

# Post-eclosion behaviour in the rose chafer *Cetonia aurata* (Coleoptera: Scarabaeidae: Cetoniinae)

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## KEY WORDS

Diuresis, genitalia, haemolymph, mycangium, pupa

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Some insects exhibit unique behaviours related to the vertical transmission of their symbionts. For example, in the stag beetles (Scarabaeoidea: Lucanidae) soon after post-eclosion the female, with the abdomen fully distended, sweeps the pupal chamber with the mycangium, several times for a long period; this in order to retrieve the fungal symbionts left by the larva before metamorphosis; whereas the male ecloses with everted genitalia and retreats it shortly afterwards. This remarkable sexually dimorphic behaviour is accompanied by a great loss of fluid. The object of this study was to find out if these observations were unique to the Lucanidae's post-eclosion. As of the Scarabaeoidea the Lucanidae is the only family who possess a mycangium, a related scarab was chosen: *Cetonia aurata* (Scarabaeidae: Cetoniinae). Six male and six female pupae were followed. There was a perfect overlap with the stag beetles regarding their wing development and exudation of fluid due to diuresis - a normal phenomenon during post-eclosion. All individuals, except one male, excreted droplets visible with the naked eye soon after eclosion and until the hindwings were folded, some six hours later. All underwent a marked weight loss (20-30%) during that stage; by the end of the study this reached 43%, roughly the same as with stag beetles. The rose chafers did not exhibit sexual dimorphism; it took both males and females a few hours to retract the tip of their abdomen; about the same time that it took the male stag beetle to retract its genitalia. Thus during post-eclosion only the female stag beetle mycangium-related behaviour is related to the acquisition of their symbionts - unique to the Lucanidae.

## Introduction

Some insects exhibit unique behaviours related to the vertical transmission to their symbionts (Hosokawa & Fukatsu 2020). For example, during the past ten years some remarkable behaviours have been discovered regarding the vertical transmission of external yeast-like symbiotic fungi in beetles with an ovipositor associated mycangium (a structure that carries viable fungal inoculum), first from larva to adult, then from adult to egg. It was only recently discovered how a lizard beetle and stag beetles retrieved the symbionts left by the larva, soon after eclosion (Tanahashi *et al.* 2015, Toki *et al.* 2012). A female stag beetle ecloses with the ninth abdominal segment everted, but soon afterwards unfolds the mycangium, which is in a fold under the eight tergite (Tanahashi *et al.* 2010), and sweeps the pupal chamber. This is in order to retrieve the xylose-fermenting yeasts left in the pupal chamber when the larva emptied its gut before moulting; this behaviour is repeated several times (Fremlin & Tanahashi 2015). Later, it is understood that during oviposition the female will swab the area near the egg with the mycangium to inoculate it with the symbionts, thus facilitating the larval development. This maternal behaviour has been described for the lizard beetle *Doubledaya bucculenta* Lewis (Toki *et al.* 2012), but only

observed once with a stag beetle (Tanahashi *et al.* 2010). From then on the symbionts will become part of the larvae's digestive system and also the substrate; so every time that the larvae moult and shed their hindgut, which is part of the exoskeleton, they can easily be retrieved, except when they moult into a pupa. Since the diet in the adult stage is based on sugary liquids devoid of such symbionts, the teneral female has to retrieve them from the pupal chamber and the cycle will start again. This remarkable post-eclosion mycangium-related behaviour was first described in several Japanese species (Tanahashi *et al.* 2015) and later was confirmed for the European stag beetle *Lucanus cervus* Linnaeus females during post-eclosion (Fremlin & Tanahashi 2015). When studying the males of *L. cervus*, it was discovered that they emerged with the everted genitalia, but retracted it shortly afterwards (Fremlin & Tanahashi 2015). In an identical study with the lesser stag beetles *Dorcus parallelipipedus* Linnaeus I observed the same behaviour for the males and females (M. Fremlin personal observations). Moreover, both species, regardless of their sex, exuded droplets of clear fluid, not just during eclosion, but during pupation as well; their pupal chamber became very moist and they lost about 40% of their weight. This raised several questions, the most important being the source and adaptive function of the



1. *Cetonia aurata* imagos foraging on a cardoon *Cynara cardunculus* flower. Photo: Maria Fremlin

1. Adulten van *Cetonia aurata* foeragerend op een bloem van kardoen *Cynara cardunculus*.

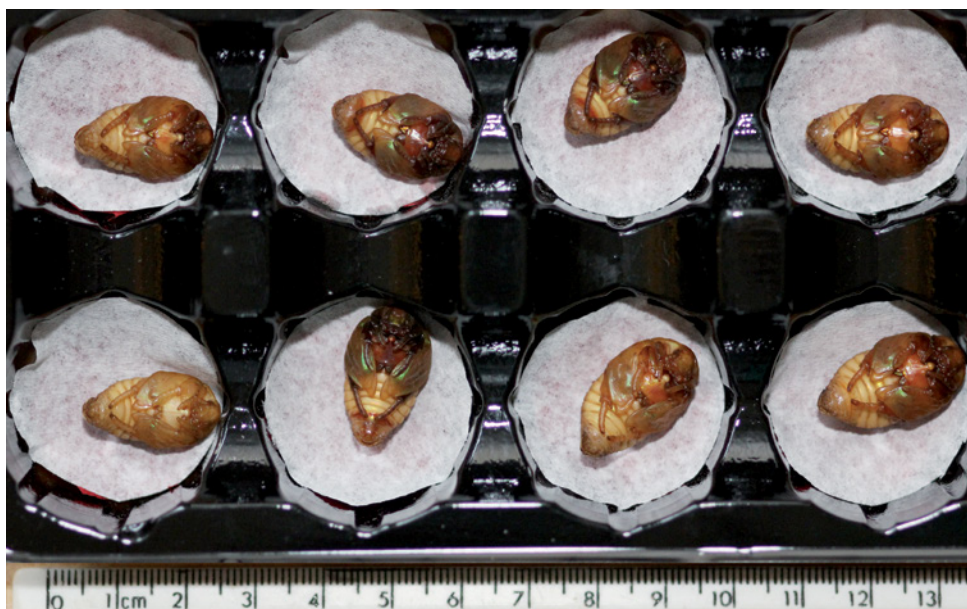
exudation of this fluid; and whether the male behaviour was linked to the female's. Therefore the object of this study was to monitor the post-eclosion of a scarab beetle in another family in order to find out if these observations were unique to their post-eclosion behaviour. As of the Scarabaeoidea the Lucanidae are the only family to possess a mycangium, I chose the locally abundant rose chafer *Cetonia aurata* Linnaeus (Scarabaeidae: Cetoniinae) (Fremlin 2018) (figure 1). This species compared to the stag beetles has very slight sexual dimorphism during the adult stage. The most obvious is that the ventral view of the abdomen in males has a depression running through the middle, but is convex in the females (Cristóvão *et al.* 2020). The Cetoniinae have been the object of many studies (for example, Micó *et al.* 2008, Šípek *et al.* 2012a, 2012b, Vendl *et al.* 2016, 2018), but not yet of their post-eclosion. This is the first report of that behaviour and weight loss compared with the Lucanidae.

## Materials and methods

This study was the result of a sudden inspiration. I had just finished some post-eclosion experiments with stag beetles when it

occurred to me to do the same with the rose chafer *C. aurata*. This species also breeds in our garden and as I had been studying their larval stage I had easy access to specimens, which had been kept in terraria indoors. I had to act promptly because the season was already quite advanced and, like the stag beetles, they pupate in the summer. The next day, 23 July 2015, I collected eight cocoons from a terrarium with leaf mould; all of them were still in their pupal stage, fortunately. From then on I had to improvise, as I had never done this before with this species. The pupae fit very closely inside their hard-shelled cocoons, so had to be extracted very carefully in order not to damage them. I decided to place them in the moulded cavities of an old chocolate box, with the bottom lined with three layers of paper, starting from the bottom: transparent printing paper, red dyed paper, paper towel (figure 2).

The use of red dyed paper was to indicate if the top layer had been wetted. This was inspired by the experiments described in Thomas Eisner's book 'For love of insects' (2004). Chemically defended insects, like bombardier beetles and cockroaches, were placed on indicator paper to illustrate the sprays from their glands when stimulated. A while ago I had prepared it by wetting tissue paper with diluted red food dye, and then drying it up;



2. Late *Cetonia aurata* pupae relocated from their cocoons to a chocolate box. Photo: Maria Fremlin

2. Ver ontwikkelde *Cetonia aurata*-poppen vanuit hun cocons geplaatst in een chocoladedoos.





**3.** *Cetonia aurata* pupae and post-eclosion imagos. First row: (a) Ventral view of early stage pupae, sexually dimorphic in the 9th sternite (s9): female medial genital pore, left, male genital ampulla, right. (b) Pharate female imago inside the pupa cuticle (note the uneven melanisation). (c) Dorsal view of a female imago soon after the wings appear from under the pale elytra. Second row: teneral imagos exuding droplets; the abdomen is distended and the ninth segment is clearly visible. (d) Male. (e) Female with hindwings fully expanded. (f) Female on a red stained tissue, note the exuvia sticking to the left hindwing. Third row: two teneral imagos exuding droplets at a more advanced stage; note the last abdominal segments are no longer visible. (g) Male with a damaged left hindwing. (h) Male with folded hindwings. (i) Dorsal view of the same male half an hour later. Abbreviations: d, droplet; s8, 8th sternite; s9, 9th sternite; t8, 8th tergite. Photos: Maria Fremlin

**3.** *Cetonia aurata*-poppen en juist ontpopte adulten. Eerste rij: (a) jonge poppen, ventraal; in het negende sterniet (s) van het vrouwtje huist de mediale genitaalporie, links, en bij het mannetje de genitale ampulla, rechts. (b) Vrouwtje vlak voor de verpopping in de cuticula van de pop (let op de kleurverschillen van het lichaam). (c) Dorsaal zicht op een vrouwelijke adult, kort nadat de vleugels verschijnen van onder de bleke dekschilden. Tweede rij: niet-uitgeharde adulten die druppels afscheiden; het abdomen is opgezwollen en het negende segment is goed te zien. (d) Mannetje. (e) Vrouwtje met volledig gestrekte vleugels. (f) Vrouwtje op een tissue met rode vlekken, zie dat de exuvia plakt aan de linker-vleugel. Derde rij: twee niet-uitgeharde adulten die druppels uitscheiden in een verder gevorderd stadium waarbij de laatste sternieten niet meer zichtbaar zijn. (g) Mannetje met een beschadigde linkervleugel. (h) Mannetje met gevouwen vleugels. (i) Dorsaal zicht op hetzelfde mannetje een half uur later. Afkortingen: d, druppel; s8, 8e sterniet; s9, 9e sterniet; t8, 8e tergiet.

I have used it successfully with the stag beetles (M. Fremlin unpublished). After the first two eclosions, the use of the red dyed paper became redundant because the droplets were visible with the naked eye; from then on I replaced it with plain tissue paper.

One week later, it became clear that I had three males and five females. The pupae have ten abdominal segments and can be sexed by the dimorphism on ninth abdominal ventrite (Sousa et al. 2018). In the male it houses the genital ampula, clearly visible, and in the female the genital pore has two tubercles; the tenth segment is barely visible (figure 3a). In order to balance their sex ratio, I added three males and one female pupae to another box prepared the same way. Three of them were sourced from another terrarium in the garage and the other one was from a cocoon found in our garden compost, which is a natural habitat for this species.

All individuals were weighed promptly after they were removed from their cocoons and thereafter, whenever possible, soon after eclosion; plus at the end of the study. I used a Scalix CB-310 electronic scale, to 0.01 g accuracy. Unfortunately, the final weight of most of the females could not be tracked down properly because they had escaped from their cells, which should have had a cover. Undisturbed teneral imagos remain quiescent in their cocoons for a while.

The chocolate box set-up only allowed photography of the top view of the specimens. To allow for a good rear view of the post-eclosed specimens' genitalia I moved some to another place: a florist's oasis block with open-ended depressions at the edge, lined with tissue paper, and covered them with a Perspex lid, hoping that this set-up would facilitate the movements of the specimens during eclosion. I used an EOS 550D SLR Canon camera fitted with a Sigma 105 mm objective, and a flash Canon Speedlight 430EXII. The camera was either set on a Jessops copy stand or a Velbon tripod (PH-248 Head).

## Results

*Cetonia aurata* pupae are a pale colour during the early stage (figure 3a), but gradually become darker as they age. All pupae in this study were quite dark already (figure 2) and moulted soon after they were taken out of their cocoons, from 26 July till 10 August (n=14).

### Post-eclosion behaviour

Eclosion started when the pupal cuticle (exuvia) split and the imago escaped from it (figure 3b). This was followed by a series of clockwork events. The first most noticeable event was the expansion of the elytra and their rotation to the back of the abdomen, accompanied by pulsating movements of a distended abdomen. The elytra were rather pale, but soon started melanising. Soon after that the tips of the hindwings appeared from underneath the elytra (figure 3c). Within 20 to 40 minutes of their appearance, the hindwings inflated (figure 3d-f) and were eventually folded under the elytra at about seven to eight hours from the start of eclosion; by then the elytra were golden brown (figure 3g-i).

However, it became clear that some teneral imagos were having trouble shedding the exuvia, which interfered with the expansion of the hindwings. In order to avoid this, in some cases, I removed the remains of the exuvia by hand, as delicately as possible. Even so, four imagos were unable to fold one hindwing.

The other most noticeable event was that the teneral imagos while moving exuded clear liquid droplets from the tip of the ninth abdominal segment, intermittently. The ventral side of this segment is dimorphic in the pupa (figure 3a), but now only a small area was visible in both males and females (figure 3d-e). Later the ninth segment folded completely and from then on

the droplets appeared at the gap in the last visible abdominal segment - between the eighth sternite and the eighth tergite (figure 3g-h). This loss of fluid lasted until the hindwings were folded, about eight hours from the start of the eclosion (figure 3i). All except one male (number 9) exuded copious droplets visible with the naked eye. As mentioned before, this loss of fluid was clearly demonstrated with the imagos on underlined red dyed paper (figure 3f), but dried without leaving a stain in the other cells.

### Weight monitoring

At the beginning of the study the average pupa weight was  $0.90 \pm 0.09$  g for the males (n=6) and  $1.00 \pm 0.19$  g for the females (n=8). There was a steady weight loss, which was faster around eclosion. Shortly after the hindwings were folded this weight loss measured between 20 and 30% (figure 4); even male number 9, who produced no noticeable droplets, lost 18.3% of its weight. Afterwards the weight loss was not so marked, between 15-20%; except for male number 11, who lost more. At the end, the male and female imagos weighed respectively  $0.51 \pm 0.09$  g and  $0.57 \pm 0.12$  g; the weight loss averaged roughly the same for males and females: 43.7% and 43.1%. The male and female imagos' body length, with the head down, was respectively  $16.68 \pm 0.48$  mm and  $17.09 \pm 1.09$  mm.

The beetles were released in our back garden, under logs; except for the four individuals, which had been unable to fold one hind wing. Those were sent for genetics research to Matthias Shiedel, Charles University, Prague, Czech Republic.

## Discussion

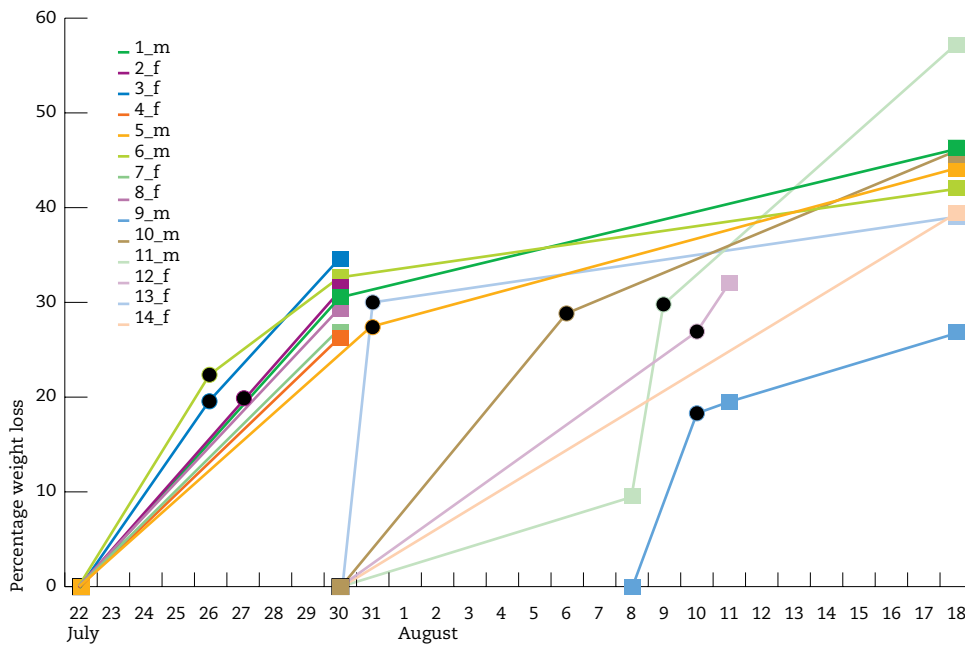
Eclosion studies inside the privacy of a pupal chamber pose a great challenge because it is impossible to observe, even through a window in it, what is going on at the tip of the abdomen of an individual, particularly when its wings are expanded. Relocation to wider cells worked, but it caused several issues in this study; mostly that the teneral imagos were unable to turn on their front because they could not grip the side walls and at the same time were unable to get rid of the exuvia. Consequently, the hindwings sometimes got trapped in it and could not expand properly. Therefore, in most cases, I had to remove the exuvia by hand, but this didn't seem to interfere with their development.

### Post-eclosion behaviour

Post-eclosion development of rose chafers was very reproducible. It followed a similar pattern to the stag beetles. This is not surprising because eclosion triggers a cascade hormone regulating developmental changes, as Peabody & White (2013) showed for the model species *Drosophila melanogaster* Meigen. The wing development of *Drosophila* is similar to the hindwings development in these beetles.

There was no noticeable sexual dimorphism in their behaviour. As we have seen in *C. aurata* sexual dimorphism is just detectable in the pupae (Sousa et al. 2018) (figure 3a); but during post-eclosion it was not possible to distinguish any genitalia differences. This observation helped to explain the puzzling observations during the stag beetles post-eclosion. Stag beetles, unlike *C. aurata*, are highly dimorphic in the pupal stage already, not just in the head, but in their genitalia as well (Klausnitzer & Sprecher-Uebersax 2008; Lai & Hsin-ping 2008). For example, the flagellum is clearly visible in a male *L. cervus* pupa (Fremlin & Tanahashi 2015). The male stag beetle emerges with the abdomen fully distended, hence displaying the genitalia, and it takes





4. Percentage weight loss from late pupa to teneral imago of fourteen individuals. ● - individuals weighed shortly after the hindwings were folded: 2\_f, 3\_f, 5\_m, 6\_m, 9\_m, 10\_m, 11\_m, 12\_f and 13\_f. Abbreviations: f = female; m = male.

4. Percentage gewichtsverlies van pop tot adult voor veertien individuen. ● - individuen gewogen kort nadat de vleugels waren gevouwen: 2\_f, 3\_f, 5\_m, 6\_m, 9\_m, 10\_m, 11\_m, 12\_f and 13\_f. Afkortingen: f = vrouwtje; m = mannetje.

up to four hours to retract that it; about the same time that it took *C. aurata* to do so. By contrast, the mycangium related post-eclosion behaviour of a female *L. cervus* occurs for the first time about two and half hours but lasts for several hours, intermittently (Fremlin & Tanahashi 2015). Thus the conclusion is that in stag beetles only the post-eclosion behaviour of the female seems to be related to the acquisition of the symbionts.

### Loss of weight

Remarkably, the exudation of droplets followed the same pattern as in the male stag beetles and had about the same duration coinciding with the wings development. By the time that the hindwings were folded under the elytra it had practically stopped. This was accompanied by a sharp loss of weight: about 20-30%. Post-eclosion diuresis is also a widespread phenomenon noted across aquatic, terrestrial and flightless insects (as in Beyenbach 2016) and the Lucanidae and Cetoniinae are no exception. Indeed there are some unreported observations of fluid droplets at the abdominal tip of various teneral scarabs. For example: Cetoniinae: *Mecynorrhina polyphemus* Fabricius (M. Smith personal communication); Dynastinae: *Xylotrupes gideon* Linnaeus (D. Scaccini personal communication); *Dynastes hyllus* Chevrolat and *Strategus aloeus* Linnaeus (M. A. Morón Rios personal communication); and Rutelinae: *Chrysinia macropus* Francillon (M. A. Morón Rios personal communication). This phenomenon has been studied in the Lepidoptera; for example, a newly emerged butterfly, the corn earworm *Helicoverpa zea* Boddie, loses 20% of its weight during the first six hours after eclosion (Bushman *et al.* 1986), due to loss of haemolymph. At this stage it is secreted very fast by the Malpighian tubules into the hindgut and then excreted (Nicolson 1976a). Individuals with a blocked anus did not lose weight. This needs to be studied further in the Cetoniinae and more importantly in the Lucanidae; in the latter it was hypothesised that the discharge was not from the anus, but this might be wrong and needs to be investigated further.

The haemolymph is discarded in order to regulate the homeostatic pressure of the newly formed adult as a result of its internal and external reorganisation during eclosion; it is a hormone-regulated phenomenon (Kataoka *et al.* 1989, Nicolson 1976b, 1976c). However this loss of weight didn't seem to be confined to that short period as by the end of the study it had reached 43.3%. This compares very well with the stag beetles' weight loss from pupa to teneral imago: 40.3% for *L. cervus* (n=3)

and 40.2% for *D. parallelipedus* (n=2) (Fremlin & Tanahashi 2015, Hendriks & Fremlin 2012, M. Fremlin unpublished data).

This is even more complicated because in stag beetles loss of fluid also occurs in the previous moult and thereafter the pupal chambers are very moist especially near eclosion (Fremlin & Tanahashi 2015). Unlike the hormone regulated developmental changes, which are triggered only after eclosion (Peabody & White 2013), the loss of weight seems to be an on-going process from the time that the larvae empty their gut. This also needs to be studied further; perhaps it would be not too challenging with other scarabs popular with hobbyists.

### Conclusion

With this study I found out that the loss of fluid during post-eclosion is a widespread phenomenon. It seems to have been under-reported in the Scarabaeoidea possibly because they hide themselves far too well inside their pupal chambers. By studying the post eclosion in a Cetoniinae in order to compare it with the Lucanidae, I proved that in the latter only the post-eclosion mycangium-related behaviour of the female stag beetle is unique to the Lucanidae. It seems a great advantage to be synchronised with a period when there is such high moisture inside the pupal chamber - perfect timing.

Since the Lucanidae are the only Scarabaeoidea to have evolved a mycangium, this raises some questions. When did this happen? Their mycangium-related behaviour is also very reproducible and probably pre-programmed (Tanahashi & Fukatsu 2015; Fremlin & Tanahashi 2015). Is it triggered by a new set of specific hormones? This would be well worth studying further as it would shed light on this evolutionary puzzle. In the Scarabaeoidea the study of the last two moults and oviposition is an open field of research to amateurs and professionals alike - there is a lot to find out.

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## Samenvatting

**Gedrag direct na de ontpopping van de gouden tor *Cetonia aurata* (Coleoptera: Scarabaeidae: Cetoniinae)**

Sommige insecten vertonen opmerkelijk gedrag ten aanzien van de verticale transmissie van hun symbionten. Het bijzondere gedrag van het vliegend hert *Lucanus cervus* (Scarabaeoidea: Lucanidae) direct na ontpopping is reeds onderzocht: de mannetjes keren hun genitale capsule en de vrouwtjes hun mycangium binnenstebuiten om zo schimmelsymbionten binnen te krijgen die de larve achterliet voor de verpopping. Van de Scarabaeoidea bezitten alleen de Lucanidae een mycangium. Het doel van de hier beschreven studie was om het gedrag van de verwante gouden tor *Cetonia aurata* (Scarabaeoidea: Scarabaeidae: Cetoniinae) te volgen. Zes mannelijke en zes vrouwelijke poppen werden gevolgd. In hun ontwikkeling volgden zij hetzelfde patroon als het vliegend hert en net als het vliegend hert scheidden alle twaalf (behalve één mannetje) een helder vocht af direct na ontpopping totdat de vleugels waren gevouwen, zo'n zes uur later. Ze ondergingen alle een duidelijk gewichtsverlies (20-30%) gedurende dat stadium; maar aan het eind van deze studie was dit 43% en grotendeels vergelijkbaar met het vliegend hert. Maar in tegenstelling tot het vliegend hert vertoonden de gouden torren in hun gedrag geen verschil tussen de seksen. Het kostte zowel de mannetjes als de vrouwtjes enkele uren om hun abdomenpunt in te trekken, ongeveer gelijk aan dat van het terugtrekken van het genitaal van de mannetjes van het vliegend hert. Hiermee blijft alleen het binnenstebuiten keren van het mycangium om symbionten te verkrijgen uniek voor de Lucanidae.

