Costly sexual dimorphism in *Cyclommatus metallifer* stag beetles

Jana Goyens*1,2, Joris Dirckx2 and Peter Aerts2,3

1Laboratory of Functional Morphology, University of Antwerp, Universiteitsplein 1, Antwerpen, B-2610 Belgium; 2Laboratory of Biophysics and Biomedical Physics, University of Antwerp, Groenenborgerlaan 171, Antwerpen, B-2020 Belgium; and 3Department of Movement and Sport Sciences, Ghent University, Ghent, 9000 Belgium

Summary

1. In many animal species, male armature has evolved through sexual selection. This male weaponry can increase reproductive success, but only if the owner overcomes the associated costs.

2. Male stag beetles bear one of the most extreme examples of male weaponry: their mandibles can be almost as long as their own body. We question whether the armature of male *Cyclommatus metallifer* negatively affects terrestrial locomotion (stability and cost). If so, we investigate whether these effects are potentially compensated by morphological and/or behavioural features, as seen in other specialized insect species. Conspecific females are used to represent the non-dimorphed condition.

3. The presence of the huge male mandibular apparatus shifts the body centre of mass (bCOM) anteriorly. Concomitantly, the male fore limbs are 28% longer and are systematically positioned in a more anterior angular sector than in females. Thus, the rostral border of the support area of the leg tripod also moves forward. This suggests a stability enhancing mechanism. However, in contrast to load-carrying ants, the anteriorly placed bCOM still creates two pronounced statically instable periods each locomotor cycle.

4. Due to the static instability, males must adjust their locomotor behaviour: they walk at higher cycle frequencies when compared to females of the same size, to ensure they proceed to the next stance before bumping to the ground with their heavy heads.

5. Contrary to other specialized load-carrying insect species, the (muscle) mass specific mechanical cost of transport of males exceeds that of females by 40%.

6. Since neither stability nor cost of transport benefit from the male forelimb size and positioning, their role in guaranteeing adequate terrestrial locomotion while carrying an enlarged mandibular apparatus seems doubtful. Instead, the long limbs are themselves functional in fights, by pitching the body upwards in order to throw opponents backwards.

7. The oversized male stag beetle armature comes at high ecological costs: locomotion economics as well as stability clearly suffer from the large mandibles. The observed limb length dimorphism does not prevent this, but should probably be considered part of sexual selection, rather than a compensation for its consequences.

Key-words: cost of transport, leg length, locomotion, *Lucanidae*, mass specific energy, sexual selection, stability

Introduction

Sexual selection has caused the emergence of a wide variety of bodily structures (mandibles, claws, cerci, horns, tusks, antlers, etc.) that are used competitively by males for access to females [for a review, see e.g. Emlen (2008)]. These weapons should provide substantial reproductive benefits, to outweigh the considerable costs of producing and bearing them (Grafen 1990; Iwasa & Pomiankowski 1991). In most cases, this reproductive benefit is the possession of critical resources (which are directly or indirectly related to mating opportunities) or the ability to defend females. A well-known example of the latter are red deer (*Cervus elaphus*), where males defend harems of females by...
fighting rival stags with their antlers (Kruuk et al. 2002). On a much smaller scale, male stag beetles fight each other to obtain mating opportunities (Darwin 1872; Emlen 2008). In these stag beetles, ‘weapons’ emerge as extremely enlarged mandibles, equipped with variable claw-like ornaments (see Fig. 1) (Darwin 1872; Kawano 2000; Kodric-Brown, Sibly & Brown 2006). Kawano (1997, 2000) showed an inverse relationship between the size of these male mandibles and wing size, which was interpreted as a developmental cost related to the sexual dimorphism. This probably results in reduced flight capacity, potentially implying an ecological cost for the males.

In addition to such indirect developmental conflicts, moving around with an extremely large mandibular apparatus may also hamper locomotor performance in a direct way. Large mandibles will hinder when moving on or through a structurally complex terrain. Additionally, altered body proportions and mass distributions will probably influence balance and its intrinsic control, potentially affecting cost and stability of locomotion in both aerial and terrestrial modes (Madewell & Brown 2007). Although this probably applies to many ornamented animals, so far the consequences of weaponry or ornamentation as a whole on locomotion remain poorly understood (Trappett et al. 2013).

In this study, we focus on Cyclommatus metallifer, an Asian stag beetle species that lives in the tropical forests of Sulawesi (Schoolmeesters 2013). The male mandibles are extremely enlarged in this species and their wing size is small (Kawano 1997). Our preliminary observations confirm Kawano’s finding that species with these morphological characteristics are seldom seen flying. Indeed, only very short, clumsy flight bouts are observed for C. metallifer, strongly suggesting the greater importance of terrestrial locomotion in its ecology. In order to assess whether the presence of the enlarged mandibles affects terrestrial locomotion capability, one should ideally compare with non-dimorph conspecific males. In the case of (stag) beetles, females can be used as a proxy for such a hypothetical non-dimorph male (after proper normalization, see further), since males and females are morphologically very similar to each other in stag beetle species without sexual dimorphism (except for the reproductive organs) (Hosoya & Araya 2005; Kawano 2006). We hypothesize that terrestrial locomotion in the dimorphed males will be more costly (mechanically) and less stable than in the non-dimorphed females. Alternatively, males could compensate behaviourally [collectively reflected in the spatio-temporal locomotor variables cf. e.g. (Aerts et al. 2000; Nishikawa et al. 2007)], which may, or may not, be coupled to subtle differences in the locomotor apparatus (e.g. limb lengths).

In order to test this hypothesis, we compare males and females of Cyclommatus metallifer. We measure morphometrics and spatio-temporal variables over a range of speeds, and estimate cost of locomotion (mechanically) and stability.

Materials and methods

**STUDY SPECIES**

Twenty adult *Cyclommatus metallifer* individuals (10 males and 10 females) were obtained from a commercial dealer (Kingdom of Beetle, Taiwan). This species was chosen because of its large mandible size and eagerness to engage in male-male combats. The range of body lengths (including mandibles) was 4.5–7.0 cm for males and 2.4–2.7 cm for females. The animals were individually housed in plastic containers (length 39 × width 28 × height 14 cm), at a temperature of between 20 and 25 °C. They were fed beetle jelly and water *ad libitum*.

**MORPHOLOGY AND MORPHOMETRICS**

After the kinematics experiments (see further), the specimens were sacrificed. Total body mass and segmental masses [head+prothorax (without limbs, HP), meso- & metathorax + abdomen (without limbs, MMA), limb segments; see Fig. 1] were obtained by means of an analytical microbalance (Mettler Toledo MT5; precision: 5 μg). Afterwards, the specimens were fixed in Bouin’s solution (Sigma-Aldrich, St. Louis, MO, USA). Linear measurements [length, width and height of head, prothorax, meso-metathorax and abdomen; femoral, tibial and tarsal length of fore, mid and hind limb; position of limb attachments (coxa-body joints) relative to the long axis of body] were obtained with a digital caliper (Mitutoyo; precision: 0.01 mm) and from high resolution digital photographs using GIMP.
(GNU Image Manipulation Program, free software, www.gimp.org; average precision males: 30 µm pixel⁻¹, average precision females: 20 µm pixel⁻¹). Because the head size differs between sexes, linear measurements were normalized to MMA length (see Fig. 1) to exclude overall size effects. All calculations were performed in MATLAB (MATLAB R2012a, 7.14.0.739, 64-bit version, Natick, MA, USA).

For three male and female specimens, the positions of the centre of mass (COM) of HP and MMA were determined as the point of intersection of the lines of gravity when suspended in three different orientations. The 3D position of segmental COM was expressed relative to the length axis of the according segment (from posterior to anterior) and were averaged for the three specimens for further use in the kinematical analyses.

RECORDINGS AND DIGITIZATION

The beetles were encouraged to run through an open top corridor (length 0.5 m, width 0.1 m, height 0.4 m) by someone following them with a twig. The bottom of the corridor was covered with smooth cork and the walls were made of PMMA (perspex). This setup enabled the simultaneous recording of dorsal and lateral views at 125 frames s⁻¹ (Redlake HR1000 & Redlake Motion PRO cameras, Redlake Digital Imaging Systems, IDT Vision, Tallahasssee, FL, USA; spatial resolution: 1280 × 1024 pixels). We recorded 0.2 m of the corridor, centrally placed at 40 cm distance from the lens (see Video S1 in Supporting Information for a video example). Two sequences for each individual of each sex, consisting of a minimum of two complete consecutive locomotor cycles (strides), were retained for further analysis. A broad velocity range for the species was obtained in this way. 2D digitizations (one per view), calibration (using a 0.04 m cube) and conversion to 3D-coordinates were carried out by means of the video analysis tool DLTvb5 by T. Hedrick [MATLAB application; open source software; (Hedrick 2008)]. The coordinates of twelve anatomical reference points were retrieved frame by frame in each view (see Fig. S1 in Supporting Information for a video example). Two sequences for each individual of each sex, consisting of a minimum of two complete consecutive locomotor cycles (strides), were retained for further analysis. A broad velocity range for the species was obtained in this way. 2D digitizations (one per view), calibration (using a 0.04 m cube) and conversion to 3D-coordinates were carried out by means of the video analysis tool DLTvb5 by T. Hedrick [MATLAB application; open source software; (Hedrick 2008)]. The coordinates of twelve anatomical reference points were retrieved frame by frame in each view (see Fig. S1 in Supporting Information for a video example). Two sequences for each individual of each sex, consisting of a minimum of two complete consecutive locomotor cycles (strides), were retained for further analysis. A broad velocity range for the species was obtained in this way. 2D digitizations (one per view), calibration (using a 0.04 m cube) and conversion to 3D-coordinates were carried out by means of the video analysis tool DLTvb5 by T. Hedrick [MATLAB application; open source software; (Hedrick 2008)]. The coordinates of twelve anatomical reference points were retrieved frame by frame in each view (see Fig. S1 in Supporting Information for a video example). Two sequences for each individual of each sex, consisting of a minimum of two complete consecutive locomotor cycles (strides), were retained for further analysis. A broad velocity range for the species was obtained in this way. 2D digitizations (one per view), calibration (using a 0.04 m cube) and conversion to 3D-coordinates were carried out by means of the video analysis tool DLTvb5 by T. Hedrick [MATLAB application; open source software; (Hedrick 2008)].

Based on the coordinates of the points D, E, F (cf. Fig. 1), the 3D positions of the segmental COMs of the HP and the MMA were determined for each frame. Since the combined limb masses constitute only 8–7% (males) or 11–0% (females) of the total body mass, each limb was represented by a single point mass situated halfway between the tarsi and the place of limb attachment. The weighted mean of the eight point masses (HP, MMA, six limbs) gave us an estimate of the 3D position of the total body centre of mass (bCOM) for each frame. First and second derivatives yield instantaneous linear velocities and accelerations of the bCOM in all three directions.

Spatio-temporal variables

The slope of the regression of the X position of bCOM against time yields locomotion speed (V). Cycle (stride) frequencies (SF) and lengths (SL; forward displacement of the bCOM during one cycle) and step lengths (SP; forward displacement of the bCOM during limb contact) were obtained. As a measure for limb protraction, the maximal protracted position of the distal ends of the tibias with respect to the coxa-body joints (e.g. distance p in Fig. 1) was used. To calculate the dimensionless speed [Vdim = V (lLG⁻¹)] and dimensionless frequency and length [of steps and strides; SFdim = SF(lL/g)¹/²; SLdim = SL/lL; SPdim = SP/lL], we used tibia length (lL) of the fore limb as the locomotion relevant length [cf. (Alexander 1977; Aerts et al. 2000); g = 9.81 m s⁻²].

Energetics

The total mechanical energy level of a body is the sum of the potential and mechanical energy components. When external forces (other than gravity) perform work on that (multi-segmented) body, its total mechanical energy changes. As a simple estimation of the cost of transport (J kg⁻¹ m⁻¹), we assume all of this work derives from the locomotor muscles (i.e. no temporary storage and recoil of strain energy in elastic structures). Potential energy was obtained from the vertical position of the bCOM (Ep = mgh). The translational kinetic energy (Ekx,埃 = 0.5 mVx,y,z²) was calculated from the linear velocities of the bCOM, with m the total body mass. To obtain rotational kinetic energy (Er = 0.5 Ix,y,z²), angular velocities (ω) and moments of inertia (I) were calculated with respect to a local frame of reference (x, y, z) attached to the bCOM. The x-axis coincided with the long body axis (axis DF in Fig. 1), the z-axis laid in the midsagittal plane perpendicular to x (pointing upwards) and y = z × x. Roll (rotation about x) was determined based on the 3D position of the eye relative to the x-axis. Displacements of the long body axis DF were used for pitch & yaw (rotation about y and z axes respectively). To estimate instantaneous I for male and female specimens, their body was modelled as an ellipsoid with an homogenous mass distribution. The semi-principal axis in x direction was half of the long body axis (DF in Fig. 1). The half of the maximal width and height of the body were taken as the semi-principal axes in y and z direction. Leg masses were not subsumed in the calculation of I, as their masses are small compared to body mass and are distributed evenly around the body.

The positive increments of the external mechanical energy fluctuations over time were summed for all components (3 × translational, 3 × rotational and potential), thus no energy transfer between segments was assumed. Divided by the thoracic mass and the distance covered, this yields (muscle) mass specific cost of transport (COT; J kg⁻¹ m⁻¹). Thoracic mass (and not total body mass) was used for this normalization, as only the thorax contains the locomotor muscles (DuPorte 1964).

The descriptive gait variables % recovery and phase shift assess the occurrence of gait changes over the speed range. % recovery quantifies the energy exchange between kinetic and potential energy, and hence determines whether locomotion is pendulum like (as in walking) or more bouncing like (as in running). We calculated % recovery using the formula of Blickhan and Full (Blickhan & Full 1987):

$$\% \text{ recovery} = \frac{\sum \Delta E_k + \sum \Delta E_p - \sum \Delta E_t}{\sum \Delta E_k + \sum \Delta E_p}$$

with $\sum \Delta E_k$, $\sum \Delta E_p$ and $\sum \Delta E_t$ the sums of the positive changes in respectively the translational kinetic, potential and total energy of the bCOM. The phase shift between the maxima of the kinetic and potential energy within one stride also distinguishes between walking (phase shift close to 180°) and running (phase shift close to 0°) gaits. Following Full and Tu (Full & Tu 1990), we calculated phase shift as the time-delay between maximal kinetic and potential energy, relative to stride duration. Bandpass filtered values of $E_k$, and $E_p$ (50% around step frequency) were used to reduce noise.
**Static stability**

Based on limb contacts, alternating supporting tripods were determined throughout the locomotion bouts (Hughes 1952; Reinhardt, Weihmann & Blickhan 2009) (see Fig. 2). As this tripod mode turned out to be systematically used (relative phase of the limbs is not significantly different from perfect tripod gait; sign test: $P = 0.60$), the vertical projection of the position of the bCOM with respect to these triangles was used as a measure for static stability (Ting, Blickhan & Full 1994). This was quantified by the stability margin (SM, see Fig. 1); the distance of the vertical projection of the bCOM to the closest edge of the support triangle and, therefore, the animal is statically instable. The SM was normalized as the percentage of the maximal instantaneous stability margin (SM, see Fig. 1): the distance of the vertical projection of the bCOM to the support triangle and females respectively. Taking size into account, similar dimensionless velocity for all limbs (‘slope’, Table 3, and % recovery and phase shift are low, the beetles neither differ significantly between slow and fast trials (Wilcoxon rank sum test, see Fig. 3 and Table 2). Together, these observations indicate that, although quite variable, males and females move in a dynamically similar way. Phase shift and % recovery are similarly distributed between slow and fast trials (Wilcoxon rank sum test, see Fig. 3 and Table 2). Moreover, since % recovery and phase shift are low, the beetles can therefore be considered to be dynamically running (bouncing gait) over the entire speed range, which is consistent with what is found for other insects (Blickhan & Full 1987; Full & Tu 1990). Average locomotion velocity is $4.22 \pm 0.88$ cm s$^{-1}$ and $2.73 \pm 0.59$ cm s$^{-1}$ for males and females respectively. Taking size into account, similar ranges of dimensionless velocity are observed (males: $0.095 - 0.19$; females: $0.079 - 0.18$). Dimensionless stride lengths and frequencies are positively correlated with dimensionless velocity for all limbs (‘slope’, Table 3, and Fig. 4, ANOVA). The same holds true for the fore limb step length only. Mid and hind limb step lengths show high variability. Dimensionless, for any speed in the observed

**Results**

**MORPHOLOGY AND MORPHOMETRICS**

Table 1 provides mean and standard deviations of male and female morphometrical measurements, and the $P$-value of a Wilcoxon rank sum test with null hypothesis that males and females have equal medians. Males are considerably larger than females. In particular their heads and mandibles are much larger and differ in shape, also when normalized to the MMA size. In contrast, the shape of the MMA does not differ between males and females (aspect ratio = length/width; Wilcoxon rank sum test: $P = 0.52$; see also Fig. 1). This supports our normalization of the linear morphometrics to MMA length.

The heavy male head shifts the bCOM anteriorly when compared to females. As a result, the bCOM lies in front of the body attachment of the middle limbs, instead of behind them (as is the case in females). Males have longer limbs, but the difference with female limb length sequentially decreases from the anterior to the posterior.

**KINEMATICS AND DYNAMICS**

Male and female stag beetles both run with an alternating tripod gait (see Fig. 2). Additionally, the gait variability does not differ significantly between sexes (two-sample $F$-test, $P = 0.57$) and no significant differences in % recovery or phase shift are found (Wilcoxon rank sum test, see Fig. 3 and Table 2). Together, these observations indicate that, although quite variable, males and females move in a dynamically similar way. Phase shift and % recovery do not differ significantly between slow and fast trials (Wilcoxon rank sum test, see Fig. 3 and Table 2). Moreover, since % recovery and phase shift are low, the beetles can therefore be considered to be dynamically running (bouncing gait) over the entire speed range, which is consistent with what is found for other insects (Blickhan & Full 1987; Full & Tu 1990). Average locomotion velocity is $4.22 \pm 0.88$ cm s$^{-1}$ and $2.73 \pm 0.59$ cm s$^{-1}$ for males and females respectively. Taking size into account, similar ranges of dimensionless velocity are observed (males: $0.095 - 0.19$; females: $0.079 - 0.18$). Dimensionless stride lengths and frequencies are positively correlated with dimensionless velocity for all limbs (‘slope’, Table 3, and Fig. 4, ANOVA). The same holds true for the fore limb step length only. Mid and hind limb step lengths show high variability. Dimensionless, for any speed in the observed

---

**Table 1.** Mean and standard deviations of morphological measurements, its male to female ratio and the $P$ value of Wilcoxon rank sum tests ($N = 10$). The length variables are normalized to meso-metathorax and abdomen length.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean $\pm$ standard deviation</th>
<th>Male/female ratio</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass ($kg \ 10^{-3}$)</td>
<td>$1.36 \pm 0.28$ $0.55 \pm 0.10$</td>
<td>2.47</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>MMA length ($m \ 10^{-3}$)</td>
<td>$1.96 \pm 0.15$ $1.47 \pm 0.05$</td>
<td>1.33</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Normalized body length</td>
<td>$1.67 \pm 0.03$ $1.52 \pm 0.03$</td>
<td>1.10</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Normalized mandible length</td>
<td>$1.30 \pm 0.21$ $0.20 \pm 0.02$</td>
<td>6.57</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Normalized head width</td>
<td>$0.51 \pm 0.03$ $0.40 \pm 0.03$</td>
<td>1.27</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Normalized head length</td>
<td>$0.38 \pm 0.02$ $0.22 \pm 0.01$</td>
<td>1.74</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Normalized fore limb length*</td>
<td>$1.33 \pm 0.06$ $0.97 \pm 0.02$</td>
<td>1.38</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Normalized mid limb length*</td>
<td>$1.17 \pm 0.05$ $0.93 \pm 0.05$</td>
<td>1.26</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Normalized hind limb length*</td>
<td>$1.27 \pm 0.05$ $1.08 \pm 0.04$</td>
<td>1.18</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Relative position bCOM (%)</td>
<td>$0.58 \pm 0.04$ $0.49 \pm 0.01$</td>
<td>1.18</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Relative position attachment middle legs (%)</td>
<td>$0.50 \pm 0.02$ $0.54 \pm 0.01$</td>
<td>0.93</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

*Limb lengths $=$ femoral + tibial + tarsal lengths.
range, males use shorter strides at higher frequencies than females do.

Only the fore limb is protracted further in males compared to females \( (P < 0.001; \text{males}: 0.59 \pm 0.08 \text{ abdomen–thorax lengths}; \text{females}: 0.33 \pm 0.09 \text{ abdomen–thorax lengths}).

Mass specific cost of transport is not significantly related with dimensionless speed (Table 3). However, on average mass specific COT is 38% higher for males than for females (males: 4.06 ± 1.12 Jm\(^{-1}\)kg\(^{-1}\); females: 2.53 ± 0.76 Jm\(^{-1}\)kg\(^{-1}\)).

**Static stability**

In Fig. 5, the mean % SM of the tripod (indicated in Fig. 1) is represented for males and females. The results of the other tripod have the same profile, but are in antiphase. Fast running males have a distinct unstable phase at the end of each tripod stance (negative % SM values). When the bCOM lies outside the support triangle, always the diagonal frontal edge has been crossed (v; cf. Fig. 1). This causes a combination of head-down pitch and lateral
roll towards the side of the body with only one limb on the substrate. The average time profiles of females always stay positive, but like males, minimal stability is reached just before the next tripod is placed on the ground. These results are presented separately for high and low velocities (above and below mean dimensionless velocity respectively), because the separated male $R^2$ values were higher than the $R^2$ of all trails combined (see Fig. 5). The influence of velocity on static stability is also clearly visible in Fig. 6. The analysis of covariance (ANCOVA, Table 3) confirms this: both sexes are statically more instable at higher speeds. Additionally, it reinforces the fact that males are less stable than females. Large males are slightly less stable than males with a shorter than average body length, but the difference is considerably smaller than between fast and slow males. Males do not enhance their static stability by increasing their sprawl (Wilcoxon rank sum test, see Table 4).

Fig. 4. Correlations between dimensionless gait variables [step length ($SpL_{dim}$), stride length ($SL_{dim}$) and stride frequency ($SF_{dim}$)] and dimensionless velocity. Results are given for males (filled circles) and females (open circles) and separately for fore, middle and hind limbs. Also, the linear regressions of the ANCOVA’s are shown (solid lines for males and dashed lines for females).

Fig. 5. Mean and standard deviation of the percent stability margin of one tripod for males (a) and females (b). Negative values indicate static instability. Results are shown separately for speeds higher (horizontal lines) and lower (vertical lines) than average speed. During the grey coloured part of the stride, the tripod does not stand on the substrate. The coefficients of determination of the fast trails ($R^2_f$), slow trails ($R^2_s$) and all trails together ($R^2_a$) are given for males and females.

Fig. 6. Linear regression between the percent stability margin and dimensionless velocity, for males (solid circles and solid line) and females (open circles and dashed line).
Discussion

Sexually selected ornaments have been shown to be costly, for example, by increasing the predation rate [e.g. barn swallows Hirundo rustica; (Moller & Nielsen 1997)], causing developmental constrains [e.g. a wide range of stag beetle species (Kawano 1997) and trade-off with eye, antennae or wing size in the dung beetle genus Onthophagus (Emlen 2001; Emlen et al. 2005)], limiting mating success [e.g. stag beetle species Lucanus cervus (Harvey & Gange 2006) and dung beetle species Euoniticellus intermedius (Pomfret & Knell 2006)], and impairing locomotor performance [e.g. scarlet-tufted malachite sunbirds Nectarinia johnstoni (Evans & Thomas 1992)]. The present paper aims to evaluate the effect of sexual dimorphism on the cost of transport as well as on the stability of terrestrial locomotion in stag beetles. It was assumed that the large mandibular system of males would come at a cost, yet postulated to be possibly compensated by their locomotor behaviour and/or locomotor morphology.

In C. metallifer, sexual selection not only increased total body weight in males, but also changed the mass distribution. Due to the enlarged mandibles, but primarily because of the disproportionally large head housing the jaw muscles (Shiokawa & Iwahashi 2000; Goyens et al. 2014), the bCOM shifts 9% more anteriorly. Consequently, the male bCOM lies in front of the attachment of the middle legs, instead of behind them. This obviously results in an increased risk for static instability as the bCOM passes the frontal margin of the supporting triangles more readily. The observed longer length and increased protraction of the fore limbs may suggest morphological and behavioural compensations in males to improve stability. As with stick insects (Cruse 1976), the fore limbs are the longest limbs in our male stag beetles, while this is not always the case in insects [e.g. cockroaches (Ting, Blickhan & Full 1994) and wood and fire ants [Fig. 1 in (Tschinkel, Mikeyev & Storz 2003; Reinhardt, Weihmann & Blickhan 2009)]. Even more importantly, these fore limbs are further protracted when initiating stance, thus shifting the frontal margin of the support triangles forward. This strategy to improve stability is observed in desert ants that carry loads with their mandibles (Zollikofer 1994). Nevertheless, male stag beetles are far less statically stable than females (see Figs 5 and 6). The observed male instability is also more distinct than that measured for other insects (Ting, Blickhan & Full 1994; Zollikofer 1994). Consistent with other insects, static stability decreases with increasing locomotor speed and reaches a minimum immediately before the second leg tripods lands, which initiates the double stance period [e.g. cockroaches (Ting, Blickhan & Full 1994) and desert ants (Zollikofer 1994)]. A comparison between large and small males revealed a trend towards lower stability in larger males. Although our data cannot prove it statistically, this finding suggests that the consequences of the weaponry increase with size, which may put a direct evolutionary constraint on weaponry size. Ting et al. (Ting, Blickhan & Full 1994) showed that static instability is dynamically compensated in fast running cockroaches. This could occur in our male stag beetles when horizontal instantaneous accelerations during stance are large enough to support upwards pitching [cf. for instance (Aerts et al. 2003)]. To determine this in a reliable way, 3D ground reaction forces should be measured. Although such recordings are not available, it seems very unlikely that dynamic stabilization plays an important role. First, horizontal accelerations (as deduced from the bCOM displacements; second time derivative) are on average almost four orders of magnitude smaller ($a_{horizontal} = 1.3 \times 10^{-3} \pm 0.8 \times 10^{-3} \text{ms}^{-2}$) than the gravitational accelerations (the latter causing downwards pitching and rolling as soon as a male becomes statically unstable). Secondly, late in stance (i.e. the statically unstable period), horizontal accelerations seem to point primarily backwards, hence increasing the destabilizing effect. Thus, lacking static stability in 33.3% of stance and without evidence for dynamic stabilization, fast running male stag beetles risk falling over. They do not sprawl out their limbs further to enhance their static stability. Instead, their only strategy, to avoid tipping over and touching the ground with their bodies or head during running, appears to be the timely planting of the next tripod. Indeed, at any given dimensionless speed, higher dimensionless stride frequencies are observed in males. Yet, given the fact that double stance phase remains rather short in males, it can be assumed that erroneous forward toppling will occur during terrestrial locomotion bouts. This is confirmed by our occasional observations of males bouncing against the ground with their head when running at high speed.

If not for stability, the question remains regarding what function the longer male limbs have. In our working hypothesis, it was also postulated that differences in locomotor behaviour and morphological characteristics (besides the mandibular apparatus) may also compensate for presumed effects on the cost of transport. Again, this is not confirmed by the present findings. The long legs hardly counterbalance (if at all) the effects of sexual selection on the (muscle) mass specific cost of transport: the COT is almost 40% higher for males than for females. This might seem to contradict the trend of lower COT in larger animals [including arthropods; (Blickhan & Full 1993)]. However, in male stag beetles the disproportionally heavy heads and mandibles account for the largest part of the extra weight, and do not promote locomotor performance.

<table>
<thead>
<tr>
<th></th>
<th>Males (10^3)</th>
<th>Females (10^3)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fore limb</td>
<td>1.26 ± 0.30</td>
<td>1.50 ± 0.46</td>
<td>0.10</td>
</tr>
<tr>
<td>Mid limb</td>
<td>1.71 ± 0.27</td>
<td>1.86 ± 0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>Hind limb</td>
<td>1.09 ± 0.18</td>
<td>0.96 ± 0.18</td>
<td>0.20</td>
</tr>
</tbody>
</table>

in any way. So despite permanently carrying their ‘load’ permanently, these beetles apparently do not have the reduced load carriage cost seen in some other specialized species [e.g. certain rhinoceros beetles, cockroaches and ants (Lighton, Weier & Feener 1993; Kram 1996)]. It can be remarked that estimated costs are maximal in that no energy transfer is assumed and no potential energy recovery mechanisms are taken into account. However, since similar simplifications are applied to both the males and females (representing the non-dimorphed condition), it can safely be assumed that percent difference between both sexes also remains largely identical.

Despite observed morphological and behavioural differences of the locomotor apparatus, male cost of transport and stability actually do suffer from the sexually selected dimorphism (i.e. the enlarged manibular system). It is not yet known though, how this performance drawback translates into fitness. Therefore, we do not know whether the reproductive advantages of the sexually selected armatures are actually balanced by natural selection. Impaired locomotion could increase predation rate, or hamper males in finding mates or oviposition sites. For example for the European stag beetle species Lucanus cervus, predators (mainly birds) are an important cause of death and males search actively for females and their nest sites (Percy 1998; Fremlin 2009; Harvey & Gange 2011). In this species, females are known to run further and more frequently than males (Rink & Sinsch 2007; Fremlin 2009). If the same could be said for Cyclomatus mettallifer beetles (which is, as yet, unexplored in the literature), this could be the consequence of their impaired locomotion, but the lessened importance of locomotion in male ecology may as well explain why males can cope with this impediment.

So, again, why do males have the longer limbs? And why does this difference between males and females sequentially increase from the posterior to the anterior limbs? An answer can possibly be found in the sexual selection for the male–male combat itself: fighting males grip each other with the mandibles and attempt to throw their opponent upwards and backwards over their own body. To enable this action, the body pitches upwards by subsequently extending the long fore and middle limbs (own observations). Alternatively, the longer legs may play a role in mating stability. From both points of view, the limb dimorphism seems not to be a consequence of sexual selection in the sense that it compensates for negative effects, but it is rather an integral part of it.

Acknowledgements

We thank Ms. Josie Meaney for English proof reading. This study was funded by BOF grant [ID BOF UA 2011-445-a] of the Research Council of University of Antwerp.

Conflict of interests

No conflict of interests declared.

References


*Received 21 June 2013; accepted 30 April 2014*

**Handling Editor:** Goggy Davidowitz

**Supporting Information**

Additional Supporting information may be found in the online version of this article:

**Video S1.** High speed video of a running male and female stag beetle.