Date of preparation: 25 November 2016

**Use of novel attraction compounds increases monitoring success of a rare beetle, *Elater ferrugineus.***

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**Abstract**.

1. The use of pheromones to determine distributions of rare saproxylic insects is an increasingly popular technique. However, pheromones may also be used to elucidate the biology of these cryptic species, a vital requirement if they are to be accurately monitored and conserved.
2. We used non-invasive aerial trapping to compare the effectiveness of chemicals produced by *Elater ferrugineus* L (Coleoptera: Elateridae), namely 7-methyloctyl (Z)-4-decenoate (the female produced sex pheromone), and male compounds (geranyl and neryl acetone and 6-methyl- 5- heptene- 2- one).The male compounds were identified using head space analysis by solid phase micro-extraction gas chromatography-mass spectrometry.
3. We discovered that, when attracted to the female compound, males reciprocally produce these compounds which then serve as attraction pheromones to other males.
4. Such compounds do not appear to attract females but for a species that has a short activity period and is non-feeding in the adult stage, may ensure breeding success when populations are low.
5. By marking all beetles caught, we were able to demonstrate that recapture rate using this method is low (approximately 11% of total captures annually) and therefore does not limit dispersal or breeding opportunities, making it a valuable tool for monitoring endangered saproxylic beetle species.

**Keywords**. Aggregation, allee effect, *Elater ferrugineus*, monitoring, pheromones, polyandry, saproxylic.

**Introduction**

The European Red List of Saproxylic Beetles (Nieto & Alexander, 2010) stated that, of 436 species native to Europe, it was impossible to determine the status of 122 species (28%) since there was not enough scientific knowledge to determine their risk of extinction. Furthermore, of those assessed, insufficient understanding of the biology of these important decomposers and nutrient recyclers means that the population trends for 57% (249 species) remain unknown. Given the importance of dead wood as a habitat for forest biodiversity and ecosystem function, it is critical that studies address not just the status of this habitat, but also of the species themselves (Seibold *et al.*, 2015).

The majority of assessments of these species have, in the United Kingdom at least, been carried out by professional and amateur entomologists performing habitat surveys. This has undoubtedly led to a broad base of knowledge. However, such work requires expert identification skills and is very time consuming, and furthermore, since there is no obligation to record sightings, it is difficult to build up an authentic inventory of the species. Also, if a rare species is recorded in a particular habitat, further searches could be restricted to similar areas, limiting knowledge of actual habitat in favour of perceived habitat (Gaston, 1994). Perhaps most worrying is the decline in identification skills; with the removal of much entomology and taxonomy from the curriculum in schools, the numbers of potential ‘traditional’ entomologists are decreasing (e.g. Leather, 2007, 2009; Leather & Quicke, 2009). This may result in fewer accurate monitoring schemes carried out and fewer species defined even to presence level within the country.

One method which can help resolve these issues but which, to date, has not been widely employed either in the UK or mainland Europe, to both monitor and understand the biology of saproxylic species is the use of the volatile chemicals produced by the insects themselves. The varied roles of these chemicals in insects are well established, with examples including aggregation pheromones (Byers *et al.*, 2013) and sex pheromones (Musa *et al.*, 2013; Andersson *et al.*, 2014; Zauli *et al.*, 2014), with much of the research having been (perhaps justifiably because of their economic impact) concentrated on pest species (Ukeh *et al.*, 2012; Benelli *et al.*, 2014; Duménil *et al.*, 2014; Han *et al.*, 2014; Vuts *et al.*, 2014). In the UK, the lack of demonstrable economic importance of saproxylic species has resulted in favouring the more charismatic, easily recognisable members of the group, such as the stag beetle, *Lucanus cervus* L*.* This species, which has done much to raise the profile of saproxylic beetles, has had several regional and three national surveys conducted to determine its distribution (Percy *et al.*, 2000; Smith, 2003, 2011). These schemes have used members of the public to monitor this largely urban species (Percy *et al.*, 2000; Smith, 2003, 2011), something which may not be possible for forest species. Furthermore, such surveys may lead to over-estimation of numbers of the species if individuals are counted more than once. They also rely on the species being easily recognisable and on the physical presence of monitors.

During the last 10 years, work has been carried out across Europe to identify volatiles produced by *L. cervus* (Chapman *et al.*, 2002; Harvey *et al.*, 2011) and some of the more elusive species (e.g. *Elater ferrugineus* L.*,* Tolasch *et al.* 2007; *Osmoderma eremita* Scop.*,* Larsson *et al.*, 2003) which has enabled monitoring and presence/absence studies to be carried out (Svensson *et al.*, 2012; Musa *et al.*, 2013; Andersson *et al.*, 2014; Kadej *et al.*, 2014; Zauli *et al.*, 2014). For the latter two species (*E.* *ferrugineus* and *O. eremita*) in particular, the identified chemicals have been utilised to provide pheromone-baited traps aimed at capturing adults of the species (Larsson & Svensson, 2009; Svensson *et al.*, 2012; Kadej *et al.*, 2014). The foremost advantage of using pheromones is the high detection probability and cost/benefit ratio compared to other survey methods (Andersson *et al.*, 2014; Kadej *et al.*, 2014). Furthermore, there is less risk of habitat destruction and disturbance (Musa *et al.*, 2013), and less reliance on a need for expert identification skills. Indeed, visual guides such as photographs can be given to monitors and rapidly confirmed by those conducting the survey, so non-specialist monitors can be employed, and larger areas can be surveyed by fewer monitors over a shorter time period. Furthermore, pheromones can be used to increase our knowledge of these enigmatic species to build up a more accurate picture of their habitat, distribution and biology.

*E. ferrugineus* is an inhabitant of hollow deciduous trees including oak, elm, willow and ash (Allen, 1966; Barševskis & Nitcis, 2011). Larvae are believed to be facultative predators (Larsson & Svensson, 2009) with a life cycle of 4-6 years depending on food sources, which can include wood mould (Andersson *et al.*, 2014; Oleksa *et al.*, 2015), and the eggs and larvae of Scarabaeidae and Lucanidae including *O. eremita, Gnorimus sp., Dorcus sp.*, and *Protaetia sp.* (Schimmel & Tarnawski, 2010; Barševskis & Nitcis, 2011). The adult phase of the life cycle lasts approximately 6 weeks post emergence and adults do not feed (Svensson *et al.*, 2012). This click beetle has been deemed Near Threatened across much of Europe (Nieto & Alexander, 2010). Such status is attributed to a decline in veteran trees (i.e. those with features, including rot sites, holes and water pockets, dead wood, hollowing, and fungal fruit bodies; Fay, 2007). Once lost, such a habitat is virtually impossible to recreate, and so any decline is likely to impact upon rare saproxylic insects. *E. ferrugineus* is extremely rare in the UK, with only 100 records from 11of the 10 km grid squares in the national biodiversity gateway dataset. This is despite its charismatic and unmistakeable (bright red) appearance and size (adults are 17 – 24 mm long). It is thus a perfect candidate for the development of a trapping programme that could use citizen science assistance to monitor its status and that of its threatened habitat.

The aim of this study was to examine the feasibility of using pheromones for monitoring the status of *E. ferrugineus* in the UK.Four compounds produced by the female were identified and have been used for presence/ absence monitoring in Germany (Tolasch *et al.*, 2007). However, Svensson *et al.* (2012) showed that just one of these components, 7- methyloctyl (Z)-4-decenoate (hereafter termed ‘female pheromone’), was the most active in generating an antennal response and was sufficient alone as a lure. Our first objective was to test the use of this compound in the UK. Furthermore, Toth (2013) suggested that some (unspecified) click beetles may produce aggregation pheromones and so our second objective was to examine whether this occurs in *E. ferrugineus.* To date, no male-produced compound has been recorded in this species and we hypothesised that if one does exist, then it could be used to enhance the trapping efficacy (and mating probability) in this extreme low-density beetle.

**Materials and methods**

Field collection was carried out similarly in all years 2013-15. Permission was granted from the Crown Estate to sample beetles on their land and regular checking of traps ensured there were no detrimental effects on population level of the species: traps were monitored daily, with captured beetles removed, sexed and marked on the elytra before being released at least 10m from the trap from which the specimen was caught. This allowed us to verify that the same beetles were not repeatedly recaptured. In each year, preliminary traps were placed at the end of June and monitored daily until beetles appeared, at which point full trials were commenced with fresh traps (lured as described below).

*2013 season experiments*

*Field Collection of beetles 2013*

In July 2013, five pairs of aerial traps, with a minimum distance of 450m between pairs, were placed within a site measuring 1.38km2, in Windsor Great Park, Berkshire, UK (SU 93577 73856), an area of mixed woodland with beech (*Fagus sylvatica)* and veteran oak trees (*Quercus robur and petraea*). Aerial flight interception traps (described in Harvey *et al*., 2011) were each baited with 2µl of pure 7-methyloctyl (Z)-4-decenoate (Svensson *et al.*, 2012) synthesised using the method described in Tolasch *et al.* (2007). The chemical was presented in a 0.2ml PCR tube (Starlab l1402-8100) pierced horizontally through the body of the tube with a small needle and suspended from copper wire through the closed lid. Lured traps were sited in pairs (with 10m separation between traps) to determine whether there was any aggregation effect in trap captures. Additionally, an empty control trap was placed equidistant from the two lured traps at each of the five locations.

Beetles were collected daily, sexed by applying gentle pressure on the abdomen to expose the genitalia. In this and subsequent years, beetles were marked on the right elytron with the trap number using a Crayola® metallic marker. This was done to distinguish between ‘new’ and ‘repeated’ captures and to verify that the same individuals were not repeatedly drawn back to traps, reducing their chances of mating success. Beetles were then released at least 10m from the capture trap.

*Headspace Volatiles Analysis*

On five occasions when a trap captured over 10 individuals more than its partner trap, these beetles (identified as all male) were collected and returned to the laboratory where they were used for head space analysis experiments to determine any differences in volatile profile between beetles from these traps compared to those from traps where less than ten were captured. Specimens were placed in a glass vessel (270cm3) fitted with a septum in the lid and the headspace sampled with a divinylbenzene-carboxen-PDMS 100µl solid phase micro-extraction (SPME) fibre for 1.5 hours. Compounds adsorbed by the fibre were analysed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 5890 Series II gas chromatograph fitted with a 30 m x 0.25 mm i.d. DB5 column with 0.25μm film, interfaced to a Hewlett Packard 5970 mass sensitive detector (Agilent Technologies, Stockport). The oven temperature was programmed as follows: temperature gradient of the oven started at 50oC, held for 2 minutes, then increased to 320oC at 2 degmin-1, where it was held for 2 minutes. The injector temperature was 250oC, with a fibre desorption time and vent delay time of 1min, and the interface temperature was 285oC. Mass spectra were acquired in total ion current (TIC) mode from 33-450amu at 1.3Hz. The mass spectra of chromatographic peaks were extracted using AMDIS (Automated Mass Spectral Deconvolution & Identification System, U.S. Department of Commerce National Institute of Standards and Technology (NIST) Standard Reference Data Program Gaithersburg, MD 20899) and searched in the NIST08 library of mass spectra. Chromatograms and spectra were examined and two potential attractant compounds identified. The retention times and mass spectra of identified compounds were compared to standards to confirm identity. A control of a blank fibre and empty vials, as well as a column blank, were also carried out using the same method and the chromatograms examined for the potential attractant compounds.

*Release rate of combination lure.*

Following the protocol of Svensson *et al*. (2012), the field trials for 2014 and 2015 field experiments were carried out with lures presented as above. Release rate of the combined lures was tested by placing 2µl of 7- methyloctyl (Z)-4-decenoate ii) 4µl of **6,10-dimethyl-5,9-undecadien-2-one** (both diastereoisomers) and 8µl of 6-methyl- 5- heptene- 2- one into a PCR tube. The tube was weighed, left for 24 hours at a constant temperature of 21oC and reweighed. Three repetitions were carried out and a mean calculated.

*2014 and 2015 Season experiments*

Following identification of potential attraction compounds in 2013, we carried out a small pilot study at the end of the season to determine the optimal composition and dosage of the identified male compounds for use as lures. Based on these initial trials, we then carried out a more comprehensive series of field trials and biochemical analyses in 2014-15 to further investigate the role of these compounds in nature and to assess their effect in monitoring of the species.

*Field trapping one, 2014*

In July 2014, 18 traps were set up as above across six locations, with a minimum distance of 450m between pairs, within SU 93577 73856. As in 2013, the traps were set in triplets of a control plus two baited traps at each site: i) 7- methyloctyl (Z)-4-decenoate (2µl, pure) and ii) a combined lure of the potential male attraction pheromones (pure), as identified by head space analysis (see below): 4µl **6,10-dimethyl-5,9-undecadien-2-one,** 97%, (5E,geranyl, 53% + 5Z,neryl, 47%; Alfa Aesar UK cat. no. A19184) and 8 µl 6-methyl- 5- heptene- 2- one (Sigma Aldrich UK cat. no. W270733), to determine the attractive potential of both sets of chemicals individually. Lures were presented in PCR tubes as above.

*Field trapping two, 2014*

Once it was confirmed that the male compounds did serve as an attractant in the field, a second study was conducted to determine the efficacy of the female pheromone with and without the male-produced compounds. This was conducted after a gap of a week, to enable any previously trapped insects to disperse in the local population. All previous traps were removed and new sets each of three lured traps set: i) 2µl of 7- methyloctyl (Z)-4-decenoate ii) 4µl geranyl acetone (both diastereoisomers) and 8µl of 6-methyl- 5- heptene- 2- one and a combination of the two lure types (i.e. female- (i) and male- (ii) produced compounds) were set to investigate any additive or synergistic effects of these compounds. These were placed with a control, across 5 sites, giving 20 traps in total. Traps were placed in the same woodland as above, though not in the exact same locations, in a further attempt to avoid trap bias. Traps were monitored until captures dropped below 10 for two successive days over the 20 traps.

*Field trapping 2015*

In 2015, 20 traps were set up as in 2014 in the same woodland, with three lured traps and a control placed at each of 5 sites. These were baited with the female and male compounds, singly and in combination, in the same quantities as in 2014, above. The traps were monitored daily and captured beetles treated as in previous years.

*Volatile analysis*

To determine whether males produced the identified mixture of chemicals when exposed to female pheromone, we took 10 males collected from all traps across two days in 2014, placed them as a group in a glass container (270cm3) and placed a SPME fibre in the headspace for 30 minutes. We then carried out GC-MS using the method detailed previously for 2013. We repeated this process with two further groups of 14 and 25 beetles, using fresh specimens on each occasion. The same groups of beetles were then exposed separately to the female pheromone for 30 minutes, the beetles placed in a fresh container and any volatiles produced were collected on a SPME fibre over 30 minute periods and examined by GC-MS, as above.

Results of the field collection in 2013 were analysed for spatial aggregation using the negative binomial model (Shaw & Dobson, 1995), with trap number as a fixed effect and captures as the response variable. Within-pair differences were determined using paired t-tests.

Differences in the numbers of beetles caught per day by the female pheromone and the male compounds were examined with a t-test (2014 trial 1) because control traps caught no beetles and thus the normal assumptions of ANOVA were violated. Meanwhile, two factor Analysis of Variance, following square root transformation of the data was used for 2014 trial 2 and 2015 trapping. Statistical analyses were carried out using SPSS v 21 (IBM Corp 2012) and RStudio 0.98.1091 software.

**Results**

*Field collection of beetles 2013*

Pairs of traps lured with each baited with 2µl of female pheromone 7-methyloctyl (Z)-4-decenoate presented in a 0.2ml PCR tube recorded different numbers throughout the season, with all but one set having significantly more catches in one of the two baited traps (see Fig. 1). However, the trap within the pair catching the greater number of specimens varied over the trapping period, suggesting that differences were not simply due to trap orientation (Fig. 1). The highest number of beetles captured on one occasion in one trap (27th July) was 54, with none caught in the trap partner. Within- pair comparisons demonstrated significantly more beetles captured by one trap of each pair throughout the trapping period except at site 4 (shown in Table 1). Analysis of the capture data for aggregation suggested that the beetles were aggregated (*k* = 0.2307). The 5 control traps collected 10 beetles overall throughout the trapping period. Recapture of beetles for the 2013 trials was 11% across all lures.

[Figure 1 around here]

Table 1. Comparisons of total number of captured beetles between pairs of traps at each site baited with 2µl of 7-methyloctyl-(Z)-4-decenoate (1-5) in 2013. Degrees of freedom in brackets

|  |  |  |
| --- | --- | --- |
| Trap pair | t | p |
| 1a and 1b | 3.974 (29.34) | <0.001 |
| 2a and 2b  | 2.376 (33.166) | <0.05 |
| 3a and 3b | 2.013 (35.07) | 0.05 |
| 4a and 4b | 0.319 (58) | NS |
| 5a and 5b | -2.957 (35.87) | 0.005 |

Note: df adjusted for equal variances not assumed (Levene’s Test for Equality of Variances *P*<0.05)

*Identification of Male Volatiles*

Examination of five chromatograms from the headspace of male *E. ferrugineus* adults collected from high trap numbers repeatedly found three compounds above background, which were identified as both diastereoisomers of **6,10-dimethyl-5,9-undecadien-2-one** (geranyl acetone (E) (Kovats Index 1449) and neryl acetone(Z) (Kovats Index 1424) and 6 methyl- 5- heptene- 2- one (MHK,Kovats index 987). These compounds were not found where the numbers of beetles were lower, nor in fibre blanks as detailed above. On the chromatogram, peak areas for geranyl acetone(GA) and neryl acetone (NA) and 6-methyl-5-heptene-2-one were in the ratio for GA&NA (E + Z): MHK of 1:2. This ratio was therefore used in determining lure composition. The mean release rate for this lure was 0.45mghr-1.

Most importantly, however, males subjected to headspace analysis without exposure to female pheromone showed no production of geranyl and neryl acetone or 6 methyl- 5- heptene- 2- one in any of the trials. After exposure to the female pheromone, all samples of headspace over males examined by SPME GC-MS contained both the 6-methyl-5-hepten-2-one and and **6,10-dimethyl-5,9-undecadien-2-one** (E) and (Z) diastereoisomers.

(Figure 2 around here)

*Field trapping one, 2014*

No beetles were caught in control traps. There was a tendency for the number of beetles captured in traps lured with 7-methyloctyl (Z)-4-decenoate (female pheromone) to be higher than with the mixture of male-produced compounds 6 methyl-5-heptene-2-one and geranyl and neryl acetone, but this was not significant (t(5) = 1.72, *P* = 0.07: Fig. 3).

[Figure 3 around here]

*Field trapping two, 2014*

Small numbers of beetles were caught in control traps (Fig. 3), but those baited with the female pheromone trapped significantly more (*F*1,44 = 92.3, *P* < 0.001). There was a highly significant interaction term (*F*1,44 = 10.4, *P* < 0.01), because traps with the mixture of compounds appeared to show a synergistic effect and caught considerably more beetles (Fig. 4).

In the 2014 trapping season, for all lures, 28% of beetles were recaptured.

 [Figure 4 around here]

*Field trapping 2015*

Trapping results in 2015 were broadly similar to those in 2014, though the female pheromone alone did not catch greater numbers of beetles than the male compound (Fig. 5). However, there was again a significant interaction between the two lures, as the mixed lure caught large numbers of beetles per day (*F*1,120 = 32.1, *P* < 0.001). In these trials 11% of beetles were recaptured by all lures.

[Figure 5 around here]

**Discussion**

The use of pheromones has been established as an effective technique for monitoring saproxylic beetle species (Harvey *et al.*, 2011; Svensson *et al.*, 2012; Chiari *et al.*, 2013; Kadej *et al.*, 2014). Here we show that monitoring using pheromones can reveal more than just population numbers: it can also give an insight into species’ biology and behaviour. We have shown that male *E. ferrugineus* attracted by a female lure respond by releasing male compounds that serve to attract further males to the site. When the two sets of compounds were baited together, they acted synergistically to capture large numbers of beetles in two successive years. In this species, we have identified three compounds that appear to work in concert namely geranyl and neryl acetone and 6 methyl- 5- heptene- 2- one. In *E. ferrugineus*,these compounds attract other males only in the presence of female pheromone. This may ensure that attraction of males only occurs in the presence of females of the species, resulting in increased mating opportunities.

Insects are exposed to many volatiles providing both useful signals, which impart cues vital for the successful completion of the life cycle and other, much larger, chemical profiles, such as plant volatiles and other environmental chemicals. Since reproductive pheromones are not without metabolic cost to the producer and can be released in extremely small quantities, in species where plant volatiles do not serve to aggregate the species, or the plant host is abundant and the prospective mates less so, a further stimulus, such as a pheromone, can aid in mate seeking behaviour (Byers & Birgersson, 2012; Pajares *et al.,* 2013; Byers *et al.,* 2013).

The production of aggregation pheromones in insects is well known (Byers & Birgersson, 2012; Byers *et al.*, 2013; Pajares *et al.*, 2013; Wheeler & Cardé, 2013) and is often associated with attraction of both sexes. Here, however, we suggest that the males of the species produce aggregation compounds that attract further males to a female. The production of aggregation pheromones by the male of the species is not unique (e.g. in the Cerambycidae including *Neoclytus acuminatus*; Lacey *et al.*, 2004, Hanks & Millar, 2016); the bug *Narnia femorata;* Addesso *et al.,* 2014*;*and a weevil *Cyrtomon luridus;* Kamiya *et al.*, 2014). Although such chemicals usually attract both sexes of a species , Leal *et al.* (1996) demonstrated a pheromone mix (2-(E)-octenyl acetate and octanol) produced by both sexes of rice bug (*Leptocorisa chinesis*) that attracts males in the field and not females, and does not elicit a sexual response in males. Eavesdropping or exploitation of a signal which is directed towards attracting females to the sender are often used to explain attraction of males to signals compounds produced by other males (Landolt & Phillips, 1997; Cardé, 2014). However, this is not the case here , since it is clear from our field studies that the male compound does not serve to attract females to the site. Since so little of the biology of this species has been established, further work is needed to determine whether this effect is only elicited in response to multiple females, which the lure of 7-methyloctyl (Z)-4-decenoate may represent. Chance alone will determine whether a male happens to detect a pheromone plume, find a female and mate. This is a risky strategy since, especially in a species where life span is limited to a few weeks, weather conditions are not always optimum for mating and a female may fail to successfully attract males before she dies. Such aggregation behaviour in males may increase the chances of males finding mates(Wertheim *et al.*, 2005; Halliday & Blouin-Demers, 2016). In systems where multiple mating occurs, where the female lays many clutches throughout the season, or where mating stimulates further ovulation, attraction of males may not reduce the overall success of the species, although the individual benefit to males is difficult to define. It is unknown if *E. ferrugineus* exhibits polyandry, but other low-density saproxylic beetles that do not feed in the adult stage do so (Harvey & Gange, 2006). Multiple mating can be costly, but can also result in a variety of fitness gains, such as egg viability, offspring fitness and attractiveness (García-González & Simmons, 2007; Okada *et al.*, 2015). We suggest that polyandry is likely in *E. ferrugineus* and would be a good strategy for ensuring population persistence in the fragmented habitats in which it occurs.

Most species’ ejaculate contains seminal fluid proteins which are able to influence mating outcome by increasing oviposition and ovulation in females, reducing receptivity to other males and increasing displacement of other males’ sperm (Dhole & Servedio, 2014). In *Drosophila*, increased mating increases oogenesis (Soller *et al.*, 1997, 1999) and it also increases ovulation (Heifetz *et al.*, 2000; Chapman & Davies, 2004). Therefore, multiple mating in *E. ferrugineus* initiated by production of attraction compounds by males of the species could serve to increase overall population success and also increase female oogenesis and oviposition. However, until further research elucidates more about the mating behaviour of the species it is not possible to draw any firm conclusions here and further work on the biology of the female is required.

The allee effect is defined as ‘a positive relationship between any component of individual fitness and either numbers or density of conspecifics’ (Stephens *et al.*, 1999). This can be further refined to demographic allee effect, which rather than concentrating on the fitness of an individual (which decreases as population increases as a result of increased competition) considers the fitness of the population as a whole, in relation to population density (Courchamp *et al.*, 2008). It can be seen that if a population becomes very small, then the risk of extinction of the population rises as chances of finding a mate fall. Demographic allee effects may be weak if the population growth remains positive even though population density is low; however, if the population drops below a threshold, then the effect becomes strong and extinction may follow. Production of an attraction compound by males of a species may, by as yet unknown mechanisms, enable fragmented populations of *E. ferrugineus* to survive, even at low densities.

At this point we cannot say that only the males of the species produce the compounds identified by us here, as we have not been able to test females for the production of volatiles. We can only say that it does not seem to act as an attractant to females. We speculate that attraction of large numbers of females to a food source for larvae would be a strategy of dubious value, since it would increase intra-specific competition for resources between larvae that would result in an overall lowering of the fitness of the species and hence would result in negative selection pressure. What is clear, however, is that further work will need to be done in this area to elucidate the chemo-ecology of the female of the species.

Therefore, although only slowly becoming part of the toolset used in the monitoring of rare saproxylic species, we have shown here that pheromones play a vital role not only in identifying presence of such species but also identifying hitherto unknown aspects of their biology that will help to complete the picture of these elusive species and increase the likelihood of their preservation.

Acknowledgements

We are grateful to Mattias Larsson and Glen Svensson for their willingness to share their knowledge and for reading this manuscript and commenting on it. We thank the People’s Trust for Endangered Species for sponsoring the purchase of lures and traps and Arran Folly and Jack Whitehouse for help in monitoring traps.

**References**

Addesso, K., Short, K., Moore, A. & Miller, C. (2014) Context-dependent female mate preferences in leaf-footed cactus bugs. *Behaviour*, **151**, 479–492.

Allen, A. (1966) The rarer Sternoxia (Col.) of Windsor Forest. *Entomologist’s Records*, **78**, 14–23.

Andersson, K., Bergman, K., Andersson, F., Hedenström, E., Jansson, N., Burman, J., et al. (2014) High-accuracy sampling of saproxylic diversity indicators at regional scales with pheromones: The case of *Elater ferrugineus*. *Biological Conservation*, **171**, 156–166.

Barševskis, A. & Nitcis, M. (2011) *Elater ferrugineus* linnaeus, 1758- a new species for the fauna of Latvia. *Baltic Journal of Coleopterology*, **11**, 187–196.

Benelli, G., Carpita, A., Simoncini, S., Raspi, A. & Canale, A. (2014) For sex and more: attraction of the tephritid parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae) to male sex pheromone of the olive fruit fly, *Bactrocera oleae*. *Journal of Pest Science*, **87**, 449–457.

Byers, J.A. & Birgersson, G. (2012) Host tree monoterpenes and biosynthesis of aggregation pheromones in the bark beetle *Ips paraconfusus*. *Psyche*, **2012**, 1–10.

Byers, J.A., Birgersson, G. & Francke, W. (2013) Aggregation pheromones of bark beetles, *Pityogenes quadridens* and *P. bidentatus*, colonizing scotch pine: Olfactory avoidance of interspecific mating and competition. *Chemoecology*, **23**, 251–261.

Cardé, R.T. (2014) Defining attraction and aggregation pheromones: teleological versus functional perspectives. *Journal of Chemical Ecology*, **40**, 519–520.

Chapman, J., Birkett, M., Pickett, J., Woodcock, C.M. & Wood, P.J. (2002) Chemical ecology and conservation of the stag beetle, *Lucanus cervus*. Chemical communication: from hormones to semiochemicals. *Comparative Biochemistry and Physiology. Part A*, **132**, 63–70.

Chapman, T. & Davies, S.J. (2004) Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides*, **25**, 1477–90.

Chiari, S., Bardiani, M., Zauli, A., Hardersen, S., Mason, F., Spada, L., et al. (2013) Monitoring of the saproxylic beetle *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) with freshly cut log piles. *Journal of Insect Conservation*, **17**, 1255–1265.

Courchamp, F., Berec, L. & Gascoigne, J. (2008) *Allee effects in ecology and conservation*. Oxford University Press, Oxford.

Dhole, S. & Servedio, M.R. (2014) Sperm competition and the evolution of seminal fluid composition. *Evolution*, **68**, 3008–3019.

Duménil, C., Judd, G., Bosch, D., Baldessari, M., Gemeno, C. & Groot, A. (2014) Intraspecific variation in female sex pheromone of the codling moth *Cydia pomonella*. *Insects*, **5**, 705–721.

Fay, N. (2007) *Defining and surveying veteran and ancient trees*. *UK Biodiversity Action Plan*. Bristol.

García-González, F. & Simmons, L. (2007) Paternal indirect genetic effects on offspring viability and the benefits of polyandry. *Current Biology,* **17**, 32–36.

Gaston, K.J. (1994) *Rarity*. 1st edn. Springer, Falmouth.

Halliday, W.D. & Blouin-Demers, G. (2016) Male aggregation pheromones inhibit ideal free habitat selection in red flour beetles (*Tribolium castaneum*). *Journal of Insect Behavior*, **29**, 355–367.

Han, B.-Y., Wang, M.-X., Zheng, Y.-C., Niu, Y.-Q., Pan, C., Cui, L., et al. (2014) Sex pheromone of the tea aphid, *Toxoptera aurantii* (Boyer de Fonscolombe) (Hemiptera: Aphididae). *Chemoecology*, **24**, 179–187.

Hanks, L.M. & Millar, J.G. (2016) Sex and aggregation-sex pheromones of cerambycid beetles: Basic science and practical applications. *Journal of Chemical Ecology*, 1-24.

Harvey, D. & Gange, A. (2006) Size variation and mating success in the stag beetle, *Lucanus cervus*. *Physiological Entomology,* **31**, 218–226.

Harvey, D., Hawes, C., Gange, A., Finch, P., Chesmore, D. & Farr, I. (2011) Development of non-invasive monitoring methods for larvae and adults of the stag beetle, *Lucanus cervus*. *Insect Conservation and Diversity*, **4**, 4–14.

Heifetz, Y., Lung, O., Jr, E.A.F. & Wolfner, M.F. (2000) The Drosophila seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. *Current Biology*, **10**, 99–102.

IBM Corp. (2012) *IBM SPSS Statistics for Windows*, Version 21.0.

Kadej, M., Zając, K., Ruta, R., Gutowski, J.M., Tarnawski, D., Smolis, A., et al. (2014) Sex pheromones as a tool to overcome the Wallacean shortfall in conservation biology: a case of *Elater ferrugineus* Linnaeus, 1758. *Journal of Insect Conservation*, **19**, 25–32.

Kamiya, A.C., Silva, W.D., Wadt, L., Maurício, J. & Bento, S. (2014) Mating behavior and evidence for male-produced aggregation pheromone in *Cyrtomon luridus* (Boheman) (Coleoptera: Curculionidae: Entiminae). *Journal of Insect Behaviour*, **28**(1), 1402–1409.

Lacey, E.S., Ginzel, M.D., Millar, J.G. & Hanks, L.M. (2004) Male-produced aggregation pheromone of the Cerambycid beetle *Neoclytus acuminatus acuminatus*. *Journal of Chemical Ecology*, **30**, 1493–1507.

Landolt, P.J. & Phillips, T.W. (1997) Host plant influences on sex pheromone behavior of phytophagous insects 1. *Annual Review of Entomology*, **42**, 371–91.

Larsson, M., Hedin, J., Svensson, G., Tolasch, T. & Francke, W. (2003) Characteristic odor of *Osmoderma eremita* identified as a male-released pheromone. *Journal of Chemical Ecology*, **29**, 575–587.

Larsson, M. & Svensson, G. (2009) Pheromone monitoring of rare and threatened insects: exploiting a pheromone-kairomone system to estimate prey and predator abundance. *Conservation Biology*, **23**, 1516–25.

Leal, W.S., Ueda, Y. & Ono, M. (1996) Attractant pheromone for male rice bug, *Leptocorisa chinensis*: Semiochemicals produced by both male and female. *Journal of Chemical Ecology*, **22**, 1429–1437.

Leather, S. (2007) British entomology in terminal decline. *Antenna*, **31**, 192–193.

Leather, S.R. (2009) Taxonomic chauvinism threatens the future of entomology. *Biologist*, **56**, 10–13.

Leather, S.R. & Quicke, D.J.L. (2009) Where would Darwin have been without taxonomy? *Journal of Biological Education*, **43**, 51–52.

Leftwich, P.T., Edward, D. a., Alphey, L., Gage, M.J.G. & Chapman, T. (2012) Variation in adult sex ratio alters the association between courtship, mating frequency and paternity in the lek-forming fruitfly *Ceratitis capitata*. *Journal of Evolutionary Biology*, **25**, 1732–1740.

Musa, N., Andersson, K., Burman, J., Andersson, F., Hedenström, E., Jansson, N., et al. (2013) Using sex pheromone and a multi-scale approach to predict the distribution of a rare saproxylic beetle. *PLoS ONE*, **8**, e66149.

Nieto, A. & Alexander, K. (2010) *European Red List of Saproxylic Beetles*. IUCN Species Programme. Publications Office of the European Union, Luxembourg.

Okada, K., Archer, C.R.C., Katsuki, M., Suzaki, Y., Sharma, M.D., House, C.M., et al. (2015) Polyandry and fitness in female horned flour beetles, *Gnatocerus cornutus*. *Animal Behaviour*, **106**, 11–16.

Oleksa, A., Chybicki, I.J., Larsson, M., Svensson, G.P. & Gawroński, R. (2015) Rural avenues as dispersal corridors for the vulnerable saproxylic beetle *Elater ferrugineus* in a fragmented agricultural landscape. *Journal of Insect Conservation*, **19**, 567–580.

Pajares, J., Álvarez, G., Hall, D.R., Douglas, P., Centeno, F., Ibarra, N., et al. (2013) 2-(Undecyloxy)-ethanol is a major component of the male-produced aggregation pheromone of *Monochamus sutor*. *Entomologia Experimentalis et Applicata*, **149**, 118–127.

Percy, C., Bassford, G., Keeble, V. & Robb, C. (2000) *Findings of the 1998 national stag beetle survey*. People’s Trust for Endangered Species, London.

Schimmel, R. & Tarnawski, D. (2010) Monograph of the subtribe Elaterina (Insecta: Coleoptera: Elateridae: Elaterinae). *Genus*, **21**, 325–487.

Seibold, S., Bässler, C. & Brandl, R. (2015) Experimental studies of dead-wood biodiversity—A review identifying global gaps in knowledge. *Biological Conservation*, **191**, 139–149.

Shaw, D.J. & Dobson, A.P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology*, **111 Suppl**, S111-27.

Smith, M. (2003) *National Stag Beetle Survey 2002*. People’s Trust for Endangered Species. People’s Trust for Endangered Species, London.

Smith, M. (2011) *Great Stag Hunt III: National stag beetle survey 2006-2007*. People’s Trust for Endangered Species, London.

Soller, M., Bownes, M. & Kubli, E. (1997) Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Drosophila melanogaster*. *European Journal of Biochemistry*, **243**, 732–738.

Soller, M., Bownes, M. & Kubli, E. (1999) Control of oocyte maturation in sexually mature Drosophila females. *Developmental Biology*, **208**, 337–351.

Stephens, P.A., Sutherland, W.J. & Freckleton, R.P. (1999) What is the Allee effect? *Oikos*, **87**, 185–190.

Svensson, G., Liedtke, C., Hedenström, E., Breistein, P., Bang, J. & Larsson, M. (2012) Chemical ecology and insect conservation : optimising pheromone-based monitoring of the threatened saproxylic click beetle *Elater ferrugineus*. *Journal of Insect Conservation*, **16**, 549–555.

Tolasch, T., Fragstein, M. von & Steidle, J. (2007) Sex pheromone of *Elater ferrugineus* L. *Journal of Chemical Ecology*, **33**, 2156–66.

Ukeh, D. a, Woodcock, C.M., Pickett, J.A. & Birkett, M. (2012) Identification of host kairomones from maize, Zea mays, for the maize weevil, *Sitophilus zeamais*. *Journal of Chemical Ecology*, **38**, 1402–9.

Vuts, J., Furlan, L., Csonka, É.B., Woodcock, C.M., Caulfield, J.C., Mayon, P., et al. (2014) Development of a female attractant for the click beetle pest *Agriotes brevis*. *Pest Management Science*, **70**, 610–4.

Wertheim, B., Baalen, E.-J.A. van, Dicke, M. & Vet, L.E.M. (2005) Pheromone-mediated aggregation in nonsocial arthropods: an evolutionary ecological perspective. *Annual Review of Entomology*, **50**, 321–346.

Wheeler, C.A. & Cardé, R.T. (2013) Defensive allomones function as aggregation pheromones in diapausing Ladybird Beetles, *Hippodamia convergens*. *Journal of Chemical Ecology*, **39**, 723–32.

Zauli, A., Chiari, S., Hedenström, E., Svensson, G. & Carpaneto, G. (2014) Using odour traps for population monitoring and dispersal analysis of the threatened saproxylic beetles *Osmoderma eremita* and *Elater ferrugineus* in central Italy. *Journal of Insect Conservation*, **18**, 801–813.

**Figure legends**

**Figure 1.** Captures of male beetles in 5 pairs of traps (trap A - and trap B - ) placed in 2013 with *E. ferrugineus* female pheromone.

**Figure 2.** Chromatograms from volatiles collected from above the headspace of 10 male *E.ferrugineus* (6-methyl- 5- heptene- 2- one and **6,10-dimethyl-5,9-undecadien-2-one** (E) + (Z) peaks identified as arrows above relevant peaks.Lower chromatogram demonstrates absence of compounds when males were not exposed to 7- methyloctyl (Z)-4-decenoate before volatile collection.

**Figure 3.** Male *E. ferrugineus* captures in 2014, where lure i) contained 7- methyloctyl (Z)-4-decenoate (2µl) and lure ii) contained **6,10-dimethyl-5,9-undecadien-2-one** (E) + (Z) (4µl) and 6-methyl- 5- heptene- 2- one (8ul). Control traps were blank. Error bars show ±SE here and in all following. Letters above the bars indicate significant differences here and in all following.

**Figure 4.** Male *E. ferrugineus* captures during field collections in 2014, where lure i) contains 2µl of 7- methyloctyl (Z)-4-decenoate, lure ii) contains 4µl **6,10-dimethyl-5,9-undecadien-2-one** (both diastereoisomers) and 8µl of 6-methyl- 5- heptene- 2- one, and lure i+ii is a combination of these lure types.

**Figure 5.** Male *E. ferrugineus* captures during 2015, where lure i) contains 2µl of 7- methyloctyl- (Z)-4-decenoate, lure ii) contains 4µl **6,10-dimethyl-5,9-undecadien-2-one** (both diastereoisomers) and 8µl of 6-methyl- 5- heptene- 2- one, and lure i+ii is a combination of these lures types.