Number of instars of *Lucanus cervus* (Coleoptera: Lucanidae) larvae

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KEY WORDS
Brooks-Dyar rule, frequency distribution, head capsule width, instar determination, sexual dimorphism

The number of instars of the stag beetle *Lucanus cervus* (Coleoptera: Lucanidae) has generally been considered three, but some recent papers suggested that in the UK it may be five. In order to check this, we compared head capsule width data from field larvae in the UK (n=419) and The Netherlands (n=240). For both sets the frequency distribution of the head capsule widths shows three distinct peaks. The mean head capsule width for each peak follows the Brooks-Dyar rule and the same applied to data collected in the ‘40s by Van Emden (n=46) in the UK. These results confirm that there are only three instars in both countries. During the third instar the Dutch larvae are bigger than in the UK and have a clear head capsule width sexual dimorphism, supporting the idea that the known size difference between the two populations is already apparent in the immature stage. The third instar also seems to be the one with the longest duration in both populations.

Introduction

It has been known for a long time that the larvae of the superfamly Scarabaeoidea ‘moult three times, the third ecdysis releasing the pupa’ (Van Emden 1941). The author gives the increasing sizes for the head capsule width for the three larval instars L1- L3, for the family Lucanidae; regarding *Lucanus cervus* Linnaeus, he presents data for 46 larvae, sourced from museum records and his own. Subsequently, this data was reproduced in *Die Hirschkäfer* (Klausnitzer 1995, Klausnitzer et al. 2008) and it has been much quoted since.

For other countries in its range, the literature also confirms this number of instars for this stag beetle, for example: Italy (Franciscolo 1997) and Germany (Rink et al. 2008). However, Tochtermann in an article referred to a fifth instar *L. cervus* larva (1992; Abb 8) but the number of instars is not discussed further and it is unknown how this number was established. Harvey & Gange (2003) state that ‘there are five (larval) instars’ but this is unsupported by references, yet repeated by Hawes (2009). Harvey et al. (2011b) suggest that the number of instars may be different in various populations. However, as far as we are aware there is no published description of the differences between the third, fourth, or fifth instars, nor any published observation of a larva moulting more than three times.

In captivity, it is possible to determine the number of moults; indeed breeders know that during the larval stage there are three instars for the genus *Lucanus* (Lai et al. 2008). Both authors have reared larvae from the UK and confirmed this. The second author has found the same number of instars with larvae from The Netherlands (unpublished) and so has Radnai (1995) with French stock. To prove that there are any exceptions in the field is rather difficult due to the subterranean habit of the immature stages.

Since we collected larval biometric data ourselves in the field over the years for this species, we decided to analyse our data for the number of instars. We also included a smaller subset of captive bred Dutch larvae in our analyses.

Methods

Head capsule width (HCW)

HCW is the largest width across the head of the larva; it is relatively easy to hold a larva and measure it without causing damage because their rigid head capsule bulges out, see figure 1. Very small larvae are rather fragile so need to be held in the cup of the hand. One HCW measurement was taken per larva. The measurements were taken with digital callipers to 0.01 mm accuracy in the UK and with manual callipers to 0.1 mm accuracy in The Netherlands (figure 1).

Weight

The larvae were also weighed; in the UK with a Salter electronic diet scale, model 1250, to 0.1 g; in The Netherlands with a Maultronic S electronic scale, to 0.5 g.

Sexing

Mature stag beetle larvae can be sexed by skilful examination of their abdominal segments with a lens. The males have a cuticular dark spot in the medio ventral region of the ninth abdominal segment, the one before the last. This is the terminal ampulla, or Herold’s organ (Herold 1815) (figure 2a). It corresponds to the development of the male sexual organs; females
lack a terminal ampula (Harvey et al. 2003) (figure 2b). In the larvae of some scarab beetles both testes and ovaries are visible through the integument (skin) between the seventh and eighth segments (Martinez & Lumaret 2005). But in L. cervus only the ovaries are visible through the fat body (figure 2c).

Field work
In the UK, data from 419 larvae was collected in the Colchester area, Essex, by the first author in many urban gardens (n=18, including her own) from 2006-2013. Some of the records were collected as part of the Bury Buckets for Beetles (BB4B) project developed by the Royal Holloway University in conjunction with the People’s Trust for Endangered Species (BB4B 2003). Others were obtained via a survey in private gardens (Fremlin 2013).

In The Netherlands, data from 240 larvae was collected in the Veluwe region, i.e. Vierhouten, Elspeet and Hoog Soeren, from 2000 to 2013 by the second author.

The authors took the utmost care while handling the larvae because they are very fragile. Digging was done as gently as possible in order to avoid injuring them. Sudden mechanical impact seems to cause fatal ruptures to their digestive system (Fremlin 2013, unpublished). The larvae were put back; some of the sites were repeatedly monitored by the authors.

Captive reared larvae
In The Netherlands, the second author has reared 84 larvae in four rearing experiments on L. cervus as part of other research; their HCWs were measured and about half of them were sexed. The larvae were reared indoors in tanks with various substrates ranging from decayed wood chips to white rotted wood from several species of deciduous trees.

Data analysis
There are two basic methods for determining the number of instars. The simplest one is by analysing the HCW frequency distribution: if it consists of a series of non-overlapping peaks, each will be representing an instar (Daly 1985). The second method is to check if the Brooks-Dyar rule applies. This rule is based on the fact that in insects which undergo complete metamorphosis the ratio postmoult/premoult of the larval HCW is constant; this was observed independently by Brooks (1886) and Dyar (1890). This geometric progression follows the equation

\[ y = a e^{bx}, \quad \ln y = \ln a + bx \]  

(equation 1) where \( x \) is the instar number (1, 2, 3, etc.), \( y \) is the HCW, ‘\( a \)’ and ‘\( b \)’ are constants which vary for each species, and \( e^b \) is the constant growth rate (Dyar 1890, Floater 1996). Thus the natural logarithm of the mean HCW of each instar plotted against instar number should produce a straight line.

A deviation from a straight line indicates missing instars (Daly 1985). The Brooks-Dyar rule can also be used to compare species growth rates. It is not universal (Daly 1985), but has been successfully applied to a number of species (e.g. Dallara et al. 2012, Floater 1996, Wu et al. 2013).

Results
Head capsule width frequency
Shown in figure 3 is the frequency of stag beetle HCWs in the UK (Colchester, Essex) and The Netherlands (Vierhouten, Elspeet and Hoog Soeren, Veluwe).

The multimodal distribution of HCWs has three clear distinctive peaks, without overlaps, for both countries. The first two instars are very similar. In the third instar the Dutch larvae are bigger and have a bimodal distribution.
In order to prove this rule (equation 1), first, we calculated means of the HCWs in our data and Van Emden’s (1941) UK data for each instar, table 1.

The relationship between the larval instar of *L. cervus* and the natural logarithm of the mean HCW is linear for the two datasets (figure 4). Van Emden’s (1941) data also fall on a straight line, (ln y= 0.4013 + 0.6751x, R² = 0.9954), not shown. A linear relationship satisfies the Brooks-Dyar rule (equation 1); the values of constants ‘a’ and ‘b’ for *L. cervus* are 1.6 and 0.6, respectively.

Dutch population third instar bimodal distribution

In figure 3 there is a distinct bimodal distribution in the third peak of the HCW frequency of the Dutch larvae, which we have investigated further with a subset of larvae that had been sexed. Shown in figure 5 is the frequency of both field and captive bred larvae. There is a clear bimodal distribution in both sets: females have a smaller HCW than males.

Weight data

The heaviest Colchester L3 larva weighed 13.3 grams, HCW 11.20 mm, whereas in the field dataset from The Netherlands the maximum weight was 21.5 grams, HCW 11.95 mm. About 21 percent of the Dutch larvae had weights above 13.3 grams.

Discussion

To collect biometric data we have monitored several breeding habitats repeatedly for many years without noticeable harm to them because female stag beetles had laid eggs there in the following years. This has also been done successfully by other researchers (Rink et al. 2008). Harvey et al. (2011a) consider this type of investigation not only harmful to the beetles, but also a potential risk of destroying their habitat. Indeed this can often be the case when the habitat is inadvertently disturbed, mostly by the public (Fremlin 2013). Thus this type of monitoring should be done with great care, avoiding the summer months in order to prevent the disturbance of ovipositing females, pupae and adults in their pupal cells.

The analysis of our data, which we believe has not been done before for the immature stage of *L. cervus* or any other stag beetle, has led to several interesting results.

**Table 1.** Values of HCWs for the three instars (L1, L2 and L3) of *Lucanus cervus* larvae in mm for the UK, The Netherlands (NL) and Van Emden’s data (VE). Other abbreviations: min - minimum; max - maximum; stdev - standard deviation.

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3. Frequency distribution of *Lucanus cervus* HCW field larvae in the UK (n=419) and The Netherlands (NL), n=240.

3. Frequentieverdeling van Engelse wilde *Lucanus cervus* kopkapselbreedtes (n=419) en uit Nederland (NL), n=240.
in The Netherlands. From our HCW data the Dutch larvae are possibly growing faster; by the time that they have reached the third instar they are noticeably bigger (table 1).

Three instars seem to be the case not just within the Lucanidae but for their superfamily, Scarabaeoidea, as well. Van Emden (1941) collected HCW data for 41 species representing several families in this order – all of which produced three larval instars. Furthermore, the first author analysed the HCW frequency for another stag beetle, Dorcus parallelipipedus, and a flower chafer, Cetonia aurata, and also obtained an identical distribution – three peaks (unpublished). The second author noticed three instars in rhinoceros beetle, Oryctes nasicornis, as well (Hendriks 2007). All these beetles may share their habitat with L. cervus.

Third instar HCW sexual dimorphism

We discovered a clear bimodal distribution in the HCW for the third instar of the Dutch larvae, field and captive bred (figures 3 & 5). This bimodal distribution does not show clearly in the L3 UK data; the third peak in figure 3 has just a slight shoulder, which will be worth investigating but sex data are not available for this dataset.

There are other species with sexually dimorphic HCWs. For example, the final instars of a processionary caterpillar (Floater 1996) and a wood borer beetle (Flaherty et al. 2012) have bigger females. We found the opposite for L. cervus, the males are bigger than the females. This is clearly linked to L. cervus adult body size dimorphism, which has been much studied. First, Clark (1977) proved that males were significantly bigger than females, remarkably with a Colchester population. Later, Harvey et al. (2006) confirmed this across the UK. Furthermore, the sexual size dimorphism in the Dutch population seems to be much more pronounced than in the UK. The males are exceptionally big (Harvey et al. 2011b).

In some species with strong sexual dimorphism the number of instars may even vary between the sexes but that is not the case for L. cervus, as we have shown. Esperk et al. (2007) in an extensive review only mention one family with variable instars in the Coleoptera: the Dermestidae.

Third instar weight sexual dimorphism

In general, our weight data indicates that in UK the third instar larvae do not reach the same weight as the Dutch ones. In the

4. Relationship between larval instar and natural logarithm of the mean HCW of field L. cervus larva in the UK (n=419) and The Netherlands (n=240).
4. Relatie tussen het larvenstadium en de natuurlijke logaritme van de gemiddelde kopkapselbreedte van wilde Lucanus cervus larven in Engeland (n=419) en Nederland (n=240).

5. Frequency distribution of sexed third instar Dutch larvae. Captive reared larvae: 19 females (cf); 22 males (cm). Field larvae: 13 females (wf); 25 males (wm). Total number of sexed larvae: 79.
latter population the males are much heavier than the females. For instance, there was a remarkable difference between their maximum weights, 13.3 grams versus 21.5 grams. Both larvae HCWs were in the upper range thus suggesting that they could have been males.

This marked weight dimorphism is also evident in captive reared larvae. Heavier larvae develop into bigger adults (P. Hendriks unpublished). However, a weight sexual dimorphism analysis of our field data, taken at different periods through the year, is far too complex and outside the scope of this paper mainly because larval weight does not stay constant throughout the instar’s duration.

Third instar duration

In all the three rows of table 1, the larvae assigned to L3 greatly outnumber the others. This interesting result could be explained by the fact that the relatively smaller L1 and L2 larvae are harder to find. Alternatively, the last instar larvae take longer to develop and therefore are more likely to be encountered. This last possibility fits with the fact that mature L3 larvae are often found in the autumn with the earlier instars or at different stages of maturity (figure 6).

It also fits with our captivity studies. Once a larva reaches the third stage (which, in general may take up to one year) it has to take extra time to fatten up (figure 7). However, from our field records, the duration of L. cervus instars seem to be very variable within the same location and their analysis is far too complex. Moreover, in the literature the duration of the larval stage seems to vary widely across its range: 3 - 6 years (Harvey et al. 2011b) but there are no references to support this upper limit. In Germany, Rink et al. (2008) found a 3-year duration in field enclosures and our research in The Netherlands confirms this (Smit et al 2005; Hendriks unpublished). Sometimes it can even be shorter. Currently, there are some records for a 2-year larval stage: several in the UK (Fremlin 2010, 2012, unpublished) and one in Belgium (Arno Thomaes personal communication).

Clearly, the duration of each instar and/or larval stage needs to be investigated further in a more systematic way in order to identify the sources of this variation.

Since, in favoured areas, stag beetles seem to be attracted to freshly generated habitats (Smit et al. 2005, Fremlin 2010, 2012) this challenging task could be achieved by first determining the beginning of colonisation of new sites and then monitoring them up to the emergence of the adults. We also suggest that notes should be taken about the maturity of the larvae (figures 6-7), which would help distinguishing overlapping generations.

Conclusion

We demonstrated that, the stag beetle L. cervus undergoes the same number of larval moults both in the UK and The Netherlands. In the process we discovered that the Dutch larvae have a marked HCW dimorphism during the last larval stage, which was not apparent in the UK larvae. This study raised several interesting questions regarding HCW and weight sexual dimorphism and the duration of each instar. We hope that it will be an incentive for a much wider investigation. Therefore, we encourage other researchers to collect biometric data for the larval stages of this stag beetle throughout its range so that, in future, we could get a better understanding of its development and ecology.

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Dyar HG 1890. The number of molts of lepi- dopterous larvae. Psyche S: 420-422.


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Samenvatting

Het aantal larvenstadia van Lucanus cervus (Coleoptera: Lucanidae)

Normaliter wordt er vanuit gegaan dat het vliegend hert Lucanus cervus Linnaeus (Coleoptera: Lucanidae) drie larvenstadia heeft. In sommige recente Engelse artikelen wordt verondersteld dat er vijf larvenstadia kunnen zijn. Om dit te onderzoeken hebben we de kopkapselbreedte van Engelse vliegende hertenlarven (n=419) vergeleken met die van Nederlandse larven (n=240). Voor beide groepen larven valt de frequentieverdeling van de kopkapselbreedte in drie duidelijke groepen. De gemiddelde kopkapselbreedte van Nederlandse larven (n=240). Voor beide groepen larven valt de frequentieverdeling van de kopkapselbreedte in drie duidelijke groepen. De gemiddelde kopkapselbreedte van Nederlandse larven (n=240) vergelijk met die van Nederlandse larven (n=240). Voor beide groepen larven valt de frequentieverdeling van de kopkapselbreedte in drie duidelijke groepen. De gemiddelde kopkapselbreedte van Nederlandse larven (n=240). Voor beide groepen larven valt de frequentieverdeling van de kopkapselbreedte in drie duidelijke groepen.