The sexual maturity of female Stag Beetles *Lucanus cervus* (Col: Scarabaeoidea: Lucanidae) during the reproductive season

by Maria Fremlin (12447)

contact: mariafremlin@gmail.com

Abstract

The Stag Beetle *Lucanus cervus* (Linnaeus) is a species of conservation concern and has been studied intensively. The development of sexual maturity in females of *L. cervus* during the reproductive season has however, not yet been documented. In order to redress this I have analysed data collected between 2006 and 2022 from 36 female Stag Beetle casualties found in Colchester, Essex. The number of dead females carrying mature eggs peaked during the period from the end of June to mid-July, when females would normally be searching on foot for nesting sites. During this period three females were found each with between twelve and fourteen mature eggs in their abdomens. All these females had depleted fat reserves and very dark mycangia. The number of individuals found without eggs increased sharply during the same period and peaked a fortnight later when the season finished. This study indicates strongly that females were sexually mature towards the end of June but the period before that needs to be studied further.

Introduction

Following its listing for protection under the Bern Convention (Appendix III) in 1982, the Stag Beetle *Lucanus cervus* has been subject to especially intensive study, as reviewed by Méndez & Thomaes (2021), compared with other saproxylic insects. But to date the development of sexual maturity in the females has been overlooked.

In a recent review of 269 scientific references Méndez & Thomaes (2021), concluded that "the elusiveness and crepuscular habits of this species have hindered detailed observations of reproductive behaviour in the wild". Indeed, females are very secretive; a study of radio-tracked females found that they spent 31% of their time in oviposition and 38% in "unknown places" (Tini *et al.,* 2017). So, it is not surprising that the development of their sexual maturity has been overlooked.

This topic has been well studied in other families in the Scarabaeoidea, for example, in the Geotrupidae by Verdú *et al.* (2010), in the Passalidae by Bryan (1954), in the Scarabaeidae: Melolonthinae by Stringer (1988) and in the Scarabaeidae: Scarabaeinae by Macagno *et al.* in 2015. In the last-named

subfamily, the reproductive system has been closely studied and found to differ from all the other subfamilies as it has just one ovary with one ovariole. Nesting behaviour in the Scarabaeinae is closely linked to the physiological and developmental state of the female reproductive system (Halffter & Edmonds, 1982).

Stag beetles that belong to the subfamily Lucaninae have two ovaries with twelve ovarioles each (Ritcher & Baker, 1974). In the only reference I could find about the reproductive system of this subfamily, Reyes-Castillo *et al.* (2004), found that a freshly eclosed *Lucanus mazama* LeConte, had no oocyte development, that is, she was sexually immature. This was a highly skilled investigation as after eclosion the ovaries are very fragile and can easily be missed under the very large fat body (Anna Macagno, pers. comm.); this was also my experience. The fat body is a very important organ as it provides energy for flight and ovarian development (Arrese & Soulages, 2010). For their ovarian development female *L. cervus* may depend entirely on the resources accumulated in their fat body during the larval stage. They are not obligatory sap feeders (Harvey *et al.,* 2011), although they may come up to feed at artificial sap runs towards the end of their lives (Fremlin & Hendriks, 2011).

It has been discovered only recently that the Lucanidae possess a mycangium, an organ adapted to transport symbiotic yeasts, which is hidden in a fold under the female's eighth tergite (Tanahashi *et al.*, 2010). The female everts it for the first time during post-eclosion in order to retrieve the symbiotic yeasts on the pupal chamber left by the larva when it emptied its gut prior to pupation (Fremlin & Tanahashi, 2015). The mycangium in *L. cervus* is a very pale cream colour soon after eclosion, but it may darken with age. Later, during the breeding season when laying eggs in the humus-wood interface, it is assumed that the female swabs the area near each egg with the everted mycangium to pass on the yeast symbionts (Tanahashi *et al.*, 2010 and Hawes & Tanahashi, 2016). While studying the mycangium Hawes, (pers. comm.) detected variable oocyte development in ten female *L. cervus* as soon as they emerged above ground after overwintering in a quiescent state. One female was captured on camera with a large number of visible oocytes which indicates distinct ovarian development (Hawes, 2013).

This study is a first attempt to follow the development of the adult females' reproductive system from soon after they emerge above ground until their death. Data were obtained by recording the state of development of the egg load, fat body, mycangium and other physiological changes and were obtained almost entirely from casualties found above ground.

The breeding season for *L. cervus* starts from around the middle of May and may last until August. Typical behaviour during this period has been noted by

Bowdrey (1997), Fremlin (2009), Fremlin & Fremlin (2010), Rink (2006) and Sprecher-Uebersax (2001) amongst others. The males emerge first and disperse to potential nesting sites. When females emerge, they also disperse, and the males will fight for them either at nesting sites or in places where the females hide. Males fly far more than females but, as the season progresses, both flight activity and the interest of males in females decrease. This causes a corresponding increase in the female-to-male ratio. From mid-season onwards females no longer elicit any interest from males as they walk about in search of nesting sites, and males generally die earlier than females. Later, some females may come up above ground and die by their nesting sites (Fremlin & Hendriks, 2011).

Methods

All specimens in this study were collected in Colchester, north-east Essex (51.88262 N, 0.88312 E, 52 m asl). Private gardens in the southwest part of town are a well-known Stag Beetle hotspot which was first studied by Clark (1964, 1965), then by Bowdrey (1997) and afterwards by the author over the last 24 years.

The data for this study come from field notes made between 2006 and 2022. The majority are from a period of very intense monitoring from 2006 to 2011. I retrospectively assessed recorded data on the abdomens of the corpses examined, an ad-hoc selection from the corpses found. For the results of previous investigations see Fremlin (2009), Fremlin & Fremlin (2010), Fremlin & Hendriks (2011), Fremlin & Hendriks (2013) and Thomaes *et al.* (2017).

The corpses were examined by removing the abdomen tergites or sternites; whenever possible, I did this promptly or after a short period of refrigeration.

Photographs were taken with an EOS 550D SLR Canon camera sometimes fitted with a Sigma 105 mm objective set on a Jessops copy stand. On two occasions I examined a female with a handheld digital microscope, model 'Supereyes.'

Results

The results are discussed chronologically through the reproductive season which lasts from May to August each year, rather than through the years 2006 to 2022. To show the full data the year is added in parentheses ( ).

The combined data of 36 female corpses show that the frequency of individuals carrying eggs increased gradually as the season progressed and peaked in the last fortnight of July (figure 1).

Only two individuals without eggs were found before the second week of July but 22 such individuals were found between 20 July and 19 August.

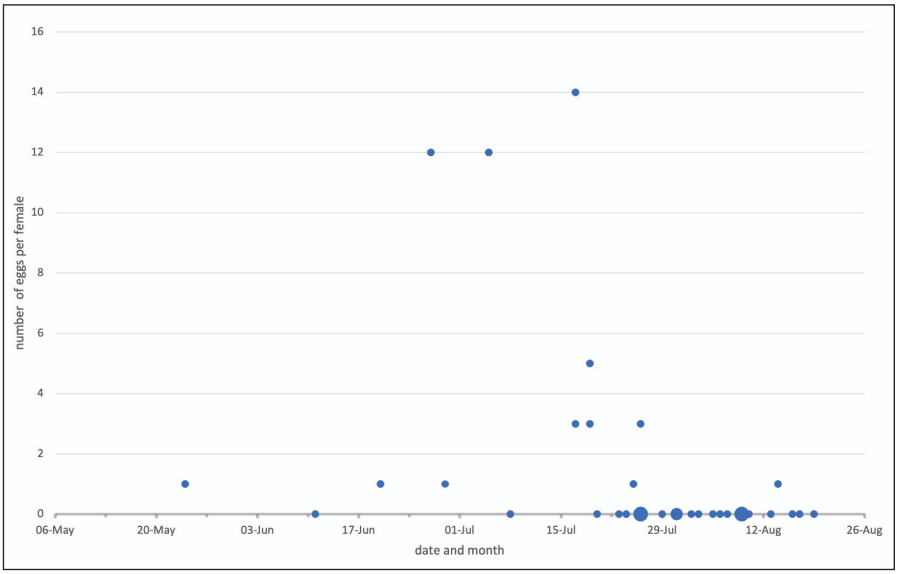

Figure 1. Number of eggs found in *Lucanus cervus* female casualties from May to August; records are ordered by day and month pooled over the period 2006 to 2022. Larger circles represent respectively two and three females.

Eggs were present in only a third of females (n = 12) and the number of eggs varied a great deal, from 1 to 14.

Females with the highest numbers of eggs were found between the end of June and the third week in July. After that, females without eggs predominated, as stated above.

During all years of the study the earliest record in the season was 24 May (2011) when an injured, but still living, female (figure 2) was collected. This female could not walk or retract her soft wings; she died two days later. During a clumsy examination of the mycangium I detected a fair-sized egg amongst a lot of fat. This was an exceptional casualty because early in the season casualties usually lack their abdomens.

On 20 June (2008) a squashed female was found which had a protruding mature egg and a great deal of fat; this individual was not dissected.

Towards the end of June (2017), a female that had been trodden on was found to have very advanced ovarian development. The abdomen contained twelve mature and several small eggs which were partially surrounded by fat. The gut contained some dark-coloured material particularly in the hindgut (figure 3a-b).

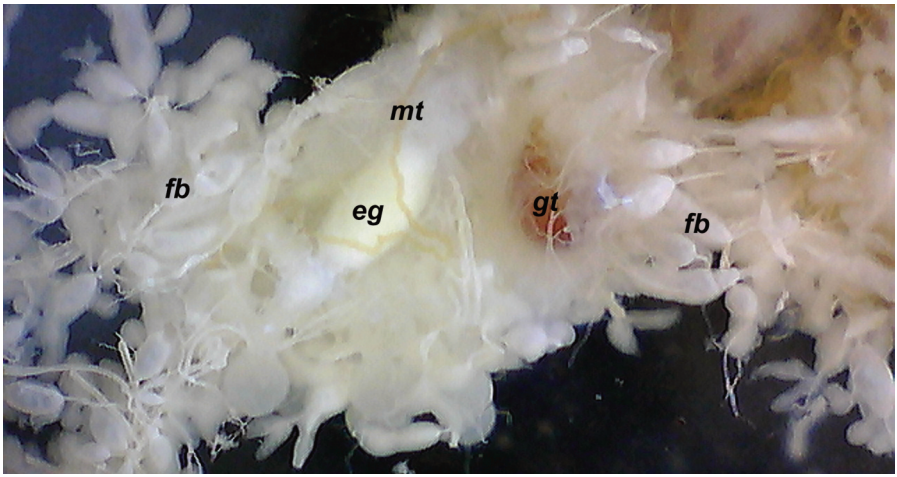

Figure 2. Close up view of the abdomen of a female *Lucanus cervus*, 24.v.2011: one large egg, surrounded by fat bodies. Abbreviations: eg: egg; fb: fat body; gt: gut; mt: Malpighian tubules.

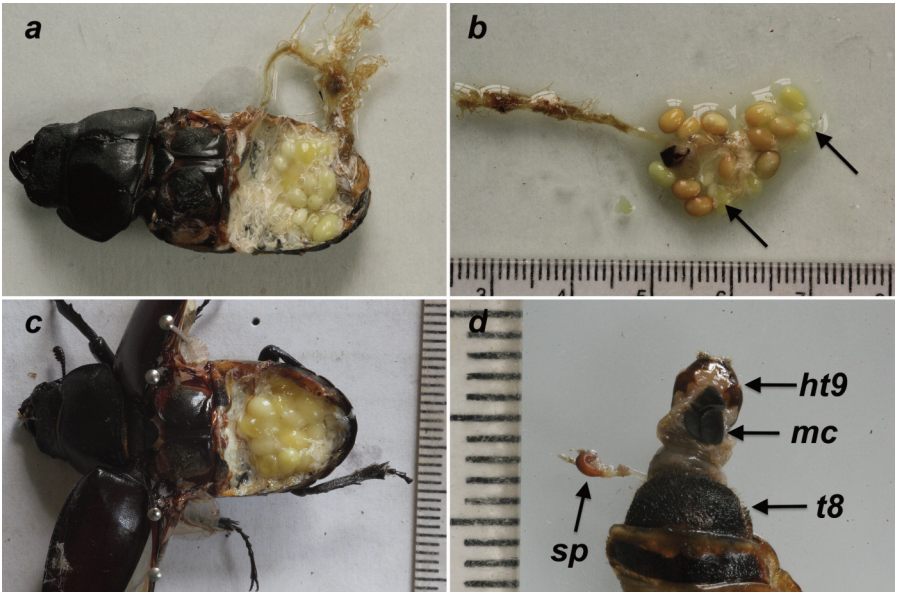

Figure 3. Females of *Lucanus cervus* with their abdomens full of eggs. Top row: trodden on female, 27.vi.2017 – (a) dorsal view of abdomen showing eggs at various stages of development; the gut has been moved upwards; (b) the hindgut, showing 12 fully developed eggs and a few smaller ones, arrowed. Lower row: trapped female, 05.vii.2022 – (c) dorsal view of the abdomen full of eggs; the gut has been removed; (d) dorsal view of the distended tip of the abdomen showing a very dark mycangium and the spermatheca. Ruler: 1-millimetre units. Abbreviations: ht9: 9th hemitergites; mc: mycangium; t8: 8th tergite; sp: spermatheca.

Shortly after that, 29 June (2006), another trodden on female was found which had a mature egg protruding from the abdomen; this individual was not dissected.

At the beginning of July (2022), a female was dissected which had been accidentally trapped in a conservatory. The abdomen was filled with ten mature eggs and two slightly smaller eggs, the fat reserves were depleted and the mycangium was very dark (figure 3c-d). The mature eggs of the last two females dissected were ovoid and measured approx. 3.6 mm × 2.5 mm.

On 15 June (2016) I started a rearing trial with a mated female but while I was absent for a few days she escaped from the terrarium and was found dead on 17 July. No eggs or larvae were found in the terrarium, but her abdomen contained fourteen mature eggs. The gut contents were dark, probably because she had been fed on beetle jelly whilst in captivity. There was very little fat left and the mycangium was ovoid, approximately 4 mm long and dark brown. For an unknown reason the eggs darkened, probably due to oxidation, as the examination proceeded (figure 4).

From 17 to 26 July (2012), several casualties were found containing between one and five eggs. The eggs were now surrounded by mostly liquefied matter. For example, a trodden on female with a very dark protruding mycangium containing three eggs is shown in figure 5a-b.

From the end of July until the end of August casualties were found near known nests. Their abdomens were practically empty and they usually exhibited worn teeth on the tibiae of their forelegs; for example, the corpses in figure 6a-b. This probably indicates that the females had made their way to the surface towards the end of their lives.

Discussion

The data show a pattern clearly linked to the behaviour of the females, but further work will be needed to determine whether dead and dying specimens are representative of the wider population in respect of sexual development. There is also a need to gather data from a much larger number of specimens, but this would be extremely difficult to obtain in the field with a species of conservation concern, which has relatively low fertility, and is very secretive.

During the period 2006 to 2022 by the third week in June only three females were available for examination; this was probably because the female-to-male ratio is very low early in the season (Fremlin, 2009). Besides, at the beginning of the season the main predators of Stag Beetles are magpies *Pica pica*. These birds selectively eat the abdomen which contains large reserves of fat (Bowdrey, 1997). Hence there were only two records of females with a single

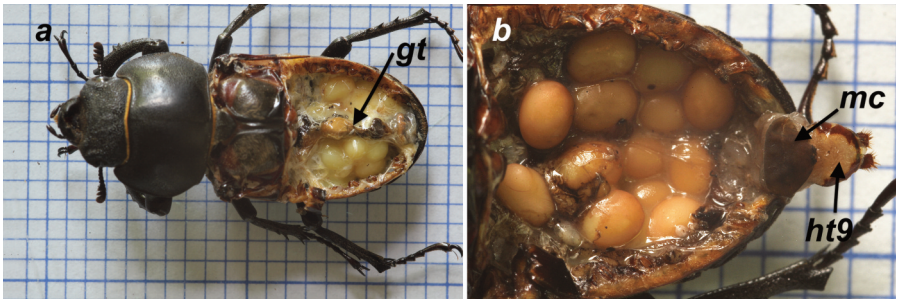

Figure 4. Dorsal view of the abdomen of a trapped female *Lucanus cervus,* 17.vii.2016: (a) abdomen containing 14 mature eggs, darkened gut and depleted fat reserves; (b) close up view after the gut was removed; note the dark mycangium. Squares = 2 mm × 2 mm. Abbreviations: gt: gut; ht9: 9th hemitergite; mc: mycangium.

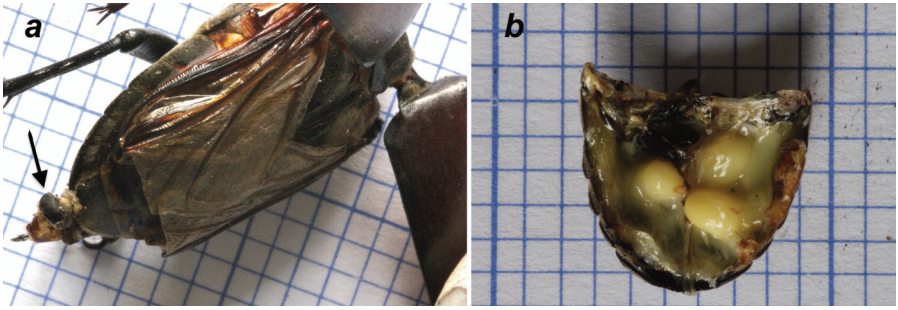

Figure 5. Trodden on *Lucanus cervus* female with protruding organs, 19.vii.2012: (a) dorsal view after the elytra were removed; note the dark mycangium, arrowed; (b) 3 mature eggs surrounded by mostly liquefied matter. Squares = 2 mm × 2 mm.

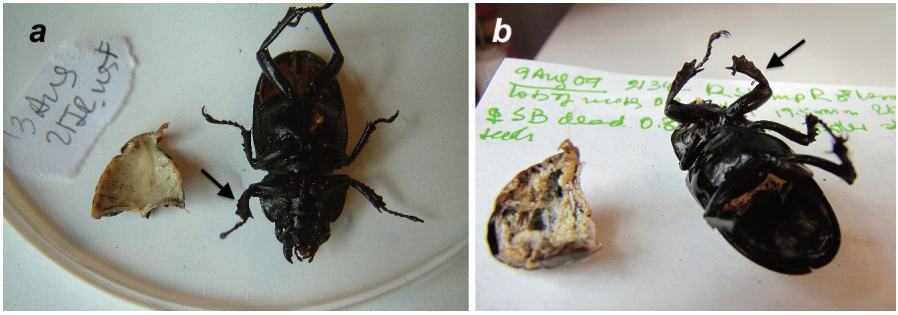

Figure 6. Dead *Lucanus cervus* females caught in the vicinity of nests. (a) female, 13.viii.2008; (b) female, 10.viii.2009. Note the worn out teeth on the tibia of the forelegs, arrowed.

egg, and both had plenty of fat (figure 2). Nevertheless, these three records provide evidence, albeit scant, that until mid-June there appears to have been some ovarian development since the females had emerged from the ground. This will be discussed further later.

By the end of June, probably after the peak of the season, there was an interesting increase in the number of specimens examined. This coincided with the females walking about as they sought suitable nesting sites and which resulted in a higher female-to-male ratio. In urban areas far more females are trodden on by humans during this stage, and many of them exhibited a cracked pronotum and protruding organs, including the mycangium (figure 5a).

Remarkably, a cluster of several females was found with their abdomens full of eggs although their fat reserves seemed to be rather depleted. The first female found in June (2017), had been trodden on and contained twelve mature eggs as well as others which were less developed (figure 3a-b). This fits in with local evidence that successful oviposition occurs from the end of June. A couple of large piles of fresh woodchips set up on 28 June (2010) were colonised by several females to judge from the number of larvae subsequently found (Fremlin, 2022).

Even more remarkable was finding two trapped females in early July (2016 and 2022), which had respectively twelve and fourteen mature eggs (figures 3c-d and figure 4). To be trapped with this many eggs is a very unusual situation, apparently fatal and unreported in sexually mature invertebrates. At about the same time of the season, there were a few records of females with very few eggs left, these were surrounded by liquefied matter and the mycangium was very dark (figure 5). Paul Hendriks (pers. comm.) reports that dead females at the end of rearing trials have been observed with a single egg each. This suggests that at the end of their lives they might not have been able to lay all their eggs. Interestingly, in the subfamily Scarabaeinae there is an egg resorption stage (Macagno *et al.,* 2015 & Tyndale Biscoe, 1978).

From the end of July onwards during the 2006 to 2022 study period, most of the female casualties examined carried no eggs at all and the contents of their abdomens had decomposed (figure 6). They were mostly found in the vicinity of nests and had apparently come up to the surface to die (Fremlin & Hendriks, 2011).

By a fortunate coincidence at end of the 2012 season, I sent a couple of rather worn-out females to Dr Tanahashi to study their mycangium yeast symbionts. These two females were found in the humus surrounding a *Eucalyptus* sp. stump together with four more females, one of which was already dead. In one of them Dr Tanahashi found no eggs but detected yellow bodies in both ovaries (Fremlin, 2012). The yellow body or *corpus luteum* is

the follicular residue of a mature egg when it passes down the oviduct, and it is taken as an indicator of ovulation (Tyndale-Biscoe, 1978). This is evidence that the female had ovulated from both ovaries by the time it reached the end of its life.

The size of a mature egg in *L. cervus* is approx. 3.6 mm × 2.5 mm and is different from the size of a freshly laid egg. Once laid, the latter starts to swell and becomes nearly spherical, approximately 3.4 mm × 3.0 mm (Sprecher-Uebersax, 2001). Interestingly, Holloway (2007), reports a New Zealand flightless stag beetle *Geodorcus sororum* Holloway, caught on 21 February 1974 with seven mature eggs. These measured approximately 3.5 mm × 2.0 mm, and one was partially extruded between the hemisternites indicating that it was in the act of being laid when the specimen was captured.

Franciscolo (1997) depicts a rather unusual dorsal view of the abdomen of the Lesser Stag Beetle *Dorcus parallelipipedus* L. with a few tergites removed to show the arrangement of about ten eggs nicely spaced out prior to egg laying. Indeed, I have come across a couple of trodden on females of this species with five and twelve mature, ovoid eggs. Remarkably, the latter female was a recapture from the previous year and had no fat left (unpublished). The Lesser Stag Beetle also has twelve ovarioles per ovary (Ritcher & Baker, 1974), so perhaps it is no coincidence that the latter also had the same number of eggs as *L. cervus*. Were they from just one ovary, or evenly distributed in both ovaries?

Given that in *L. cervus* twelve mature eggs seem to take over most of the space in the abdomen, and that in the field the average number of eggs laid is 24 (Harvey *et al.,* 2011), it seems probable that the females found with a large egg load from the end of June onwards had already laid some eggs. Earlier in the season there were already a few females with mature eggs, and this fits in with the fact that females can lay their eggs in more than one place (Rink, 2006). This hypothesis can only be proved by a systematic and skilful examination of their reproductive systems throughout the season, including the presence of yellow bodies.

Conclusion

This study, based entirely on dead specimens, indicates strongly that females *L. cervus* were sexually mature towards the end of June. A few carried a large load of mature eggs, had a very dark mycangium and depleted fat reserves; as expected, they died sometime later.

Therefore, there is still a period to document between the females emerging above ground, when they are thought to have developed oocytes, and the end of June. It is possible that the females' behaviour is linked to their sexual

maturity, as it is in the Scarabaeinae (Halffter & Edmonds, 1982). By studying their sexual maturity during this period we may find out why some of them make themselves so scarce at the beginning of the season. Is it because they are not sexually mature? When do they actually start laying their first eggs?

I hope this paper will be an incentive for people to find the answers to these questions.

Acknowledgements

I would like to thank Anna Macagno for her helpful discussion. I am also very grateful to Paul Hendriks, Davide Scaccini, Arno Thomaes, the editor, and a reviewer for their generous and constructive comments on the manuscript, which as result became greatly improved.

References

Arrese, E.L. & Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. *Annual review of entomology*, **55**: 207-225.

Bowdrey, J., 1997. The Stag Beetle *Lucanus cervus* L. (Coleoptera: Lucanidae) in north-east Essex: Results of the 1996 Colchester "Search for Stag Beetles" survey. *The Essex Naturalist*, **14**: 79-88.

Bryan, J.H., 1954. Cytological and cytochemical studies of oogenesis of *Popilius disjunctus* Illiger (Coleoptera-Polyphaga). *The Biological Bulletin*, **107**(1): 64-79.

Clark, J.T., 1964. The stag beetle in north-east Essex. *The Essex Naturalist*, **31**(3): 167-172.

Clark, J.T., 1965. The stag beetle in north-east Essex – second report. *The Essex Naturalist*, **31**(4): 267-270.

Franciscolo, M.E., 1997. *Coleoptera: Lucanidae, Fauna d'Italia*, **35**. Edizioni Calderini, Bologna, Italy.

Fremlin, M., 2009. *Stag Beetle (Lucanus cervus (L., 1758), Lucanidae) urban behaviour*, **In** Saproxylic beetles. Their role and diversity in European woodland and tree habitats. Proceedings of the 5th Symposium and Workshop on the conservation of saproxylic beetles. Sofia-Moscow: Pensoft Publishers, 161-176.

Fremlin, M., 2012. The reproductive system of female *Lucanus cervus*. (accessed 10.iv.2023) <http://maria.fremlin.de/stagbeetles/reproductive_system.html>.

Fremlin, M., 2022. The life cycle of the European stag beetle *Lucanus cervus* is three years minimum in the field (Coleoptera: Lucanidae). *entomologische berichten*, **82**(4): 138-144.

Fremlin, M. & Fremlin, D.H., 2010. Weather-dependence of *Lucanus cervus* L. (Coleoptera: Scarabaeoidea: Lucanidae) activity in a Colchester urban area. *Essex Naturalist (New Series)*, **27**: 214-230.

Fremlin, M. & Hendriks, P., 2011. Sugaring for stag beetles – different feeding strategies of *Lucanus cervus* and *Dorcus parallelipipedus*. *Bulletin of the Amateur Entomologists' Society*, **70**: 57-67.

Fremlin, M. & Hendriks, P., 2013. Lesser Stag Beetles *Dorcus parallelipipedus* (Coleoptera: Lucanidae) longevity - at least three years. *Bulletin of the Amateur Entomologists' Society*, **72**: 48-56.

Fremlin, M. & Tanahashi M., 2015. Sexually-dimorphic post-eclosion behaviour in the European stag beetle *Lucanus cervus* L. (Coleoptera: Lucanidae). *Bulletin de la Societé Entomologique Suisse*, **8**: 29-38.

Halffter, G. & Edmonds, W.D., 1982. *The ovary and nesting behaviour*. **In** The nesting behavior of dung beetles (Scarabaeinae) - An ecological and evolutive approach. México Instituto de Ecología, D. F.: 123-125

Harvey, D.J., Gange, A.C., Hawes, C.J., Rink, M., Abdehalden, M., Al-Fulaij, N., Asp, T., Ballerio, A., Bartolozzi, L., Brustel, H., Cammaerts, R., Carpaneto, G.M., Cederberg, B., Chobot, K., Cianferoni, F., Drumont, A., Ellwanger, G., Ferreira, S., Grosso-Silva, J., Gueorguiev, B., Harvey, W., Hendriks, P., Istrate, P., Jansson, N., Jelaska, L., Jendek, E., Jovic, M., Kervyn, T., Krenn, H., Kretschmer, K., Legakis, A., Lelo, S., Moretti, M., Merkl, O., Mader, D., Palma, R., Neculiseanu, Z., Rabitsch, W., Rodriguez, S., Smit, J., Smith, M., Sprecher-Uebersax, E., Telnov, D., Thomaes, A., Thomsen, P., Tykarski, P., Vrezec, A., Werner, S. & Zach, P., 2011. Bionomics and distribution of the stag beetle, *Lucanus cervus* (L.) across Europe. *Insect Conservation and Diversity*, **4**(1): 23-38.

Hawes, C.J., 2013. Discovery of a mycangium and associated yeasts in the stag beetle *Lucanus cervus* (Coleoptera: Lucanidae). *White Admiral*, **85**: 22–23.

Hawes, C.J. & Tanahashi, M., 2016. A brief review of mycangia and associated yeast symbionts in British stag beetles. *Bulletin de la Société royale belge d'Entomologie,* **152**: 174–176.

Holloway, B.A., 2007. Lucanidae (Insecta: Coleoptera). *Fauna of New Zealand*, **61**.

Macagno A.L., Beckers O.M. and Moczek, A.P., 2015. Differentiation of ovarian development and the evolution of fecundity in rapidly diverging exotic beetle populations. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **323**(9): 679-688.

Méndez, M. & Thomaes, A., 2021. Biology and conservation of the European stag beetle: Recent advances and lessons learned. *Insect Conservation and Diversity*, **14**(3): 271-284.

Reyes-Castillo, P., Martinez, M.I. & Castillo, M.L., 2004. Biological notes on Mexican *Lucanus (Pseudolucanus) mazama* LeConte (Coleoptera: Lucanidae). *Entomological news*, **114**: 138-146.

Rink, M., 2006. *Der Hirschkäfer Lucanus cervus in der Kulturlandschaft: ausbreitungsverhalten, Habitatnutzung und Reproduktionsbiologie im Flusstal*. PhD Thesis, University of Koblenz-Landau, Alf, Germany.

Ritcher, P.O. & Baker, C.O., 1974. Ovariole numbers in Scarabaeoidea (Coleoptera: Lucanidae, Passalidae, Scarabaeidae). *Proceedings of the Entomological Society of Washington*, **76**(4): 480-494.

Sprecher-Uebersax, E., 2001. *Studien zur Biologie und Phänologie des Hirschkäfer im Raum Basel: mit Empfehlungen von Schutzmassnahmen zur Erhhaltung und Förderrung des Bestandes in der Region (Coleoptera: Lucanidae, Lucanus cervus L.)*. Doctoral diss., Univ. Basel, Switzerland: 1-196.

Stringer, I.A.N., 1988. The female reproductive system of *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae: Melolonthinae). *New Zealand Journal of Zoology*, **15**(4): 513-533.

Tanahashi, M., Kubota, K., Matsushita, N. & Togashi, K., 2010. Discovery of mycangia and the associated xylose-fermenting yeasts in stag beetles (Coleoptera: Lucanidae). *Naturwissenschaften*, **97**: 311-317.

Tini, M., Bardiani, M., Campanaro, A., Chiari, S., Mason, F., Maurizi, E., Toni, I., Audisio, P. & Carpaneto, G.M., 2017. A stag beetle's life: sex-related differences in daily activity and behaviour of *Lucanus cervus* (Coleoptera: Lucanidae). *Journal of Insect Conservation*, **21**: 897-906.

Thomaes, A., Verschelde, P., Mader, D., Sprecher-Uebersax, E., Fremlin, M., Onkelinx, T. & Méndez, M. (2017) Can we successfully monitor a population density decline of elusive invertebrates? A statistical power analysis on *Lucanus cervus*. *Nature Conservation*, **19:** 1-18.

Tyndale-Biscoe, M., 1978. Physiological age-grading in females of the dung beetle *Euoniticellus intermedius* (Reiche)(Coleoptera: Scarabaeidae). *Bulletin of Entomological Research*, **68**(2): 207-217.

Verdú, J.R., Casas, J.L., Lobo, J.M. & Numa, C., 2010. Dung beetles eat acorns to increase their ovarian development and thermal tolerance. *Plos One*, **5**(4): e10114.