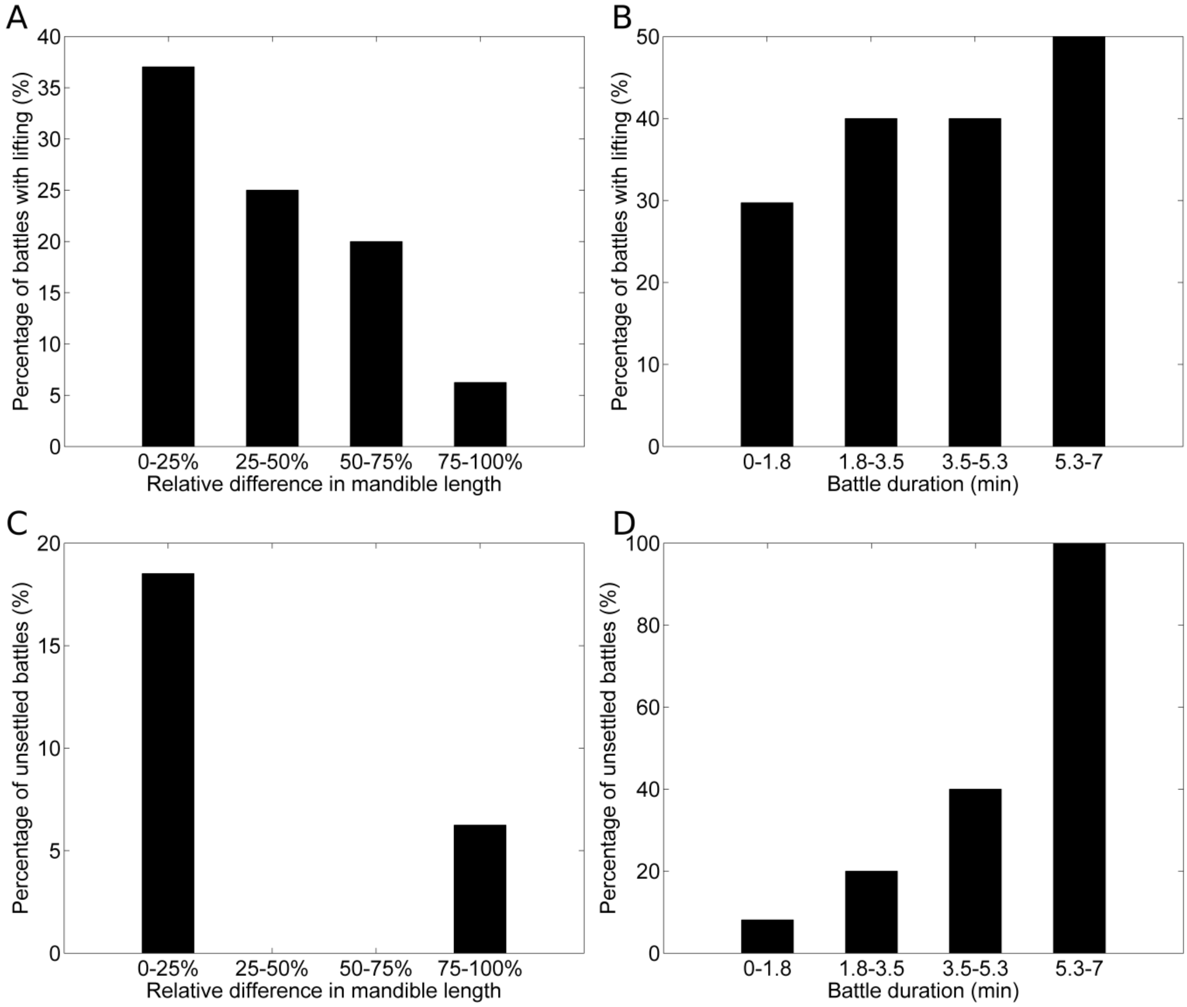

Fig. 10 Percentage of battles with lifting in relation to the relative difference in mandible length of the opponents (A) and the duration of the battle (B); and the relation of the percentage of battles without a clear winner with the relative difference in mandible length of the opponents (C) and the duration of the battle (D)

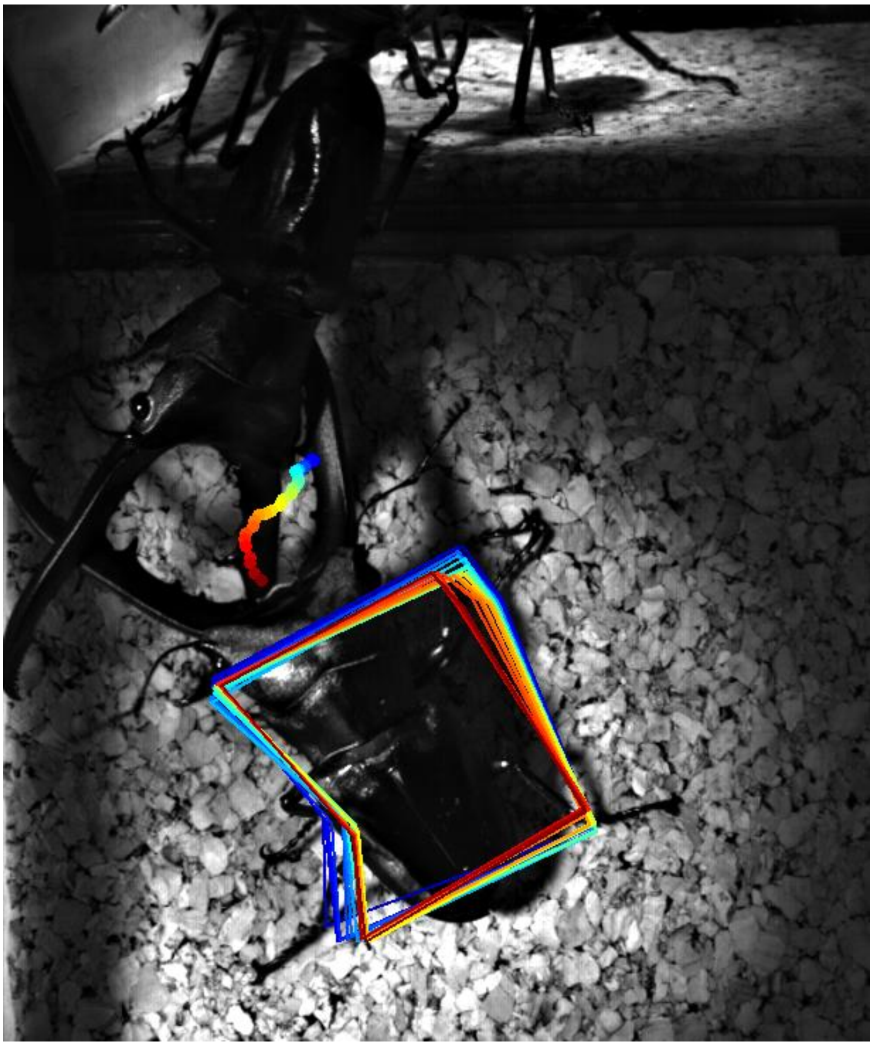

Fig 11 Stability while lifting in a battle. On the screenshot, the beetle in top left corner of the picture is being lifted by the beetle in the bottom. The leg support base of the latter is shown by a polygon, the combined body center of mass of the beetles with a circle. The polygon color gradually differs between frames (from blue over to yellow and then to red). The darkest blue drawings correspond to the depicted screenshot

TABLES

Table 1: Parameters that were scored in the fight behavior analysis.

- **Battle duration**
- **Minimal contact:** only very brief contact, often a tap with the mandibles
- **Attempt of dislodging:** grasping towards legs or body, biting, hauling, pushing, repeated short pulling motions,…
- **Lifting:** moving upwards a rival, the rival does not touch the ground
- **Mandible region used for biting**
- **Identity of winner & loser**
- **Aggressive posture:** standing posture with mandibles opened and highly raised, the midline of the body makes an angle of app. 45° with the substrate
- **Pumping:** repeatedly lifting the body up and down for a few millimeters with all legs
- **Behavior of loser:** running away, falling off tree log

Table 2: Frequencies of actions during battles (percentage and ratio). For the one-tailed paired t-tests, the p-value and test statistic are given.

Behavior	Percentage	Ratio	p-value	Test statistic
aggressive posture before first contact	43% of all battles	36/84		
aggressive posture at first contact	32% of all battles	27/84		
only minimal contact (A)	26% of all battles	22/84		
A & distinct winner (B)	82% of battles in A	18/22		
A & B & male with longest mandibles wins	89% of battles in A & B	16/18	0.0024	t_{17}=3.2
dislodging phase	29% of all battles	24/84		
lifting	33% of all battles	28/84		
distinct winner (C)	86% of all battles	72/84		
C & loser runs away	63% of battles in C	45/72		
C & loser falls	29% of battles in C	21/72		
C & loser stays nearby	8% of battles in C	6/72		
C & male with longest mandibles wins	85% of battles in C	61/72	<0.0001	t_{71}=6.5
C & male with longest body wins	75% of battles in C	54/72	<0.0001	t_{71}=4.9
pumping behavior winner	21% of all battles	18/84		
lifting in blindfolded battle	22% of blindfolded battles	4/15		
male with longest mandibles wins in blindfolded battle	47% of blindfolded battles	7/15		

Table 3: Spearman's rank correlations of the length parameters in Figures 7 and 8 with battle duration. The correlation coefficient ρ and the p value are given.

Figure	Parameter	ρ	P value
7A	Absolute difference mandible length	-0.38	<0.001
7B	Relative difference mandible length	-0.34	0.0019
8	Mandible length loser	0.239	0.030
8	Mandible length winner	-0.010	0.928