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Spatiotemporal monitoring of rare and elusive saproxylic beetles

A pheromone based approach

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Cover: Stylised montage of photos depicting the saproxylic beetles *Anaglyptus mysticus, Bostrichus capucinus*, *Plagionotus arcuatus* and *Tragosoma depsarium* together with a pheromone trap in an oak environment.

(photos: B. Eriksson)

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Abstract

Forest ecosystems are under severe pressure from global environmental changes and increasing demands of wood-related products. During the last century, intensified management regimes have led to the decline of many previously common forest habitats, with catastrophic effects for biodiversity. Many saproxylic beetles, which often play important roles as decomposers and food sources in natural ecosystems, are suffering population declines due to the losses and degradation of their forest habitats. As many of the species have become increasingly rare, or appear cryptic and elusive, it is often challenging to study their genuine distributions and habitat demands. In recent years, pheromone-based methodologies have begun to be applied for surveying such rare and threatened insects. In this thesis, I first identified the aggregation-sex pheromone components of three saproxylic beetles of Swedish conservation interest. In a second step, I utilised pheromone-based trapping methodologies to investigate how beetles with varying substrate and habitat demands fare in the Swedish forest landscape. By setting up large-scale monitoring studies across their distribution ranges in Southern Sweden, I was able to evaluate both their spatial and temporal population fluctuations. My results reveal large variability in species' responses to modern forestry practices, seemingly dependent on differences in their spatiotemporal ecology. Through the high accuracy sampling, I was significantly able to alter our perception of the presence and abundance of several species within their distribution ranges. Overall, this work demonstrates the need for efficient monitoring methodologies for conservation of forest biodiversity.

Keywords: saproxylic beetles, pheromones, Swedish forest management, dead wood, forest biodiversity, species distribution, conservation, oak, pine

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Spatiotemporal övervakning av hotade och gäckande vedlevande skalbaggar

Abstract

Skogens ekosystem är under hårt tryck från globala miljöförändringar och ökande behov av vedrelaterade produkter. Under det senaste århundradet har allt mer intensifierade skötselregimer lett till att många tidigare vanliga skogshabitatstyper har minskat, med katastrofala effekter för den biologiska mångfalden. Många vedlevande skalbaggar, som ofta har viktiga roller som nedbrytare och födokällor i naturliga ekosystem, har minskat kraftigt på grund av förluster och fragmenteringar av deras livsmiljöer. Eftersom många av arterna har blivit allt mer sällsynta, eller förefaller kryptiska och svårfångade, är deras genuina utbredning och livsmiljökrav ofta utmanande att studera på ett tillförlitligt sätt. Under de senaste åren har feromonbaserade metoder utvecklats för att noggrant och systematiskt kunna utföra storskaliga inventeringar av sällsynta och hotade insekter. I denna avhandling identifierar jag aggregation-sexferomoner hos tre vedlevande skalbaggar av svenskt naturvårdsintresse. En feromonbaserad fångstmetodik användes sedan för att utföra storskaliga övervakningsstudier över deras utbredningsområden i södra Sverige. Inom dessa studier, undersökte jag hur skalbaggar med varierande substrat- och habitatkrav klarar sig i det svenska skogslandskapet. Mina resultat avslöjar en stor variation i arters förutsättningar att överleva i det moderna skogsbruket, till stor del beroende på skillnader i deras spatiotemporala ekologi. Genom att väsentligt förändra vår uppfattning om flera arters förekomst och abundans inom sina utbredningsområden demonstreras även vikten av feromonbaserad övervakning.

Keywords: Vedlevande skalbaggar, feromoner, skogsskötsel, död ved, skogsbiodiversitet, feromoner, spatial, temporal, bevarandebiologi, ek, tall

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Dedication

To my readers.

“En händelse, som ser ut som en tanke.”
Erik Gustaf Geijer

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Molander MA*, **Eriksson B**, Winde IB, Zou Y, Millar JG, Larsson MC (2019). The aggregation-sex pheromones of the cerambycid beetles *Anaglyptus mysticus* and *Xylotrechus antilope ssp*. antilope: New model species for insect conservation through pheromone-based monitoring. Chemoecology. 29. 111-124.

II. Molander MA*¤, **Eriksson B**¤, Vestlund M, Zou Y, Millar JG, Larsson MC. Pheromone-based monitoring in oak forests reveal no effect of set-aside conservation sites, but short-term effects of logging on habitat use by saproxylic longhorn beetles Cerambycidae) with fast population dynamics. Manuscript.

III. **Eriksson B***, Westerberg L, Wallin E, Bergman KO, Larsson MC. Monitoring the true abundance of the cryptic red-listed beetle *Bostrichus capucinus* (Coleoptera: Bostrichidae) in relation to its presumed distribution. Manuscript.

IV. **Eriksson B***, Westerberg L, Bergman KO, Milberg P, Nilsson A, Wikars LO, Larsson MC. Distribution and abundance of *Tragosoma depsarium* (Coleoptera: Cerambycidae) - effects of habitat and substrate availability. Manuscript.

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The contribution of Björn Eriksson to the papers included in this thesis was as follows:

I. Conducted field work with MAM. Compiled and analysed results. Contributed to writing the manuscript with MAM and MCL.

II. Conducted field work with MAM in 2017. Planned and conducted field work in 2018. Combined and analysed data with assistance from MAM. Assisted MAM in writing the manuscript with input from co-authors.

III. Developed the questions and study design with input from co-authors. Supervised the field work. Compiled and analysed the data. Wrote the manuscript with input from co-authors.

IV. Planned the study and conducted the field work in 2019. Compiled and analysed data from 2014 and 2019. Wrote the manuscript with input from co-authors.

1. Introduction

1.1 Background

Forests cover almost a third of Earth's landmass and are crucial for the welfare of humankind (Cardinale et al. 2012). About 1.6 billion people are estimated to be directly reliant on forests (Newton et al. 2020) and many more indirectly benefit from the myriads of provided ecosystem services, including carbon sequestration, nutrient cycling and timber production. To ensure a continued supply of services, functioning ecosystems are of vital importance, which in turn require maintained forest biodiversity and species richness (Cardinale et al. 2012; García & Martínez 2012; Mori et al. 2017). This is however far from being guaranteed in modern forest ecosystems, where the previously heterogeneous forest structures are replaced by more homogeneous stands of higher economic value (Östlund et al. 1997; Shorohova et al. 2011). As managed forest stands become more similar in regard to both age and tree species (Cyr et al. 2009), many species suffer from habitat degradation and losses (Ibarra & Martin 2015; Betts et al. 2017). The resulting increasing number of threatened and red-listed species (Shortall et al. 2009; Fox et al. 2019) highlight the importance of efficient management methods for the preservation of forest biodiversity and ecosystem services.

In Sweden, forestry has for centuries been a vital part of the national economy and is today producing around 10% of the globally traded timber, pulp and paper (Helander 2015). During the last century, Swedish forestry has significantly increased its efficiency and become dominated by clear-cut loggings. Since then, most of the Swedish forests have been cut, and only small proportions remain untouched by modern forestry (Mikusiński 2006).

With modern management regimes slowly homogenizing the landscape, forest biodiversity is suffering from fragmentation, as well as from substrate and habitat losses (Bengtsson et al. 2000; Devictor et al. 2008; Paillet et al. 2010; Halme et al. 2013). For a sustainable forestry regime, a balance has to be found between the economic and societal needs of forest resources, and the retention of biodiversity and ecosystem services.

The Swedish model of sustainable forestry utilises a combination of formally protected nature reserves, as well as smaller set-aside stands of protection forests. A vast majority of logging is done within the PEFC™ and FSC® forest certification systems, with care taken to biodiversity conservation, although their positive effects are disputed (Elbakidze et al. 2011; Johansson 2012). Overall, the Swedish model has an emphasis on smaller scale preservations at the habitat level. While protected areas, such as nature reserves, have been proved beneficial for many species (e.g. Götmark & Thorell 2003; Karpiński et al. 2021), recent decades have seen an increased focus on following species at larger spatial scales (e.g. Paltto et al. 2006; Franc et al. 2007; Bergman et al. 2012; Rubene et al. 2017). With species needs and characteristics differing both within and among taxa, it is of utmost importance to consider both spatial (Devictor et al. 2008; Sverdrup-Thygeson et al. 2014b) and temporal (Boucher et al. 2012; Laaksonen et al. 2020) scales.

Successful evidence-based conservation of forest biodiversity is dependent on trustworthy information of the environmental requirements of threatened and endangered species. Unfortunately, this type of data is often difficult to obtain and still unavailable for many groups of taxa. While saproxylic (dead wood dependent) insects are well studied as groups or communities, much less is known at the species level. As many saproxylic beetles are elusive and hard to systematically sample, little is known of their true distributions and population sizes. It is not until the last decades that pheromone-based methodologies have been developed for cost- and effort-efficient directly surveys of individual species (e.g. Andersson et al. 2014; Harvey et al. 2017; Molander et al. 2019a).

1.2 Dead-wood biodiversity

Dead wood constitutes a very large and diverse group of substrates, sustaining a myriad of taxa. In natural forests, dead wood is constantly generated following disturbances, such as fire, storms or pest outbreaks (Kulakowski et al. 2017), or after the natural death of trees. In heterogeneous forests, this creates a large variety of dead-wood substrates where differing tree species of varying ages, dying from different causes, generate substrates of different qualities (Thorn et al. 2020). This variety of substrates is exploited by saproxylic insects that often specialise in different niches, such as canopy branches (Seibold et al. 2018), hollow cavities (Chiari et al. 2013) or fallen logs and stumps (Andersson et al. 2015). As the dead-wood substrates age, decomposition is facilitated by a succession of species colonising and departing, often dependent on both microclimate (Weslien et al. 2011; Thorn et al. 2020) and the surrounding landscape (Jonsell et al. 2019). These countless numbers of dead-wood dependent insects form complex ecological interactions and often become basal links in trophic chains, e.g. with woodpeckers (Roberge et al. 2008). While natural forests usually contain large amounts of dead wood (Radu 2006; Thorn et al. 2020), managed forests often hold mere fractions of the substrates (Siitonen 2001; Jonsson et al. 2016). With forest managements mainly focused on production, dead wood availability has been reduced, both indirectly, through diminished substrate generating disturbances, and through active removal (Linder 1998; Ehnström 2001; Bouget et al. 2012; Heikkala et al. 2016a). As both the quantity and variety of substrates have decreased, during the last century many saproxylic insects have become rare and threatened (e.g. Seibold et al. 2015; Calix et al. 2018).

In Sweden, there are around 3,500 known saproxylic insects. A third of these belong to the order of Coleoptera and they inhabit substrates of all native trees species (Jonsell et al. 1998; Dahlberg & Stokland 2004). Saproxylic beetles usually spend their larval stage within dead-wood substrates and play important parts as both wood decomposers (Grove 2002; Buse et al. 2008), and food sources for species at higher trophic levels (e.g. Bell et al. 2015). With the modernisation of forestry regimes, many of these beetles are today considered rare, red listed and often declining (SLU Artdatabanken 2020; Pilotto et al. 2021). To preserve saproxylic beetle diversity, national action plans have been created for species dependent on a wide range of trees (e.g. Ehnström 2005; Wikars 2006; Pettersson et al. 2013; Wikars et al. 2014). Through these and similar initiatives, much focus is today made to create and restore suitable substrates for a wide range of insects (e.g. Figure 1).

Figure 1. Boxes of mulch and wood substrates attached to oaks to mimic tree hollows. A large fauna of saproxylic biodiversity is dependent on these type of cavities that form in large veteran oaks, now rare in the modern woodland landscape. (photo: B. Eriksson)

1.3 Swedish forestry

Historically, Swedish forests have during the last millennium been dominated by mixed deciduous trees in the south and predominately pine (*Pinus sylvestris*) in the north (Lindbladh et al. 2000). During the last century's modernization of Swedish forestry, spruce (*Picea abies*) has become the by far most economically viable tree, and has slowly overtaken the others as the dominating species (Björse & Bradshaw 1998; Felton et al. 2020). The actively managed tree plantations differ significantly from natural forests, with much darker and denser even-aged tree stands. As the trees are usually cut upon reaching maturity, older veteran trees are predicted to become ever more scarce in the coming decades (Claesson et al. 2015). Within modern forests, much of the natural dynamics have been interrupted and previously common disturbances replaced by logging actions. Forest fires have historically been common in a large proportion of the Swedish forests (Figure 2), renewing older stands and generating large quantities of diverse dead wood (Eriksson et al. 2013). While larger fires can be economically devastating when hitting modern dense spruce plantations, they are now exceedingly rare. This development has seriously affected many woodland insects adapted to the previous conditions. For some species, substrates left at clear cuts can be utilised as replacements, while other see no such beneficial effects (Hjältén et al. 2010; Johansson et al. 2010).

While there is a long history of nature reserves and other formally protected areas in Sweden, it is during recent decades that production forests have seen increased efforts for biodiversity conservation. The Swedish model of sustainable forestry (Sverdrup & Stjernquist 2013) was accepted in 1993 as a revision of Sweden's previously completely production-focused forestry policy. With the new model, the production priority was relaxed and complemented with an environmental one, aiming to create a regime capable of both meeting demands of wood products and preserving high levels of biodiversity (Lindahl et al. 2017). While this shift, together with forest certifications (i.e. PEFC^{TM} and FSC$^{®}$), has led to increases in dead-wood resources, the levels are still far below those of natural forests (e.g. Fridman & Walheim 2000; Jonsson et al. 2016; Thorn et al. 2020). The increased emphasis on biodiversity conservation has also led to extensive surveys of rare and ecologically valuable habitats, classified as woodland key habitats (Nyckelbiotoper in Swedish). While these often smaller forest stands containing rare substrates or species, have no formal protection, they cannot be cut within PEFC^{TM} and FSC$^{®}$ certified forestry (Timonen et al. 2010). Woodland key biotopes have since showed positive effects for a wide range of forest taxa (Hottola & Siitonen 2008; Timonen et al. 2011; Gustafsson & Hannerz 2018).

During the last decade, Swedish forests have been put under increasing pressure. While the amount of dead-wood substrates has slowly been growing since 1993, the progress is threatened by an increasing interest in wood-based bioenergy (Riffell et al. 2011; Snäll et al. 2017). Where dead-wood removal previously was predominantly focused on the larger substrate classes, the economic interests of harvesting stumps, twigs and branches are nowadays increasing. The resulting effects of such intensified harvest on biodiversity and ecosystem services are complex (Bouget et al. 2012; Ranius et al. 2018; Eggers et al. 2020) and not much is known of how individual saproxylic species are affected.

Figure 2. Pine stand after a small forest fire. *Pinus sylvestris* are well adapted to historical fire regimes and usually survive fires. Weaker trees might however perish and large amount of dead-wood substrates are common after a forest fire. Many threatened saproxylic insects are adapted to natural fires and can cover large distances following miniscule odour plumes to the area. Controlled fires are today an important tool in the conservation of forest biodiversity. (photo: B. Eriksson)

1.4 Pheromone-based monitoring

For many insect species, chemical signals are the main method of communication, and are utilised for a wide variety of activities, such as food, host or oviposition site searching (Law & Regnier 1971). By releasing volatile organic compounds into the ambient environment, individuals can elicit physiological or behavioural changes in conspecifics (Karlson & Lüscher 1959). Such signals are known as pheromones, and can trigger many different responses, such as dispersal or aggregation, often with species specific effects. A category of pheromones that has proven extremely useful for monitoring and trapping of insects are sex pheromones (Witzgall et al. 2010). Sex pheromones are used to attract individuals of the opposite sex, and can, depending on species, be produced by either males or females (Law & Regnier 1971). For some species, males produces less specific sex pheromones, attracting both females as well as other males. These are usually called aggregation-sex pheromones (Cardé 2014).

Pheromones, especially those used for sex and aggregation, have a long history of use against pest species of a wide variety of taxa (Wright 1964; Witzgall et al. 2010; Howse et al. 2013). However, during the last few decades, sex and aggregation-sex pheromones have been identified for an ever growing number of species of conservation interest, with representatives from many taxa such as Scarabaeidae (e.g. Larsson et al. 2003), Elateridae (e.g. Tolasch et al. 2007) and Zygaenidae (e.g. Oleander et al. 2015). The pheromones of the hollow oak inhabiting hermit beetle (*Osmoderma eremita*) and rust red click beetle (*Elater ferrugineus*) (Figure 3) for example, were deployed in ecological studies, where synthetic lures proved to be effective to study local abundances and distributions (e.g. Svensson et al. 2009; Andersson 2012; Chiari et al. 2013; Musa et al. 2013). With the systematic and high accuracy monitoring offered by pheromone lures, environmental data can effectively be analysed to determine the requirements of species on both habitat and landscape levels (Larsson 2016). Within the last decade, there have been a dramatic increase in the number of identified pheromones of saproxylic species (Hanks & Millar 2016; Millar & Hanks 2017). While invasive and pest species still dominate, many identifications are also done on rare or elusive species, such as *Rosalia alpina* (Kosi et al. 2017), *Tragosoma depsarium* (Ray et al. 2012), and *Plagionotus detritus* (Molander et al. 2019b).

Although still developing, pheromone-based monitoring methods of threatened species have shown great potential. Studies of *O. eremita* have given new information of dispersal capabilities (Svensson et al. 2011:20; Zauli et al. 2014), and considerably revised our understanding of the presence and abundance of *E. ferrugineus* within European woodlands (Musa et al. 2013; Oleksa et al. 2013; Zauli et al. 2014). With effective and comparative trapping, it is possible to directly evaluate the effects of both forestry and conservation efforts on previously elusive saproxylic fauna. The identification of several thin oak dependent longhorn beetles (Molander & Larsson 2018; Imrei et al. 2019; Molander et al. 2019c; a) made it possible to directly study both distributions and forest management effects of species often previously missed (Molander 2019). Pheromone-based methodologies are continuously advancing, and have the capacity to become important parts of future biodiversity conservation toolboxes. Cost-effective monitoring schemes could directly evaluate threatened populations, and by utilising species correlations to develop indicator and umbrella species, larger groups of species could be surveyed indirectly.

Figure 3. The rust red click beetle (*E. ferrurgineus*) and hermit beetle (*O. eremita*) trapped with female produced sex pheromones, and male produced aggregation-sex pheromones, respectively. Until recent years, the vast majority of pheromone-based conservation studies have focused on these two hollow-oak dependent species. (photos: B. Eriksson)

2. Thesis aims

In the context of modern Swedish forest management, the overarching aim of this thesis was to increase our knowledge of how saproxylic beetles are affected by modern conservation and forestry regimes. While forest biodiversity is a well studied subject, there are still large gaps in our understanding of how species respond to their rapid changing environments. As many saproxylic beetles are elusive and hard to quantitatively survey using general monitoring approaches, confident estimates of species abundance and conservation status are difficult to make. To alleviate this, the present thesis aimed to utilise new pheromone-based methodologies to investigate the conservation status of saproxylic species with differing spatiotemporal population dynamics in Sweden.

The first part of the thesis focuses on the identification of pheromones for three red-listed saproxylic beetles commonly overlooked in many forest biodiversity studies. By utilising the species own aggregation-sex pheromones, the aim was to develop lures for efficient monitoring methodologies. These methods are applied for large-scale field studies in the second part of the thesis. There, studies were set up across the species' distribution ranges in Sweden, with the aim of illuminating their habitat requirements and genuine conservation statuses.

The following four specific aims addressed in the research papers were:

i) Identify the aggregation-sex pheromones and develop efficient lures for the saproxylic beetles *Anaglyptus mysticus*, *Xylotrechus antilope* and *Bostrichus capucinus* (Paper I & III).

ii) Investigate the presence and abundance of threatened species within their presumed Swedish distribution ranges (Paper II-IV).

iii) Evaluate the species' responses to different management regimes, such as managed production forests and nature reserves (Paper II-IV).

iv) Relate species occurrences to the availability of suitable substrates on habitat and landscape levels (Paper II-IV).

3. Methods

3.1 Study species

The four papers investigated the pheromones and ecology of nine saproxylic beetles in Swedish forests. Paper I and II focused on seven cerambycid species predominantly specialising on small diameter substrates, such as twigs and branches. In contrast, the studied species *Bostrichus capucinus* and *Tragosoma depsarium*, in paper III and IV respectively, utilise larger and older wood substrates less common in Swedish forests.

The seven longhorn beetles were all belonging to the subfamily Cerambycinae, but from three different tribes. *Anaglyptus mysticus* is a member of the Anaglyptini tribe while *Xylotrechus antilope* and *Plagionotus arcuatus* belong to the Clytini tribe. The remaining *Phymatodes* species; *P. alni*, *P. pusillus* and *P. testaceus* belong together with *Pyrrhidium sanguineum* to the Callidiini tribe (Danilevsky 2018). The species are mainly dependent on oak (*Quercus robur, Q. petraea*) in Sweden, although *A. mysticus* and *P. testaceus* also utilise other deciduous tree species (Ehnström & Holmer 2007). For the former, common hazel (*Corylus avellana*) is an especially important host species. The substrate requirements of the studied cerambycids are similar and overlapping, specialising on recently dead twigs and branches. The substrates are usually colonised by the beetles during the summer after dying, and only suitable for a single generation. Depending on the species and local environment, the larval development takes between one to three years before the adults emerge (Ehnström & Axelsson 2002; Ehnström & Holmer 2007). *Bostrichus capucinus* is an auger beetle in the subfamily Bostrichinae and tribe Bostrichini. As the studied longhorn beetles, it is also predominately oak dependent within Sweden, although it is sometimes found in other deciduous trees. The beetle usually colonises hard, dry and sun-exposed dead wood. The larval stage usually takes two years, whereupon the adults emerge in middle May (Ehnström & Axelsson 2002). On the contrary, *Tragosoma depsarium* is a pine (*Pinus sylvestris*) dependent longhorn beetle in the tribe Meroscelisini of subfamily Prioninae. It is specialised on bark-free logs (usually 5-10 years old) from old and large pine trees, and in the right conditions, the substrates can be suitable for decades. The larval stage usually takes at least four years and adults emerge in the middle of the summer (Ehnström & Axelsson 2002; Wikars et al. 2014).

3.2 Pheromone identification

3.2.1 Insect rearing

To collect volatile samples for pheromone identifications, adult beetles were required. To obtain these, potentially colonised dead wood substrates were collected in the field and transferred into greenhouses. For *X. antilope*, logging residues were collected on two occasions in the Ekopark Hornsö, while hazel wood inhabited by *A. mysticus* was collected in the Mittland forest in the province of Öland. Larvae of *B. capucinus* were found in the exposed roots of a wind-felled oak tree, whereupon wood samples were brought back to the SLU campus. There, all collected wood was put into plastic boxes within a greenhouse until the adult beetles started to emerge. As adults were found, they were separated, either by sex or individually, into smaller containers with pieces of fresh wood substrates. Solutions of honey and water were provided for nourishment during the collection of emerging adults.

3.2.2 Volatile collection

For collection of pheromones, the adults were subjected to headspace sampling (Figure 4). The volatile collection set-up consisted of three glass bottles connected by Teflon® tubing to an air pump, pulling ambient air through the bottles. Between the bottles and the tubes, Teflon® collectors containing the adsorbent material Porapak™ Q were placed to capture volatile compounds released by the beetles. Similarly, collectors containing granulated charcoal were put on the inflow to the bottles to minimise contaminations from the surrounding environment. At the beginning of headspace sampling, the male and female beetles were transferred into separate glass bottles with a third bottle left empty as a control. They were left with the pump running for an average time of four hours. Thereafter, the collectors between the bottles and pump were removed and eluted with hexane, causing the volatiles to release from the adsorbent. The hexane solution could then be further analysed to compare the volatiles of the bottles with male, female and no beetles. Depending on the number of emerged beetles, and the amount of volatiles released, headspace sampling was repeated several times with different durations.

The volatile extracts were analysed with Gas Chromatography – Mass Spectrometry (GC-MS), utilising both a nonpolar HP5 column and a polar DB-WAX. Chromatograms of the male, female and control extracts were then compared visually to identify potential pheromone components, using the Agilent ChemStation software (Agilent Technologies 2011). If peaks (elevated concentrations of ions) were found exclusively in the male extracts, they were noted as signs of potential pheromone components. Databases of mass spectra were then used for preliminary identifications of the compounds. For final identifications, more extensive chemical analyses were conducted by collaborators at the Millar lab, University of California, Riverside (Paper I) and the Hedenström lab at the Mid Sweden University (*B. capucinus*).

Figure 4. Glass bottles for headspace sampling of volatile compounds. Pheromones produced by beetles were pumped into collectors adsorbing the compounds. The picture displays a male and female timberman beetle (*Acanthocinus aedilis*) subjected to headspace sampling. (photo: B. Eriksson)

3.3 Pheromone-based monitoring

Similar trapping methodologies were used for all bioassays and ecological studies. Flight-intercept traps baited with pheromone lures were put up at locations of interest of the respective study. The traps were custom-made and consisted of two rectangular cross-vane panels hanged on top of a funnel protruding into an one litre collecting jar. The panels and funnels were coated with a layer of Fluon® to prevent insects from escaping the traps. The traps are described in detail in Molander et al. (2019a). Depending on the study, the trapping jars could be set up for alive captures (Paper III & IV) or be filled with propylene glycol for preservation of the insects (Paper I & II). The traps were usually hung from rebar rods on an approximate height of 1.5 metres, to ensure an optimal and systematic accessibility for the beetles. At some sites, cattle grazing made this impossible and the traps were instead hung from branches on trees (often at 2.5-4 metre height).

The pheromone lures were mixed in the lab at SLU Alnarp campus by components bought from commercial manufacturers or synthesised by collaborators in the Millar lab, University of California, Riverside (Paper I, II & IV) or the Hedenström lab at the Mid Sweden University (*B. capucinus* bioassay, Paper III). During all the studies, the pheromone aliquot were dissolved in 0.5 mL isopropanol and stored individually in Eppendorf tubes until the trap activation. The substance was then transferred into a Grippie® zip-lock bag attached to the south-facing cross-vein panel with a metal wire. To ensure continual efficient trapping, the activity periods of the species were usually divided into two terms, with an additional pheromone lure added after two to three weeks.

During the studies with killing traps, the collection jars were emptied while replenishing the lures. This was done by filtering the propylene glycol through a tea filter, allowing the preservative to be reused while bringing back the captured specimens to the lab. There, all longhorn beetles were examined and identified, predominately using the key from Ehnström & Holmer (2007).

With live captures, moistened moss or sponge cloth were added to the jars, to provide some cover and protection to captured specimens and the traps were usually revisited every third day. Captured beetles were marked individually with ink pens and released a short distance from the traps.

While the trapping sites in the ecological studies (Paper II, III & IV) contained one to three identical traps, the sites of the pheromone identification bioassays consisted of several treatments to determine attraction to synthetic compounds (Figure 5). At each site, traps were put up with the species' pheromone components, both separately and in blends of different ratios. A single trap baited with only isopropanol was used as a control. For *A. mysticus*, the replicates were spread along the hazel rich Mittland forest on the island province of Öland, while *X. antilope* and *B. capucinus* were surveyed within Ekopark Hornsö.

All studies consisted of surveys in South-Eastern Sweden (Figure 6), although *T. depsarium* was also surveyed in the county of Värmland (Paper IV).

Figure 5. Pheromone traps set up for bioassay evaluating the attraction effects of pheromone component candidates. (photo: B. Eriksson)

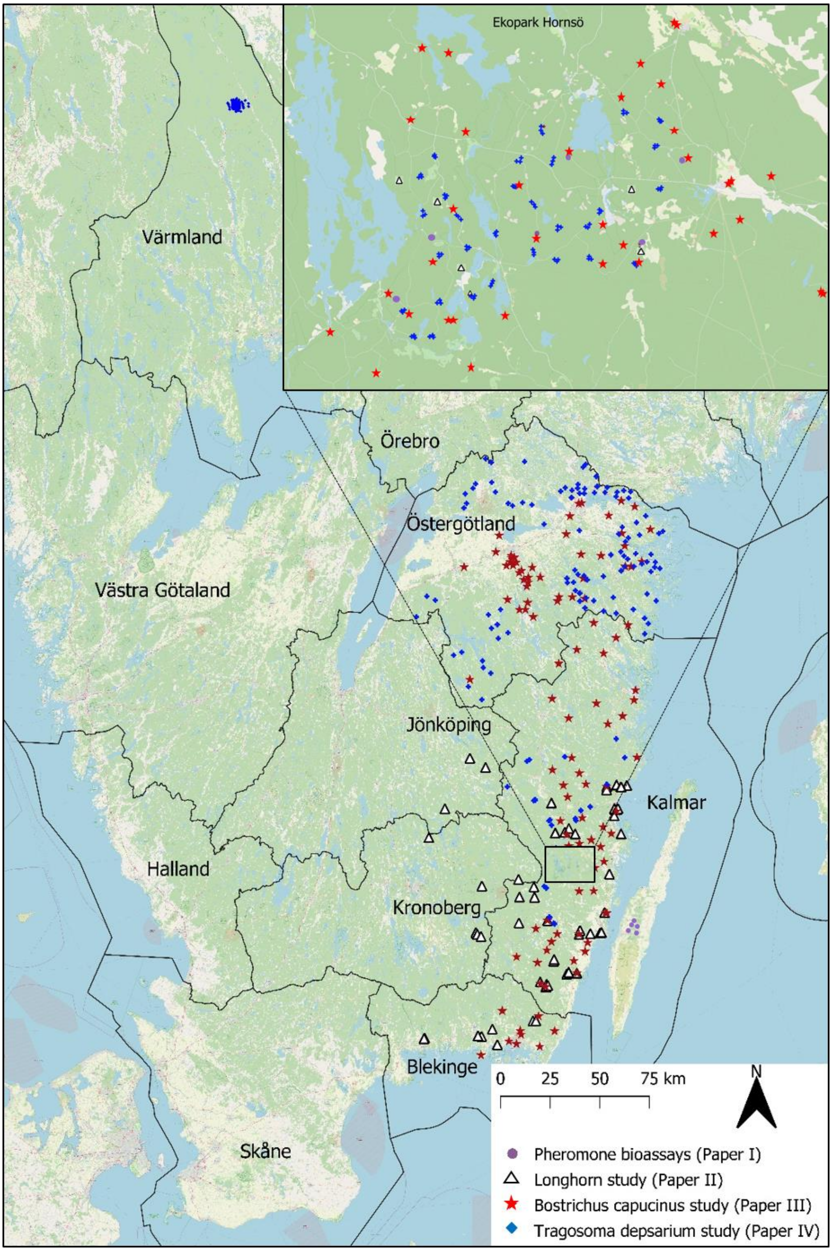

Figure 6. Summarising map of all trapping locations of the thesis. The top right map show Ekopark Hornsö, a saproxylic hotspot area surveyed in all studies. The map display the county names of Southern Sweden. Background map: OSM (© OpenStreetMap contributors)

3.4 Longhorn study (Paper II)

During 2016, pheromone traps were set up at 62 sites across the Swedish provinces of Blekinge and Småland to determine the short-term effects of forestry actions and management regimes (Figure 7). The six longhorn species with recently identified pheromones *P. sanguineum*, *P. alni*, *P. testaceus* (Molander et al. 2019c), *P. pusillus* (Molander & Larsson 2018), *P. arcuatus* (Imrei et al. 2019) and *X. antilope* (Paper I) were surveyed with three traps each at each site. Since some shared pheromone substances, or differed in activity periods, nine traps were used at each site. The monitoring started at the early May, when the first species (*P. sanguineum* and *P. pusillus*) started flying and continued until the end of July. While *P. pusillus* and *X. antilope* have Swedish distribution ranges smaller than the study area, traps were still put up for all species at all sites.

All 62 sites were oak-dominated forests or woodlands that were split into five different groups depending on management regime: three consisting of production forests, and two of set-aside or protected areas. Among the production forests, 14 sites had been logged or thinned recently before our survey (during the winter 2015-2016) and had relatively large amounts of dead-wood substrates in the form of twigs and branches. Control sites were selected in oak stands of similar age, where no forestry actions had been conducted during recent years. Among these stands, traps were put up at 14 sites within the shady interior, and at the sun-exposed forest edges respectively. The set-aside areas consisted of 10 sites of woodland key habitats (small set-aside habitats for biodiversity conservation) and 10 saproxylic hotspots (areas often formally protected of mostly veteran oaks known for very high biological values of saproxylic biodiversity). To control for spatial effects, the study aimed to cluster one of each oak habitat type together in groups of five. The study continued at the same sites during 2017, but were reduced during 2018 to just the 28 logged and production forest edge sites.

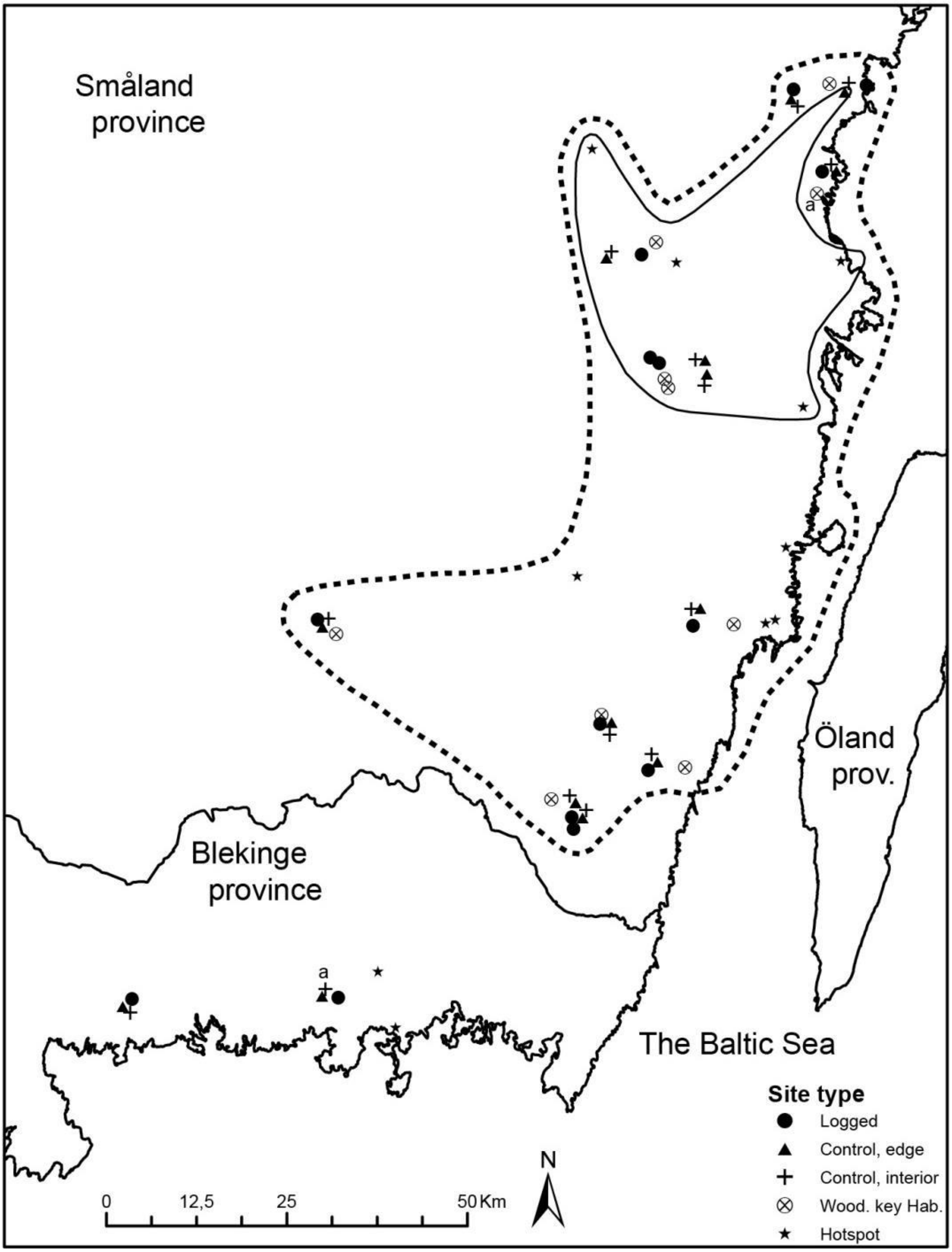

Figure 7. Map of trapping locations in the 2016-2018 study of the longhorn beetles *P. sanguineum*, *P. alni*, *P. testaceus*, *P. arcuatus*, *P. pusillus* and *X. antilope*. Two of the species had limited distribution ranges within the study area and are here represented with a hatched line (*X. antilope*) and a solid line (*P. pusillus*) respectively. Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

3.5 *Bostrichus capucinus* study (Paper III)

In May 2021, pheromone traps were put up at 105 sites within the Swedish distribution area of *B. capucinus* (Figure 8) in the counties of Blekinge, Kalmar and Östergötland (Figure 9). The sites were divided into three different categories spread across the study area. 34 of the sites were chosen nearby known observations of *B. capucinus* from the last century (SSOS 2021a). Furthermore, 26 oak hotspots were selected among areas known for their high values of saproxylic biodiversity. Several of them were the same ones monitored during the Cerambycid study (Paper II). The third category consisted of 45 oak habitats randomly selected using a national land cover map (Naturvårdsverket 2018) in the software QGIS (QGIS Development Team 2020). Of these sites, there was no prior information, except that the land was classified as deciduous hardwood. At these 85 sites, two traps were put with average distance of 85 metres between them. Finally, a focused mark-recapture study was performed within the Ekopark Hornsö, consisting of 30 sites with one trap each. These sites were closer together, with the shortest distances between the traps around 500 metres.

All sites were revisited on every third day to mark and release the captured beetles. In the first half of June, new pheromone baits were added to the traps. At the start of July, the main activity period was deemed over, and the trapping terminated.

Figure 8. Trapped and marked individuals of *B. capucinus* (to the left) and *T. depsarium* (to the right). The beetles were caught in pheromone traps in bioassays 2021 and 2019 respectively. (photos: I. Rönnqvist (*B. capucinus*) and B. Eriksson)

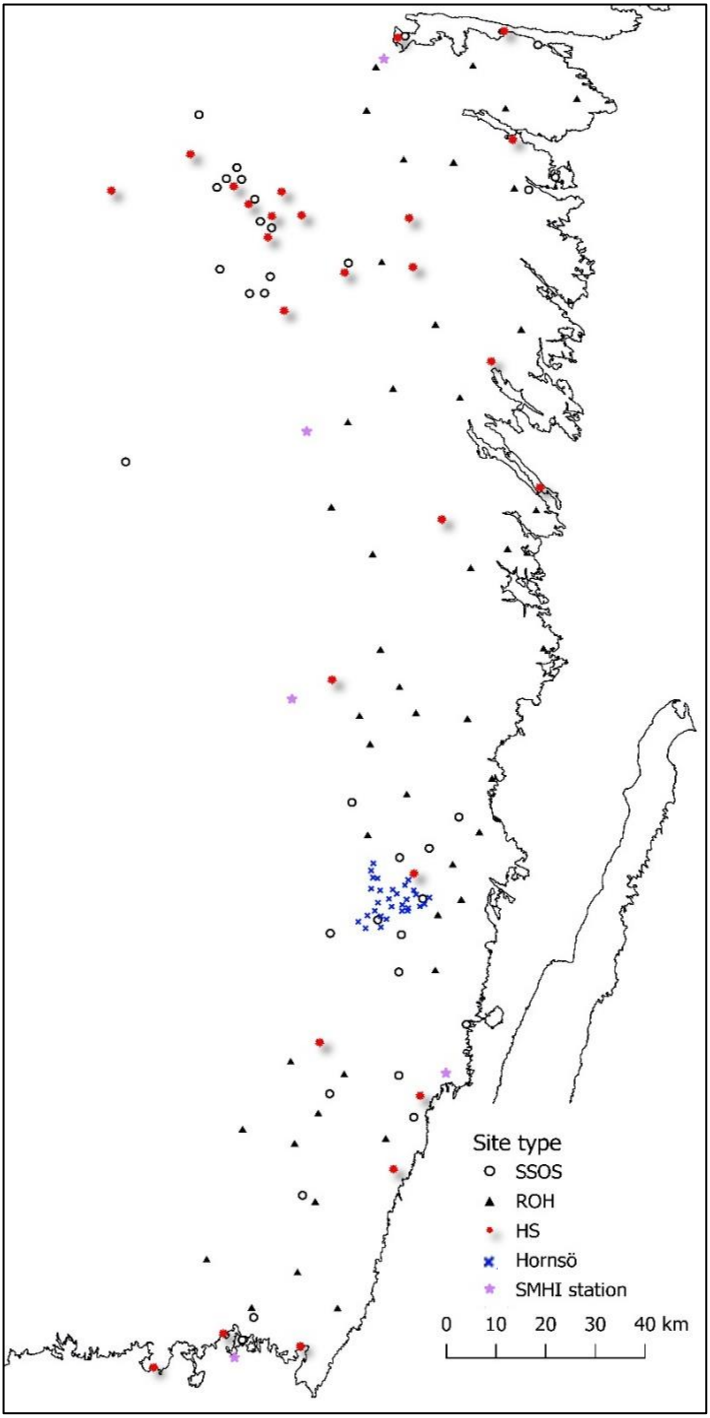

Figure 9. Map displaying the trapping sites of the *B. capucinus* study in 2021. The study was conducted in the counties of Blekinge, Kalmar and Östergötland of Sweden. Black circles (SSOS) represent sites revisited after previous findings on the Swedish species observation system; black triangles (ROH) are randomly selected trapping locations at oak woodlands; Red circles (HS) are oak hotspots, areas with known high saproxylic values and blue crosses (Hornsö) represent the mark-recapture substudy in Ekopark Hornsö. Pink suns (SMHI station) mark the weather stations measuring daily temperature and precipitation. Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

3.6 *Tragosoma depsarium* study (Paper IV)

To investigate the conservation status and habitat requirements of *T. depsarium* (Figure 8), three sub-studies were set up in the provinces of Småland and Östergötland in the summer 2014 (Figure 10). In Östergötland, 100 woodland key biotopes of suitable qualities (old sparse pine stands on dry lichen vegetated ground) were chosen and surveyed during July. For logistical reasons, not all sites could be surveyed simultaneously, and approximately a fourth of the traps were active at a time. Upon activation, a single trap was placed at the site and thereafter revisited 3 times during the following 7-10 days. If a *T. depsarium* specimen was found the trapping stopped, and the trap moved to a new site. Similarly, if no beetles were caught after three visits, the trap was moved to a new site. During the same period, 20 pine dominated nature reserves were surveyed in the province of Småland. Two or three traps were placed at each site, spread at open areas in the reserves. The traps were activated during the first two weeks of July and remained active until the end of the month.

During the same season, a mark-recapture study was set up in Ekopark Hornsö. At thirty locations, three traps were set up 50-80 metres apart. The trapping was conducted between June 30 and July 31, with a 5-day break between July 7 to 11. The species had previously been found in general invertebrate surveys in the area (Nilsson & Huggert 2001), and several trapping sites corresponded to earlier observation localities.

During 2019, an additional mark-recapture study was set up in the area of Sågtjärn, Norra Ny Parish in the province of Värmland (Figure 11). The area had for decades regularly been surveyed for *T. depsarium* through surveys of emergence holes in pine logs. In the end of June, 251 traps were put up in a grid across the area, with the traps being 220 metres apart. In the beginning of July, the traps were activated and then revisited every third day until August 1st. New pheromone lures were applied in the middle of the period. The traps were visited a final time at the second half of August, whereupon the traps were taken down. At each trap location, habitat characteristics such as canopy cover, dominating tree species and basal tree area, were noted. All pine logs (diameter > 20 cm) found while walking between traps were also noted with GPS coordinates.

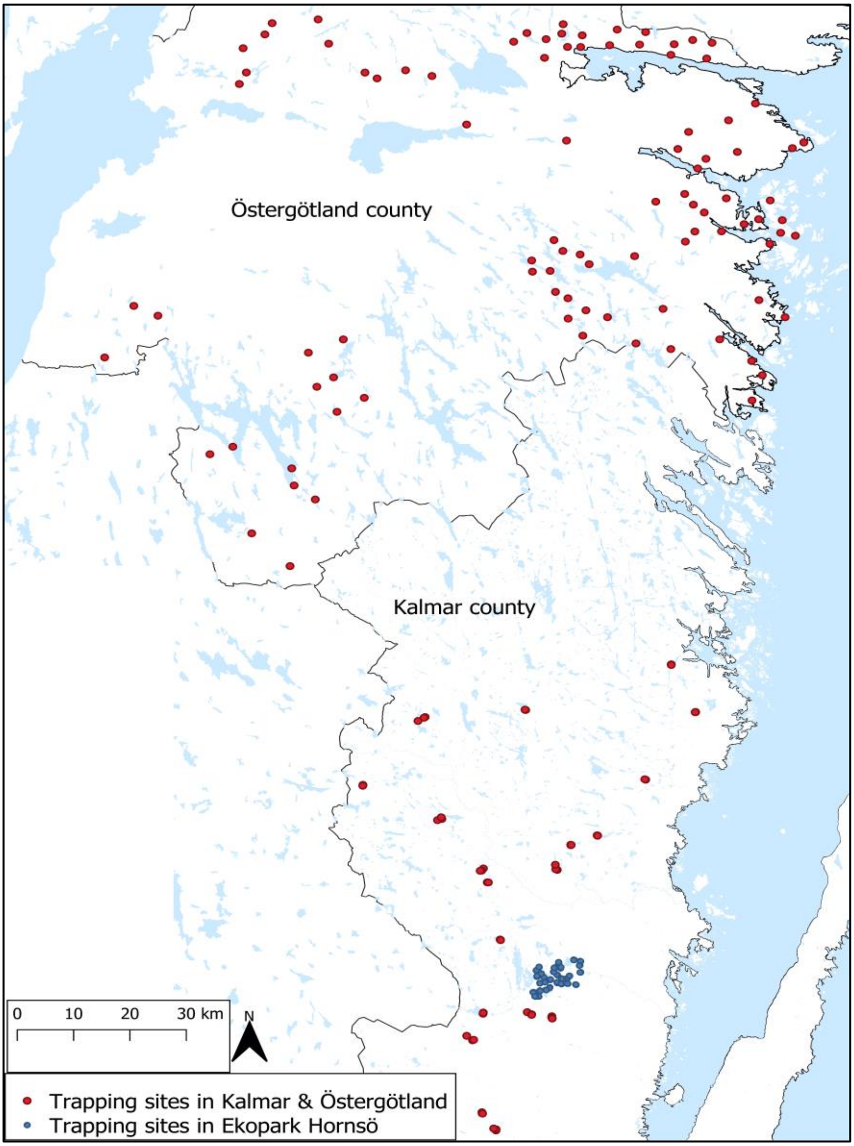

Figure 10. Map of trapping positions of *T. depsarium* in the counties of Kalmar and Östergötland in 2014. Red circles represent the 120 sites surveying the species southern distribution. Blue circles are the 90 traps in a mark-recapture study in Ekopark Hornsö. Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

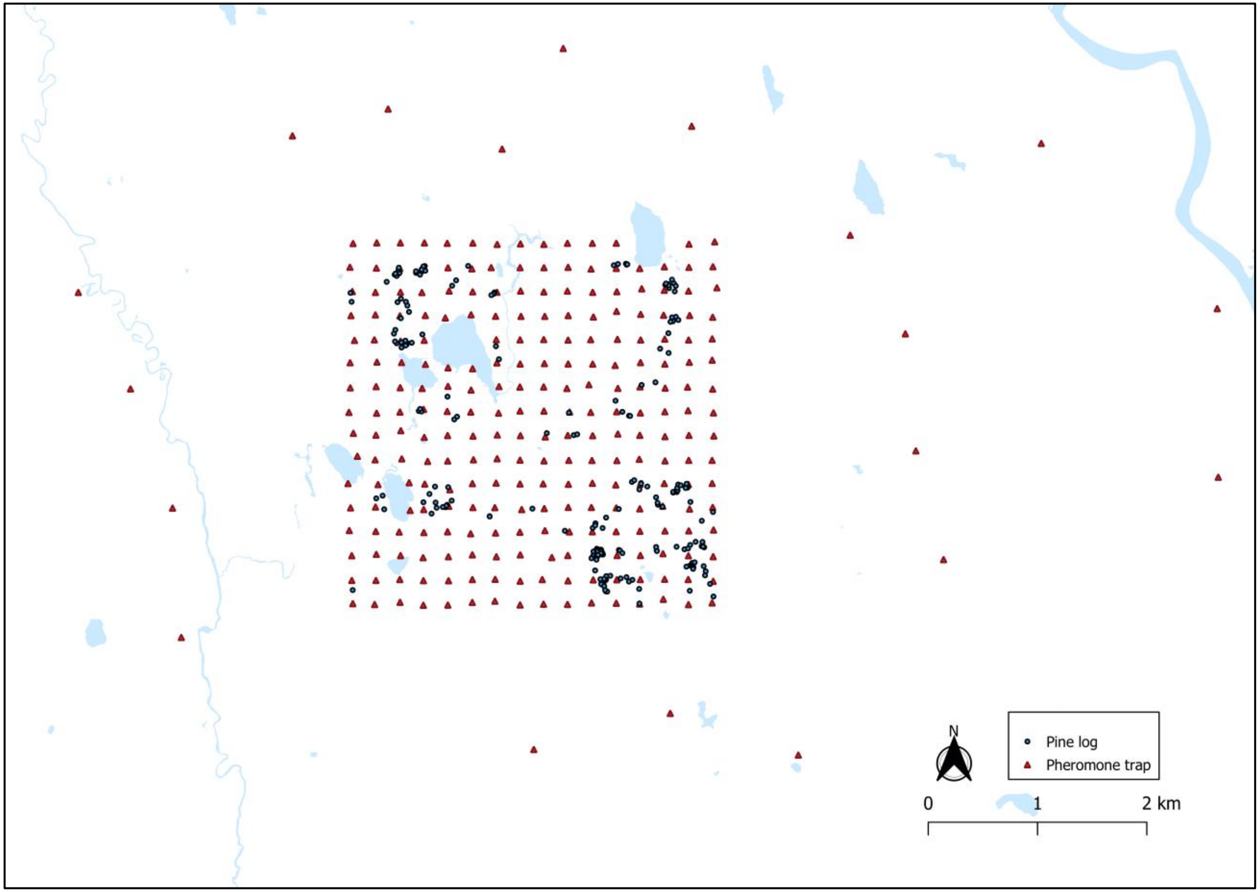

Figure 11. Map of trapping locations of *T. depsarium* in Sågtjärn 2019. The red triangles display the 251 pheromones traps put up across the area. The black circles represent the apparent suitable pine log substrates surveyed in the area. Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

4. Results and discussion

During recent decades, pheromones have proven invaluable for ecological studies into habitat and landscape requirements of a growing number of insects. In Europe, pheromone-based studies of saproxylic beetles have predominantly focused on inhabitants of old oaks, such as the hollow inhabiting hermit beetle, *Osmoderma eremita* and rusty click beetle, *Elater ferrugineus*. For these species, pheromones have played a vital role in detection of both actual occurrences and distribution limits (Chiari et al. 2013; Zauli et al. 2014; Harvey et al. 2017), but also substrate requirements on a landscape level (Bergman et al. 2012; Musa et al. 2013). In Sweden, the recent pheromone identification of the red-listed (EN) cerambycid *Plagionotus detritus* (Molander et al. 2019b), allowed efficient evaluations of national conservations schemes (Franc 2011), such as habitat restorations and re-establishments of extinct populations, to be set up (*unpublished data)*.

With the present studies, pheromone based monitoring of rare species has been broadened with surveys of saproxylic beetles with different niches. The pheromone identifications of *A. mysticus*, *X. antilope* and *B. capucinus* allowed focused studies on a new group of oak dependent species. In contrast to the hollow oak species, *B. capucinus* and the six studied cerambycids, utilise much younger and more ephemeral substrates. By investigating these species across a wide range of areas, their actual distributions could be evaluated together with the effect of dead-wood availability and management regimes. Overall, this work demonstrates the use of pheromone based monitoring methods for large-scale ecological studies of saproxylic species across a range of species and habitats.

4.1 Pheromone identifications

4.1.1 *Anaglyptus mysticus* & *Xylotrechus antilope* (Paper I)

The extracts of male *A. mysticus* contained two major pheromone components, *(R)*-3-hydroxy-2-hexanone and 2-nonanone. *(R)*-3-hydroxy-2-hexanone appeared to be the main component with a 70:100 ratio between the compounds. The bioassay showed a significant attraction to blends of the two compounds, seemingly increasing with ratios from 10:50 to 40:50 (Figure 12). On their own, no component differed significantly from the isopropanol control. Since then, *A. mysticus* specimens have been found as bycatches in traps containing *(R)*-3-hydroxy-2-hexanone during our ecological studies along both the east (Paper II) and west (*unpublished data*) coasts of Sweden. While the species was only trapped at a minority of the sites within both studies, it is probably more due to the spotty occurrences within its distribution range than to the lack of 2-nonanone. When the species was present at a site, it was usually found in several traps.

The male *X. antilope* extracts also contained two compounds; *(S)*-2-hydroxy-3-octanone and minor quantities of 2,3-octanedione. Of these, only the first proved to be active and attractive during bioassays, both racemic and pure (Figure 13). During the ecological study in South-Eastern Sweden (Paper II), the species was captured both in its specific traps, as

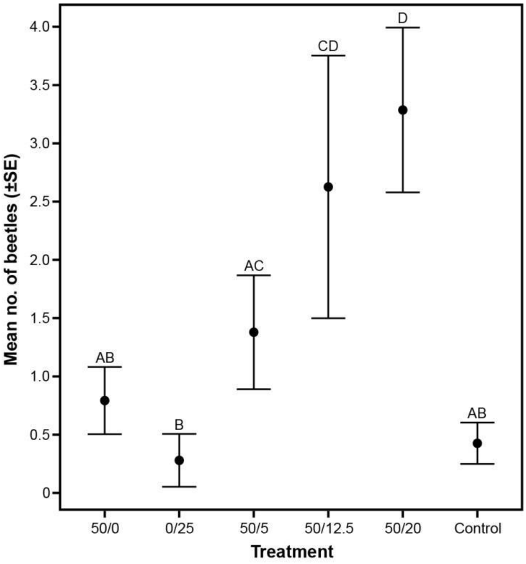

Figure 12. Mean (±SE) numbers of *A. mysticus* captured per sampling period in traps with different quantities of racemic 3-hydroxy-2-hexanone and 2-nonanone in 0.5 ml isopropanol (n=14 samples). Three blends with a variable quantity of 2-nonanone were tested, in addition to two treatments with the compounds as single components (50 mg racemic 3-hydroxy-2-hexanone and 25 mg 2-nonanone, respectively). The control was 0.5 ml isopropanol alone. Means that do not share a common letter are significantly different (*P*<0.05)

well as in the traps for *Plagionatus arcuatus* (lures of (racemic)-3-hydroxy-2-hexanone, (racemic)- 3-hydroxy-2-octanone and (racemic)-3-hydroxy-2-decanone), indicating a cross-attraction to components of other species' pheromones. The cross-attraction did however appear weaker, failing to detect the species at 26 % of the sites where the specific *(S)*-2-hydroxy-3-octanone traps captured them. At the sites where the species was caught with both lures, the actual pheromone captured on average almost eight times more individuals than the heterospecific one.

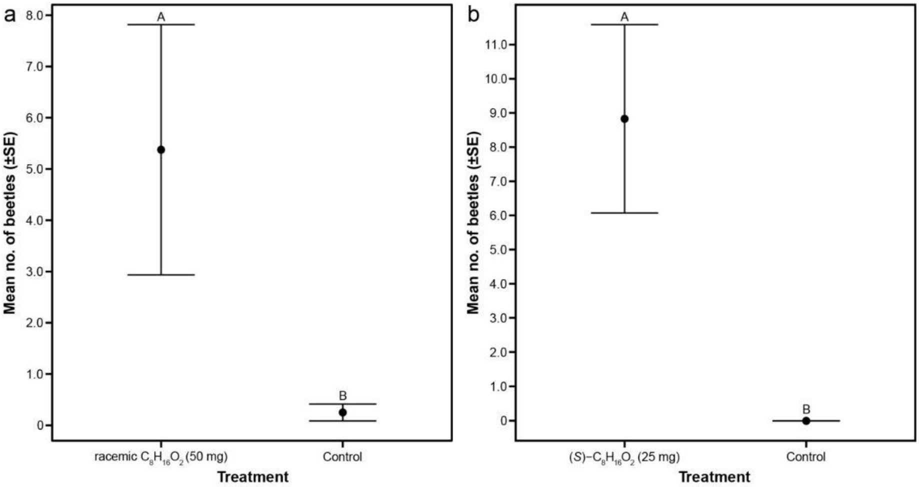

Figure 13. **a)** Mean (±SE) numbers of *X. antilope* captured per sampling period in traps with 50 mg racemic 2-hydroxy-3-octanone in 0.5 ml isopropanol (n=8 samples). The control was baited with 0.5 ml isopropanol alone. Means with different letters are significantly different (P<0.05) **b)** Mean (±SE) numbers of *X. antilope* captured per trap with 25 mg *(S)*-2-hydroxy-3-octanone in 0.5 ml isopropanol (n=5 samples). The control was baited with 0.5 ml isopropanol alone. Means with different letters are significantly different (P<0.05)

4.1.2 *Bostrichus capucinus*

The *B. capucinus* extracts contained two compounds that differed considerably between the male and female samples, 3-pentayl-octanoate and (cis)-4-3-pentanyldec-4-enoat. While the compounds appeared in all male extracts, the ratio between them varied significantly, making it impossible to determine their natural ratio. Furthermore, the antennas of both male and female beetles responded to the two compounds during electroantennography, indicating that they likely constituted pheromone components.

The preliminary bioassay revealed significant attraction of both components dissolved in 1 ml isopropanol (Figure 14). In total, 295 beetles were captured (50 mg 3-pentanyl octanoate: 115 specimens, 50 mg (cis)-4-3-pentanyldec-4-enoat: 40 specimens, blend of 50 mg each: 139 specimens, control: 1 specimen) during the two weeks of trapping. While more beetles were caught with the combination of components, the difference was not statistically significant. The preliminary study was however small, with only six replicates active during around half of the species' activity period. It is possible that a larger study would detect a greater difference between the components. Even so, the total number of captured specimens indicated a strong attraction to the pheromone components. This is highlighted by the rarity of observations of the beetle in non-specific traps. During recent decades, several studies have been conducted on saproxylic biodiversity in Ekopark Hornsö, and few *B. capucinus* are usually found (Nilsson & Huggert 2001; SSOS 2021a). Even though the same trapping locations have been utilised in both earlier pheromone identification (Molander et al. 2019b; c) as well as ecological studies (Paper II), only sporadic captures of a few *B. capucinus* were had. Even though an optimal blend of the two components might have increased the attraction, the single component of 3-pentanyl octanoate was deemed attractive enough to be used on its own during a following distribution study (Paper III).

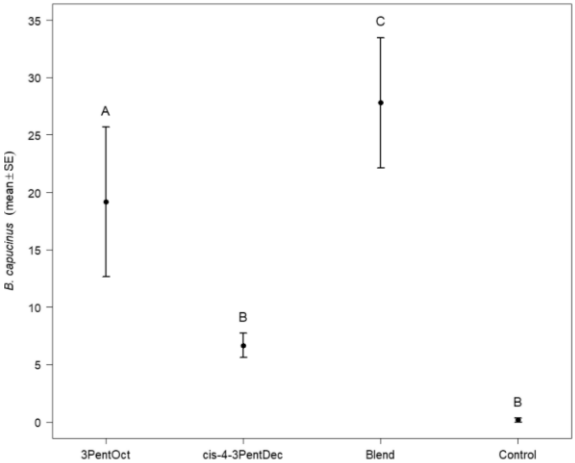

Figure 14. Mean (±SE) numbers of *B. capucinus* captured during a two week sampling period with 50 mg of 3-pentanyl octanoate, 50 mg (cis)-4-3-pentanyldec-4-enoat, a blend of 50 mg of each in 1 ml isopropanol. The control was baited with 1 ml isopropanol alone. Means with different letters are significantly different (P<0.05)

4.2 Spatial and temporal distributions

For rare insects in general, including many cryptic saproxylic species, knowledge about their true distribution and abundance remains one of the most significant obstacles to their long-term conservation. Without access to quantitative or semi-quantitative information about their geographical distribution, and how these change over time, it is difficult to assess their threat status and whether they are in decline or recovery. These are essential pieces of information to evaluate the effects of landscape and habitat changes and conservation measures on insect populations. Broader taxonomic surveys of saproxylic insects based on active sampling and trapping can often provide general information about the composition and diversity of saproxylic insect communities, and are useful for comparisons between sites, e.g. in terms of conservation value. However, most stochastic sampling methods provide uncertain estimates of the true presence or absence, let alone abundance, for many individual species.

For individual species, assessment of their true distribution, abundance and conservation status rests on a combination of known observations and estimates of sampling effort and assumed probability of detection (Jeppsson et al. 2010; Lindhe et al. 2010). Observations may come from a mixture of systematic surveys and citizen science in the form of individual observation reports and material from collections. Citizen science may be a very important source to find information about species, but translating observational records to estimates of true distributions may be challenging, especially with regards to absences.

In this thesis, the different ecological studies focused on a mix of (presumed) rare and common beetles. Four of the longhorn beetles have widespread distributions, *B. capucinus* is deemed very rare and *T. depsarium* was common historically, but might be declining. The studies in this thesis were based on the assumption that pheromone-based surveys provided close to a true estimate of presence and absence of a species, and comparable semi-quantitative estimates of abundance. Depending on the species under study, the results yielded very mixed results regarding the observed distributions of species in regards to prior estimates of their distributions based on traditional means of assessment.

4.2.1 Longhorn study (Paper II)

The six longhorn species studied in the provinces of Blekinge and Småland have previously been thought to occur sporadically within their Swedish distribution ranges (Ehnström & Axelsson 2002; Ehnström & Holmer 2007). At the time of the study, *P. pusillus* (VU), *P. sanguineum* (NT, until 2020) and *X. antilope* (NT), were red-listed (SLU Artdatabanken 2015) with suspected declines in population sizes, primarily due to deteriorations in habitat quality. In the last few years, this has started to be reassessed, however, with an increased frequency of observations, and more focused pheromone based surveys. During the last decade, three of the species, *P. sanguineum*, *P. alni* and *P. testaceus* were surveyed across Skåne, the southernmost province of Sweden, and all three, including the red-listed *P. sanguineum* were found at a majority of the 70 sites, of which many had no known prior observations (Molander 2019:2). In the present study, the pheromones of all six species were for the first time used together in a large-scale ecological survey. With trapping conducted over three subsequent flight seasons, both spatial distribution and temporal variability, as well as the effects of habitat classes, could be evaluated across the Eastern ranges of the provinces of Blekinge and Småland (Figure 6).

While all six species have nominally similar life cycles and substrate demands, two of them: *X. antilope* and *P. pusillus* have been presumed to have very limited distribution ranges within Sweden. *X. antilope* is limited to the province of Småland, while *P. pusillus* only is further restricted to the region surrounding Ekopark Hornsö (Nilsson & Huggert 2001; Molander & Larsson 2018). The other four species have a wider observed distribution range, to a large extent following oak (*Q. robur*) to its northern climatological limit (along *Limes Norrlandicus*). The present study results exhibited mixed results with regards to expectations from previous general observations about the species' distribution and abundance. As in Skåne, the species have been considered very local, with only spotty occurrences, but during the study, all six species were found at all sites within their previously recognized distribution ranges. While both absolute and relative abundances of the species shifted among the sites between the years, the species were (with very few exceptions) found in all years, indicating maintained populations. As previous general studies often have missed the species at individual localities (compare Götmark et al. 2011), it appears that they, rather than rare, should be considered cryptic or elusive, being hard to efficiently survey without very focused and specific studies. Both the present study and Skåne results indicate that the surveyed species are widespread within their respective distribution ranges, and capable of finding suitable substrates and habitats across the region. It is likely that they are part of a larger group of forest invertebrates, considered unjustifiably rare and threatened. Without authentic knowledge of distributions and abundance, it is impossible to plan efficient conservation and restoration schemes of forest biodiversity.

The limited distribution ranges of *X. antilope* and *P. pusillus* were to a large extent corroborated by the present study, although slightly expanded by the findings of individual beetles along the presumed borders. There is no clear explanation of their limited distribution, although it probably has to do with climate as the region around the South-Eastern coast contains the warmest and driest areas of Sweden. During recent years, there has been some indications of an expansion of the *X. antilope* distribution range. New specimens have been observed both to the east, on the island province of Öland, and in the north within the province of Östergötland (SSOS 2021b).

While the study was designed to capture the changes in abundance at the logged sites, as they passed from a colonisation to an emergence phase, it also captured large variations at the unmanaged sites. Depending on the year, the beetle activity changed drastically, both in number of captures and in flight period. In 2017, more beetles were caught at almost all sites than the year before, while thechanges to 2018 were less pronounced and varied between sites. There is still little known about between-year variations in abundance among saproxylic species, although it is probably to a great extent connected to temperature. Temperature is an important part of saproxylic beetles' life cycles, affecting both survival rate and emergence time for the larvae, as well as the activity of the adults. A warm temperature during trapping might lead to higher capture probabilities of the present adults, while the temperature of previous years affect the number of emerging individuals.

4.2.2 *Bostrichus capucinus* study (Paper III)

This study investigated the presence and abundance of the auger beetle *Bostrichus capucinus* within its almost entire known Swedish distribution range. Although the beetle is red-listed (VU) and deemed rare with few known localities, it was found at a majority of the study sites. This was in stark contrast to previous assumptions, as very few observations are made of the beetle every year. During the last decade some focused surveys have been conducted, lowering the IUCN red-list classification from EN to VU. Still however, the population abundance appears heavily underestimated compared to our trapping results (Figure 15). The known species distribution is almost exclusively limited to the provinces of Blekinge, Småland and Östergötland. Although potentially suitable oak habitats can be found across larger regions of Sweden, the species might have additional requirements, e.g. temperature. To a great extent, its presumed distribution area overlaps with that for *X. antilope*, and as with the longhorn beetle, it is possible that *B. capucinus* is in a stage of expansion. During the recent decade, the number of observations has increased, with 10 observations reported (apart from our own) just during the summer of 2021. A few individuals have even been found outside the previously known distribution range, close to the cities of Uppsala, Skövde and Kristianstad to the north, west and south of its presumed distribution range respectively. As no extensive surveys have previously been made to investigate the spatial limits of the species, it is impossible to tell whether these findings represent new arrivals or older, previously undiscovered populations. It is even possible that the findings are erratic individuals emerging from transported wood.

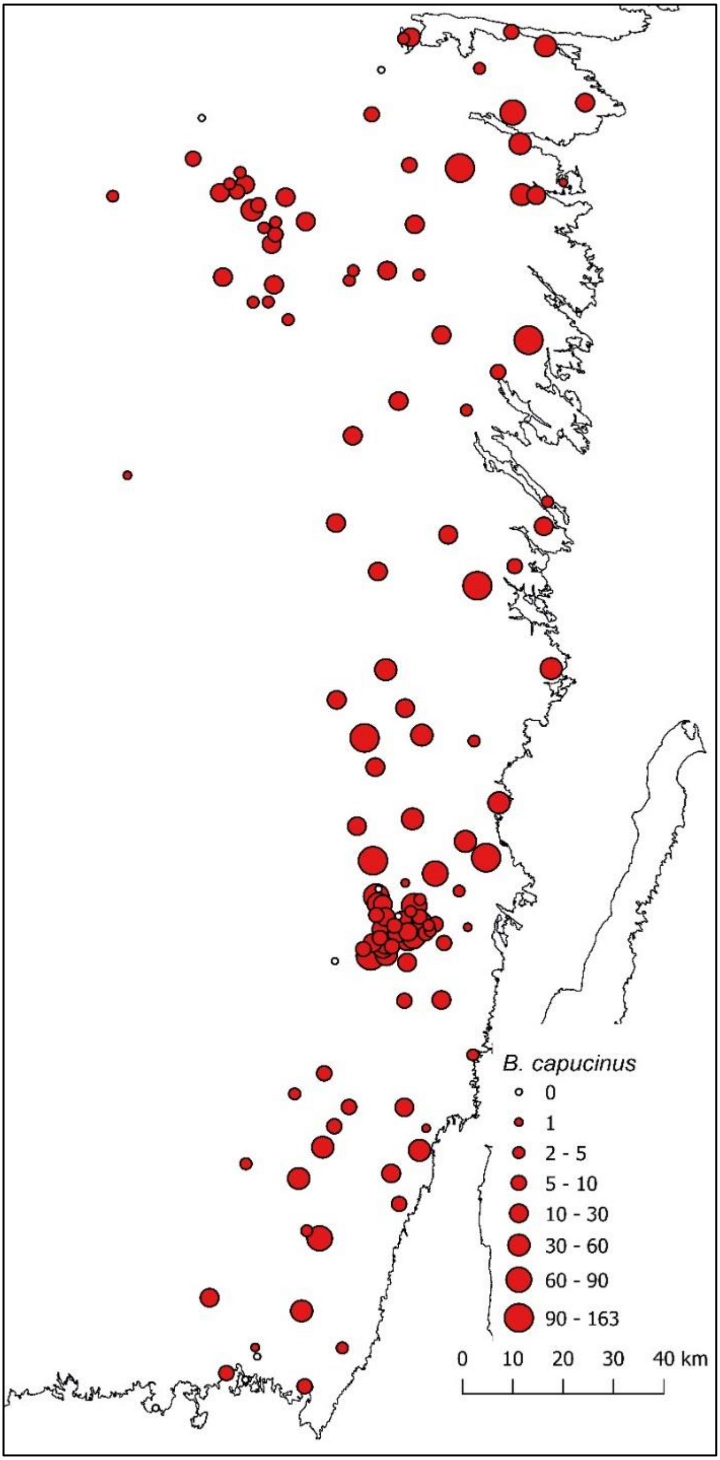

Figure 15. Map of trap captures of *B. capucinus* per site during the study 2021. The size of the red points represents the number of captured individuals. White points had no captured beetles. Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

4.2.3 *Tragosoma depsarium* study (Paper IV)

T. depsarium proved to be a contrast to the oak-dependent beetles from the other studies, with much more infrequent occurrences across its South-Eastern distribution range. With individuals observed at only eleven sites (outside Ekopark Hornsö), it appears that the presumed decline of the species in the region was well founded (Figure 16).

The sites in both the provinces of Småland and Östergötland were selected as potentially suitable habitats. In Småland, they consisted of pine dominated nature reserves, with high occurrences of pine-dominated habitats, selected with no other prior knowledge of habitat suitability or the general properties of the habitat. The Östergötland sites were, on the other hand, selected at woodland key habitats, chosen with regards to stand age, sun exposure and vegetation type. Even with the combination of a single trap and a limited trapping period, ten of the observations were made in Östergötland, indicating the importance of the specific habitat characteristics defined for these sites in supporting local populations of *T. depsarium*.

There was a large discrepancy between the trap catches in the south-eastern parts of Sweden and Sågtjärn in the western province of Värmland (Figure 17). The male population of Sågtjärn was estimated to around an order of magnitude larger than the one in Ekopark Hornsö (Figure 18), and it has previously been described as a part of a larger continuous pine forest landscape (Wikars 2004). It seems apparent that the population distribution patterns differ substantially between the regions, with a larger, more continuous population in Värmland, and very scattered, smaller and isolated populations along the southern range. The short trapping period at sites in Östergötland may have led to some false absences in the results. Generally, with longer generation times of several years, it is also possible to have missed smaller populations during the surveys of a single season. Studies of a few years would be required to really ensure the validity of reported absences at individual localities. Nevertheless, larger populations should be detected with relatively high certainty, illustrating the contrast between trapping patterns in Värmland and South-Eastern Sweden.

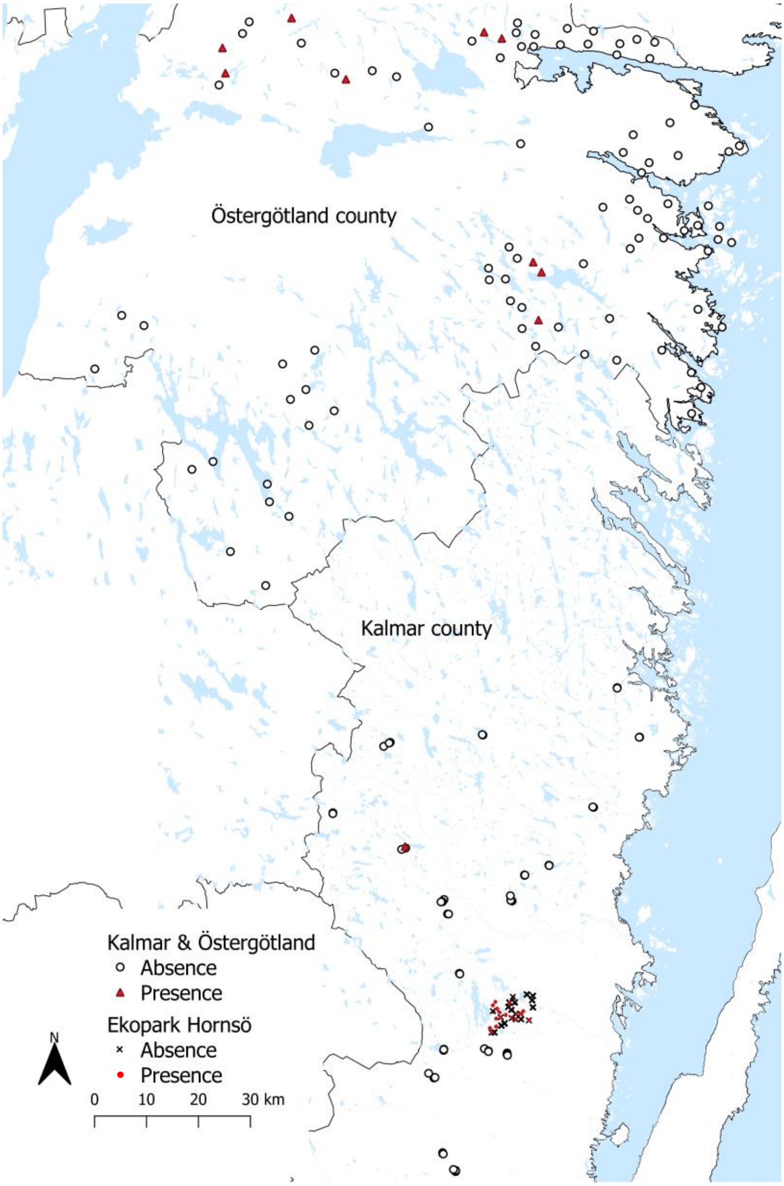

Figure 16. The study areas within the counties of Kalmar and Östergötland. White circles and red triangles denote observations of *Tragosoma depsarium* in pheromone traps at 120 sites during the study in July 2014. Red dots and black crosses indicate the presence and absence of the species in 90 traps within a sub-study within Ekopark Hornsö (see Figure 2). Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

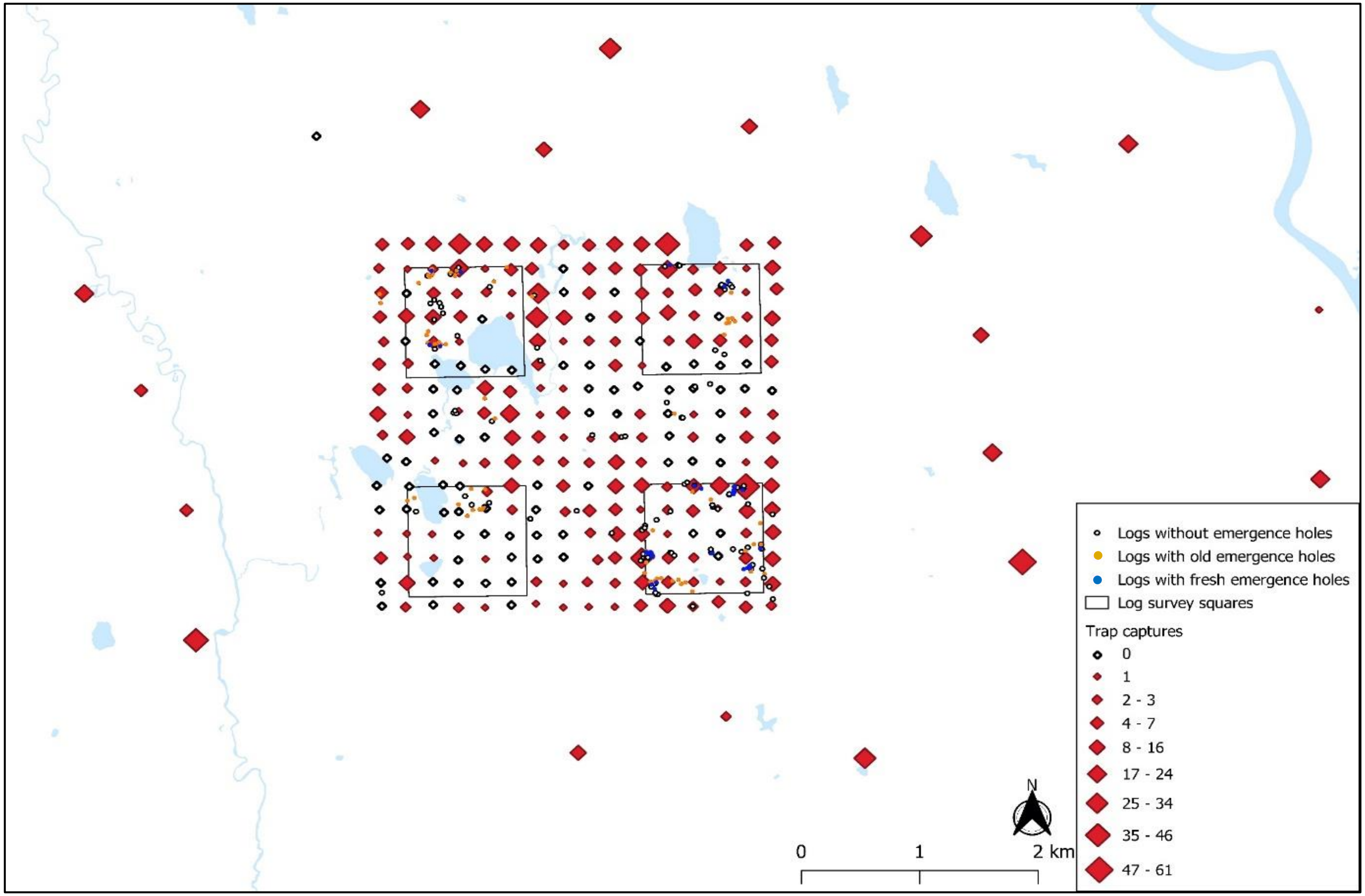

Figure 17. The study area of Sågtjärn, in the county of Värmland. The map displays *Pinus sylvestris* logs without (white circles), with fresh, less than a year old (blue circles) and older (orange circles) emergence hole from *Tragosoma depsarium*, surveyed during July and October 2019. The black outlined survey target squares (ST squares) indicate the areas surveyed continuously monitored for pine substrates (Wikars 2004). The red squares display pheromone trap placement and total number of captured *T. depsarium* in pheromone traps during the study in July-August 2019. Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

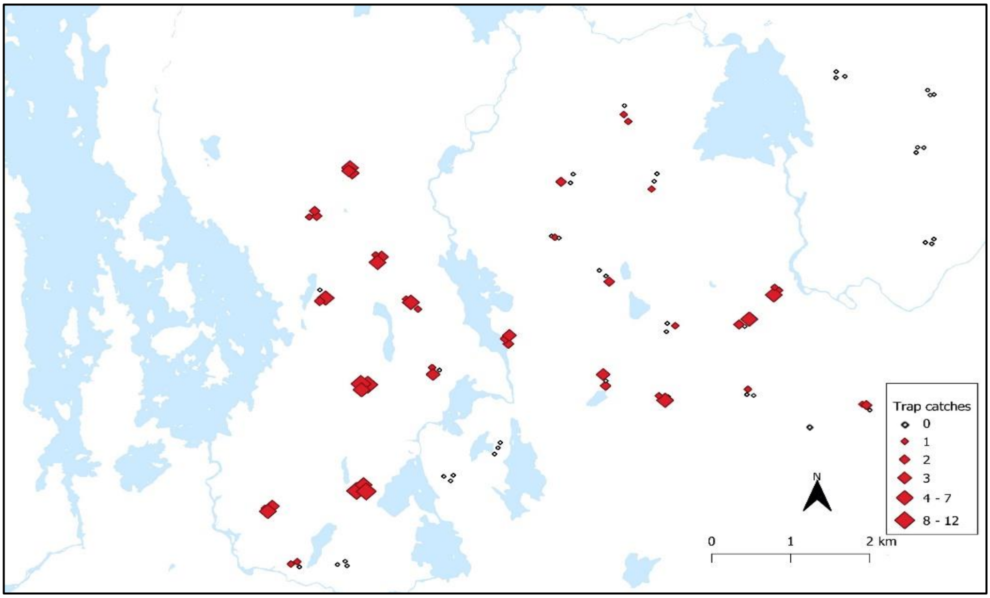

Figure 18. The study area of Ekopark Hornsö. The red squares indicate the number of *Tragosoma depsarium* caught in pheromone traps during the study in July 2014. Background map: GSD-Översiktskartan, vector ©Lantmäteriet, Gävle, Sweden

4.3 Habitat management and characteristics

Swedish forest biodiversity conservation hinges on a combination of formally protected nature reserves, set-aside areas of production stands and a general care during forestry actions, in order to maintain sufficient resources at a landscape level. For many threatened species, positive effects have been attested from both nature reserves (e.g. Bouget & Parmain 2016; Karpiński et al. 2021) and woodland key habitats (e.g. Hottola & Siitonen 2008; Timonen et al. 2011). Within a modernised forestry landscape, scattered protected stands appear critical for the survival of many species, and can constitute good general indicators of biodiversity at the landscape level (Götmark et al. 2011). Saproxylic beetles are however, a diverse group with a wide range of requirements, of which many still are poorly understood. While some species are diminishing in managed forests, others are capable of utilising the environments created by forestry activities. Dead wood left after clear cuts have been shown to have beneficial effects for some beetle assemblages (Kaila et al. 1997), whereas others suffer ecological traps as they oviposit on wood stockpiles destined for biofuel (Hedin et al. 2008).

In our studies, we have included systematic comparisons between defined habitats, in order to evaluate the effects of specific habitat type and other habitat characteristics for the distribution and abundance of various species in the landscape.

4.3.1 Oak habitats (Paper II & III)

The study of longhorn beetles utilising ephemeral oak substrates in the provinces of Blekinge and Småland showed species specific responses in regard to oak habitat type, although no obvious effects of management regime (Figure 19). *P. sanguineum* turned out to be the most generalist species with no significant differences in abundance between the habitat types. For the other species, the greatest differences were found between the edge and interior stands of the actively managed forests. *P. alni*, *X. antilope* and *P. arcuatus* preferred the sunlit edge areas, while *P. testaceus* was captured in higher abundance within the shadier interior stands. The lack of differences between passively and commercially managed habits was surprising as it is often found for many saproxylic species (cf. Djupström et al. 2012; Sverdrup-Thygeson et al. 2014; Hämäläinen et al. 2018) but is probably related to substrate demands. With species utilising coarser and rarer substrates with long generation times, there might be greater differences in availability between habitat types. The hotspot sites of the study are for example known for large, sparse stands of century old oaks, usually with hollows. While these trees generate substrates essential for a large number of species, they might not actually provide more of the thin-diameter substrates than the younger actively managed stands. With a large proportion of the substrates consisting of still attached dead wood in the canopy covers (Svensson et al. 2014; Seibold et al. 2018), it is possible that the denser production forests even generate higher quantities of suitable twigs and branches. Similarly, while woodland key habitats provide beneficial effects for general forest biodiversity (Hottola & Siitonen 2008; Timonen et al. 2011), their small sizes might limit positive effects on saproxylic beetles (Sverdrup-Thygeson 2002; Sverdrup-Thygeson et al. 2014a).

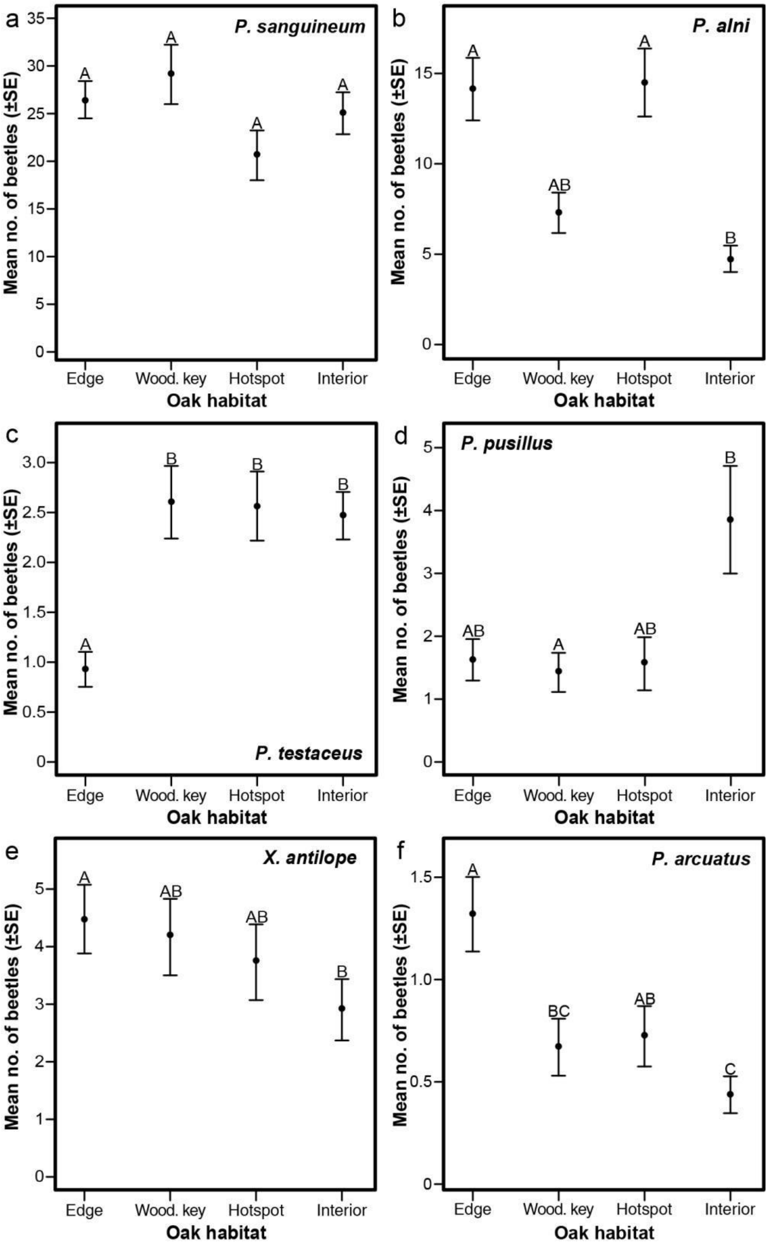

Figure 19. Mean number of beetles per pheromone trap and trapping interval of six species of longhorn beetles captured in four different types of oak habitats; the edge of production stands, woodland key habitats, hotspots and the interior of production stands. Two years (2016-2017) of repeated sampling are pooled. Habitats that do not share a letter are significantly different ($P < 0.05$)

Overall, within the region covered in the study, sun exposure and openness appeared to be more important factors for the six species than management regime. Four of the species had significant differences in the number of captured individuals between interior and edge habitats. The two exceptions , *P. sanguineum* and *P. pusillus*, also constitute the species with earliest activity periods, usually starting to emerge already in the end of April, before the oak leaves fully unfold. This, of course, reduces the contrasts in sun exposure between the habitat types, which grow more pronounced during late May and June. With the exception of *P. testaceus*, the other species were to a higher degree captured at the edge habitats. This pattern is consistent with earlier studies both within and outside of Sweden (Lindhe et al. 2005; Bouget et al. 2013; Allison et al. 2019). While open oak habitats often feature larger communities of species (Koch Widerberg et al. 2012), there are apparent successional differences (Jonsell et al. 1998). Early species are to a higher degree found in the open areas, which correspond well to the results of both this study and with *B. capucinus* (Paper III).

In a very similar fashion to the longhorn beetles (Paper II), *B. capucinus* was found along all habitat classes (Paper III). There were significant differences in abundance, however, both between the habitat types and the density of the oak stands, with the beetle preferring sun exposed traps at the randomly selected oak habitats (ROH). Unexpectedly, the average ROH site captured twice the number of beetles as the more selectively chosen hotspot and SSOS revisit sites. In contrast to the actively managed sites within the cerambycid study, the ROH sites did not consist of exclusively production forests, but were a mix of different oak dominated woodlands and pastures of potentially differing management regimes. The most important factor explaining *B. capucinus* abundance appeared to be sun exposure, with traps in open stands having much higher number of captures. However, this does not explain the lower catches at the hotspot sites, which often consisted of free standing and sun-exposed trees. Instead, the species might have an aversion for the larger veteran oaks, in favour of the younger trees in the managed production landscape. It is possible that more suitable substrates are generated among the younger trees in pastures and production forests than within the set-aside hotspots.

The number of captures was also significantly lower at the SSOS sites than at the randomly chosen oak habitats, suggesting that the species is more likely to be observed at sub-optimal sites. Previous observations often tend to be connected to sites that could be classified as hotspot sites, which are often favoured by entomologists and naturalists in general. This agrees with the limited number of observations aggregated in rather aggregated clusters. In general, much emphasis appears to have been put on observations that might mostly represent stray individuals outside of their main habitats. This demonstrates the importance of reliable systematic monitoring schemes for efficient conservation of forest biodiversity.

4.3.2 Pine habitats (Paper IV)

For *T. depsarium*, different habitat types (managed forest and set-aside or protected) were not directly compared within the same regions. In south-eastern Sweden, surveyed habitats were generally either set-aside or protected. The only exception, Ekopark Hornsö, represents an area with large fractions of actively managed pine forests, interspersed with some protected areas. The park provides relatively beneficial conditions for *T. depsarium* in the managed forest, and active measures are being taken to generate suitable substrates. The limited catches and overall absence of *T. depsarium* at a great majority of the studied sites in South-Eastern Sweden suggest that the species is generally absent from the region. This could be due to restricted availability of habitats that can support population growth at the local and landscape levels. There was however, no inclusion of commercially managed forest sites in this region, which leaves the possibility that larger populations could be found within the actively managed pine forests. Nevertheless, it would be unlikely, as the general conditions for generating suitable substrates here are much less favourable.

In Värmland, the study area was an extensive area of commercially managed pine-dominated forest, which has been characterized by relatively high proportions of mature and overmature stands and an extensive logging activity during the last few decades (Wikars 2004; Wikars et al. 2014). During this period, the combination of relatively high proportions of older age classes of trees and active disturbances appears to have been favourable, generating substantially higher population densities than in South-Eastern Sweden. It appears that managed stands can be favourable for the species, as long as the surrounding landscape remains capable of generating suitable substrates. Adapted to large scale disturbances, i.e. forest fires, it has been proposed that *T. depsarium* is capable of utilising the temporary openness and sun exposure of clear cuts, as long as stands of older pine remain nearby

(Wikars 2004). This corresponded well to the captures in Sågtjärn, where large numbers of males appeared to aggregate at older logged sites, both with or without suitable substrates. As with both the *B. capucinus* and five of the longhorn species, openness and sun exposure are probably very important factors for beetle activity.

4.4 Dead wood and substrate dynamics

For saproxylic species, the quality and quantity of dead-wood substrates are major factors determining habitat suitability and population abundances (Fridman & Walheim 2000; Gibb et al. 2006; Seibold et al. 2016). With the general decrease of dead-wood substrates in commercially managed forests (Jonsson et al. 2016; Thorn et al. 2020), many species thus face serious problems. Dead wood is however, a very broad category of substrates, with very different characteristics and dynamics. Depending on the type of dead wood utilised, some species may do very well in modern forestry regimes, while others perish. Examples of species adapted to the slowest form of dead-wood dynamics are *O. eremita* and *E. ferrugineus*, two species inhabiting hollow oaks. They both have long larval stages inside a substrate that takes centuries to form. With a general lack of ancient oaks in modern oak landscapes, the species are rare and threatened to disappear. On the other hand, they can often show very high persistence, as long as their long-lived habitats remain. Studies have also documented good agreement between habitat and substrate availability and occupancy and abundance (Larsson & Svensson 2011; Andersson et al. 2014). In contrast, the present studies concern species with entirely different substrate dynamics, ranging from very fast to intermediate. The six longhorn beetles studied utilise the ephemeral substrates, quickly generated and only suitable for a single generation. *B. capucinus* has been mostly associated with hard dead wood of mature trees. While taking much longer to form, the substrate can be utilised for several generations. *T. depsarium* has the harshest demands, and utilises decade old pine logs, from preferably old and slow-grown trees, lying in dry and sun-exposed environments.

4.4.1 Dead wood and longhorn beetles (Paper II)

Within the study of longhorn beetles breeding in ephemeral substrates (Paper II), we investigated the effects of dead wood availability, by following population dynamics in managed oak forests for three years after logging events (Figure 20).

The amounts of lying fresh dead-wood substrates were qualitatively surveyed at all sites in 2016. Compared to the recently logged sites, the undisturbed sites appeared similar, with low quantities of suitable substrates on the ground, and no correlation between dead wood and trap captures could be seen. It is possible that more precise measurements of dead-wood resources could have better explained inter-site variation, but it was not deemed feasible. As the species slightly differ in their preferred substrate size, and are known to utilise both lying and still attached twigs and branches (Ehnström & Axelsson 2002; Svensson et al. 2014), it would be overwhelmingly difficult and time consuming to conduct quantitative measurements across the sites.

As a group, the recently logged oak stands contained much larger amounts of suitable substrates during the first year. The logging residues appear to have provided the hypothesized effect and attracted beetles from the surrounding landscape, as five of the species (except *P. alni*) were caught in significantly greater numbers compared to the control sites (oak stands without recent management). With the substrates usually only lasting a single season, it is probable that emerging beetles quickly disperse to find new habitats with colonisable substrates. Depending on the life cycles of the species, the subsequent years differed. For the species with a one-year larval stage, trap captures continued to be high during the second year, while diminishing during the third summer. The remaining species, with a varied 1-2 life year cycle, continued to show an increased abundance at the logged sites during both 2017 and 2018. It is likely that an additional year of trapping would have shown the abundance of all species starting to approach that of the control sites.

Overall, the six longhorn species seemed to benefit by substrate generating processes as thinning and logging. Earlier studies have shown varied responses of saproxylic biodiversity to forest management (e.g. Johansson et al. 2007; Fedrowitz et al. 2014), often tied to species specific requirements of forest continuity (Janssen et al. 2017), successional stage (Jonsell 2008) and substrate characteristic requirements (Hjältén et al. 2012). In the case of the six studied cerambycids, they are utilising relatively thin twigs and branches of oaks, substrates quickly generated even among younger trees. In contrast to species specialising on larger and older substrates, this makes them less dependent on forest age and continuity. From a landscape perspective, the species appear to be able to sustain themselves in all the oak habitat types, and can probably easily exploit sudden increases in substrates. For species with more exclusive requirements, this might not be possible, and several studies on species communities have revealed the difficulties for red-listed species to favourably utilise areas after clearcutting (e.g. Franc & Götmark 2008; Fossestøl & Sverdrup-Thygeson 2009). Instead, more selective logging through retention cutting, is often suggested as better forest management methods for the conservation of biodiversity (Götmark 2007; Fedrowitz et al. 2014; Heikkala et al. 2016b).

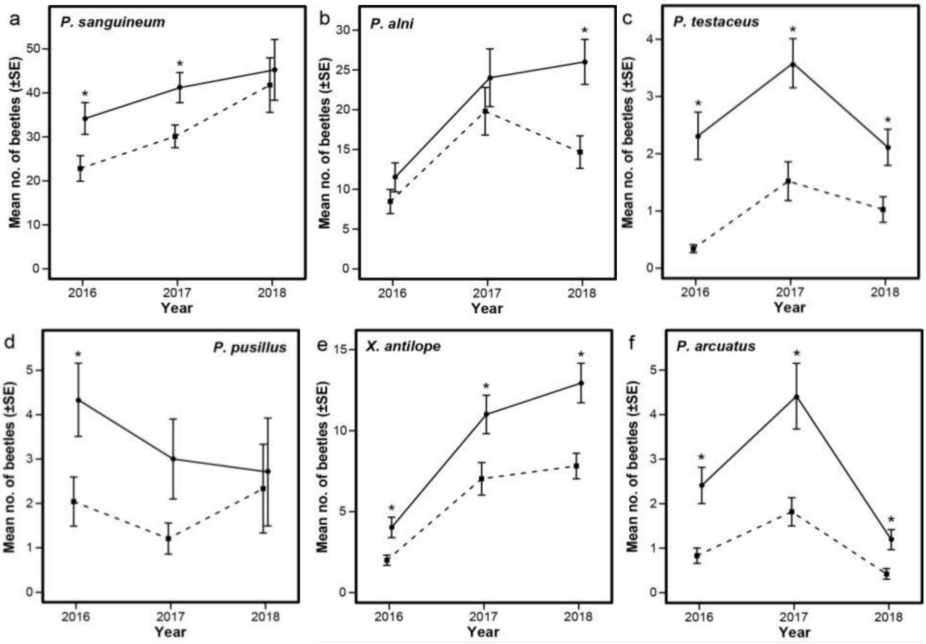

Figure 20. Mean number of beetles of six species of longhorn beetles captured per pheromone trap and trapping interval in recently logged oak production stands (connected by solid line), and matching control stands without recent logging activities (connected by hatched line). The stands were sampled for three consecutive years. Years with an asterisk indicate a significant difference ($P < 0.05$) between the logged stands and the control stands

4.4.2 Dead wood and *Bostrichus capucinus* (Paper III)

In contrast to the longhorn species breeding in oak substrates, *B. capucinus* is known to utilise older wood substrates, which is often suitable for several generations. The species has been found so rarely colonizing wood substrates, however, that we could not generate any plausible comparisons between different presumed substrates amenable to trapping studies. Instead, we have performed a general comparison between two types of habitat that could represent different substrate requirements, by contrasting hotspot sites with high densities of veteran trees to general deciduous forest landscapes. The comparison showed that, contrary to expectations, *B. capucinus* appears to be much more common in the general deciduous forest landscape than at sites characterized by considerable amounts of dry, sun-exposed wood such as veteran tree stems and branches. Beyond this observation, further studies are needed for a more in depth understanding of its actual substrate requirements.

4.4.3 Dead wood and *Tragosoma depsarium* (Paper IV)

The dead wood requirements of *T. depsarium* differ from the investigated oak species with the preference of a much rarer substrate. Large pine logs are of economic importance and generally not left behind after logging events (Fridman & Walheim 2000; Eräjää et al. 2010). Historically, the geographical distribution of *T. depsarium* has to a great extent been surveyed or noted by the presence of characteristic emergence holes. This constitutes a very favourable situation for general surveys of its geographical distribution, as easily identifiable and durable markers of its presence. However, it can be difficult to ascertain present occupation apart from fresh emergence holes. It is also desirable to evaluate the effects, in terms of changes to population size, of different measures implemented at a large scale in conservation efforts. Estimating abundance of local populations at greater scales through fresh emergence holes requires labour-intensive, thorough surveys of logs across the area of interest. This is in stark contrast to pheromone traps, which allow simultaneous and effortless monitoring of vast forest areas. By combining the two methodologies, this study aimed to establish a link between pheromone catches and substrate availability.

Among the Östergötland woodland key habitat sites, large pine logs were found at a majority of the sites (68/100), but emergence holes from the species were only present at 28 of them. While all the barkless and sun-exposed logs appeared suitable, most had apparently not been colonised by the beetle. The high number of captures at clear cuts in Sågtjärn and at an old fire site in Ekopark Hornsö indicated that the males aggregate in open areas, but there was no direct correlation to substrate availability. Instead, the number of captures were well correlated to the amount of emergence holes of logs within 200 metres of the traps. The current studies could not determine why such a large number of logs remained uncolonised, but there might be a combination of rejections and failure to find the substrates. With the large population in the Sågtjärn area, it is unlikely that substrates remain hidden from the beetles. Instead, it is probably their high substrate demands that make them reject many of the logs. Wikars (2004) discusses the importance of substrate qualities such as the proportion of heartwood and type of wood-rot, which are qualities difficult for humans to quickly assess. More thorough surveys of substrate suitability would potentially find correlations between the abundance of logs and beetles, but be too time-consuming for practical usage. In South-Eastern Sweden, it is also possible that suitable logs remain uncolonised. With smaller, potentially fragmented populations, it is likely that too few females emerge to colonise the logs, or even that potential habitats remain empty. Overall, the results demonstrate great potential for pheromone-based trapping to be used both as a direct link to absolute population density through mark-recapture studies, but also to semi-quantitative compare population abundances through trap catches. Both the presence of beetles at sites in Östergötland and local abundance in Sågtjärn could be directly related to the presence of emergence holes, further establishing a link between different levels of population surveys with different methods. As several other threatened saproxylic species utilise similar substrates as *T. depsarium* (Wikars et al. 2014), it would be of great interest for further studies to evaluate the potential of the species as an indicator for pine dead-wood availability.

5. Conclusions and future perspectives

This thesis demonstrates the general difficulties in accurately evaluating the conservation status of saproxylic beetles, but also what differences efficient pheromone-based monitoring systems can make in facilitating this process. Although the study areas have been subject to countless surveys by both researchers and amateur entomologists, the study species have often remain unobserved in large parts of their distribution range. This phenomenon is problematic, as common but elusive species might be mislabelled as rare or threatened. Without trustworthy information of species distribution and abundance, it is very difficult to conduct effective conservation efforts, and there is an imminent risk of sub-optimal utilisation of limited funds. While pheromone trapping here has proven incredible useful for accurate and comparable sampling of specific species, individual sampling of all forests insects of conservational interest is not a realistic option. It could however, be an important tool in focused surveys of especially threatened species. There, immediate evaluations of habitat restoration efforts or accurate estimations of population sizes can be invaluable. Furthermore, to maximise the benefits of pheromone-based monitoring, specific species could also be utilised as indicators of both larger insect communities and landscape parameters. By investigating correlations between the surveyed insects and the surrounding species assemblages, it could be possible to increase the information value of surveyed species dramatically. Similarly, the presence and abundance of certain species could potentially be used as indicators of important habitat or landscape characters, such as the availability of specific substrates. Useful estimates of different types of dead-wood substrates are complex and time consuming, and practically impossible in studies over larger spatial scales. With the correct tool-box of species, it could instead be possible to utilise insects as proxies for habitat qualities at a landscape scale.

An efficient future monitoring methodology of saproxylic biodiversity could potentially consist of more general window traps complemented by lures of rare and indicator species.

While their abundances sometimes varied, the oak dependent species appeared to utilise all surveyed habitat types in their distribution ranges, without much beneficial effects of management or protection. It is likely that the surrounding landscape holds enough quantities of oaks to continuously generate at least the minimum amount of suitable dead-wood substrates. With populations spread across the landscape, this community of beetles are well adapted for quickly utilising temporal boosts of substrates, such as after fires or logging activities. This is contrasted with *Tragosoma depsarium*, whose decade-old pine log substrates are much more uncommon in the modern forest landscape. With more fragmented populations in large parts of its distribution range, it represents a group of insects lacking the widespread presence to immediately capitalise on sudden substrate boosts. In general, harsher substrate demands seem to correlate well with the need for formal protection and conservation efforts. While insects utilising younger ephemeral dead wood relatively easily adapt, it is impossible for sedentary beetles such *Osmoderma eremita* and *Elater ferrugineus*, reliant on rare century-old hollow oaks, to persist in the greater production forest landscape. Together these observations highlight the importance of implementing conservation strategies on several spatial scales. As species communities substantially differ in habitat demands and dispersal capabilities, direct enrichment and restoration actions at the habitat level must be combined with long-term strategies for the surrounding landscape matrix. A complex heterogeneous forest landscape, encompassing a wide range of habitat and substrate types is of vital importance for sustainable preservation of forest biodiversity.

Even though several species shared nominally very similar substrate demands, their overall distributions differed dramatically. The South-East coast of Sweden is known to inhabit large amounts of saproxylic biodiversity, among which there are several oak dependent species not found within the rest of the country. The reasons for their limited distribution ranges are not yet known, but as the region is considered among the warmest and driest in Sweden, some combination of substrate availability and climate effects seems to be a likely explanation. With pheromones identified for *B. capucinus*, *X. antilope*, and *P. pusillus* (with progressively more narrow distribution ranges), we now have the tools for reliably evaluating such effects. Furthermore, by systematic long-term studies mapping their current and future distribution ranges, the species could provide invaluable insights into saproxylic insects' responses to climate change.

While this thesis focused on forest management and beetle conservation from a Swedish perspective, the resulting conclusions should be internationally applicable. Forest biodiversity is globally under threat from both increasingly intensive management practices and changes in climate and the environment. For future preservation of forest and woodland ecosystem functioning and biodiversity, it is of utmost importance to understand the genuine habitat demands and landscape utilisation of their inhabiting organisms. Within the EU, the Biodiversity Strategy for 2030 comprises ambitious goals for restoration and protection of natural habitats (Hermoso et al. 2022) but have been criticised for lacking much consideration of spatial connectivity and landscape perspectives (Selva et al. 2020). With a steadily growing portfolio of available species, pheromone-based methods have the potential to become an important tool for cross-continental monitoring of biodiversity. In a rapidly changing environment, systematic long-term monitoring schemes will be of increasing importance for distinguishing between natural and anthropogenic fluctuations in insect abundance.

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Popular science summary

Sweden's forests have undergone major changes over the past century. After the Second World War, forestry was rapidly mechanised and an increasingly rational and production-adapted forest industry gained momentum. The naturally diverse forests were at an increasingly faster pace replaced with dense, even-aged stands of mainly spruce. The new management regimes led to a very efficient timber production, but unfortunately also negatively affected large parts of the forest biodiversity. For millions of years, thousands of species have adapted to specific ecosystems and specialised themselves to different niches in all types of environments. When the forests, through human influences, began to homogenize it also meant that a variety of previously common habitats and substrates started to disappear. Through this, an ever-increasing number of species have ended up on the Swedish red list, and about fifty species are estimated to have gone completely extinct in Sweden. Since 1993, an increased focus has been put on the conservation of forest biodiversity in Sweden, which in today's national guidelines is set as a goal equated with that of timber production. Since then, general consideration in production stands has been combined with woodland key habitats (nyckelbiotoper in Swedish), and formally protected nature reserves to form a sustainable Swedish forestry regime. An important part of this is the conservation and restoration of dead wood. With about 7,000 deadwood-dependent species in Sweden, this group of substrates are incredibly important for forest biodiversity. Many of these species are decomposers, utilising dead-wood substrates as food sources. The largest such species group is the wood-living beetles, with about 1,200 species directly dependent on dead wood, usually mainly for their larval development. The different species are often specialised in different types of wood (e.g. tree species, thickness, age, types of fungal infestation) and can have larval stages stretching from a year to a decade. In woodland ecosystems, the beetles perform several important services, primarily as wood decomposers and as basic food for many other species, such as woodpeckers.

Despite the increased focus on biodiversity consideration, many wood-living beetles still appear rare and endangered. For many species, the trend is still even going in the wrong direction, and an increasing number of beetles are being added to the Swedish red list. To reverse the trend, many conservation initiatives are being implemented today to recreate habitats and substrates in Swedish forests. A major problem, however, is that we still lack much knowledge about the habitat requirements of wood-dwelling insects. As many species are rare or hidden, they are often difficult to systematically survey. Reliable studies have often been expensive and time-consuming, but in recent years new pheromone-based methods have been developed to compensate that. Pheromones are chemical compounds emitted by insects for communication with their species members. They are predominantly used as sexual attractants by either males or females to call members of the opposite sex. By applying such pheromones as lures in insect traps, it is possible to carry out extremely reliable surveys of both the presence and abundance of specific insects. So far, pheromone studies have mainly been used to survey pest species, but during recent years, new pheromone identifications have been made for species of conservation interest.

In this dissertation, I have developed and applied pheromone-based monitoring methodologies for wood-living beetles to assess and evaluate how they fare with modern forestry regimes. In the first part, I identified pheromones from three beetle species, the two longhorn beetles *Anaglyptus mysticus* and *Xylotrechus antilope*, and the auger beetle *Bostrichus capucinus*. All three species are found on the Swedish Red List, and are considered endangered in the Swedish forestry landscape. The work began by collecting dead wood that was suspected to have been colonized by the beetle larvae. The wood was brought to the Alnarp campus, and placed in plastic boxes in a greenhouse. There they were allowed to pupate and all emerging adults were collected and separated by sex. The beetles were then used for head-space volatile collection. This consisted of placing the beetles separated by sex in two glass containers, from which a small pump continuously sucked through air. At the outlet of each container was a filter that was capable of chemically capturing potential odour molecules in the air flow. By using chemical analysis instruments to compare which molecules that were captured in the filter for the males and the females, I was then able to identify potential pheromone components. Usually, they consisted of individual substances that were present in very large quantities in the collections from males, but were completely absent in the female one. The potential pheromone candidates were then purchased or synthetized in chemistry laboratories for field testing. During the species’ activity periods, I set up insect traps baited with the species' pheromones in the provinces of Småland and Öland. By letting the beetles choose between traps with different combinations of pheromone compounds or unbaited control traps, I was able to assess how efficient the synthetized pheromones actually were. For all three species, they proved to be very effective and capable to assess whether the species were present at a site or not with a high certainty.

In the second part of the dissertation, I used recently identified pheromones for three large scale ecological studies of presumed threatened wood beetles, in order to determine their true distributions and habitat requirements. In the first study, six longhorn species were surveyed in the Swedish provinces of Småland and Blekinge, during three summers (2016-2018). The species have overlapping habitat requirements with larvae that live in thin, recently dead twigs and branches of oak. Although the species are popular with insect collectors, they rarely appear in traps during general insect studies, and are therefore considered local and sporadic. To investigate the effects of different management regimes in oak landscapes, traps were set up in both production forests and set aside or protected oak environments. In half of the production forests, logging activities had taken place during the winter of 2015-2016, and I wanted to follow how the species' population sizes changed during the first years after logging. The remaining areas were used as controls for comparisons. At the control sites, traps were placed both on along the forest edge, and deeper inside the stands to also investigate the effect of canopy cover and sun exposure. The study showed that all six species increased in numbers of caught individuals after forestry actions, probably representing the colonisation of branches and twigs left at the sites. While the adult individuals hatched (1-3 years after colonisation, depending on the species), the population sizes continued to be high, after which they again approached those of the control forests. There also seemed to be positive effects of sun exposure for five of the species. In the comparison between production forest and protected areas, the species' preferences varied. However, all species could be found on all the surveyed sites within their respective distribution area, indicating that they were capable to survive in the production forest landscape. There is probably enough oak in the landscape for a continuous generation of sufficient amounts dead branches and twigs to sustain the beetle populations.

In the second study, I examined the distribution of the threatened (Vulnerable on the Swedish red list 2020) *B. capucinus* in the provinces of Blekinge, Småland and Östergötland. The species lives in hard dead and sunlit oak and has been considered very rare in Sweden. During the last century, relatively few observations have been made, and its active distribution range has been suspected to be shrinking. During the species' activity period in May-June 2019, I surveyed the species in 135 oak dominated habitats in South-Eastern Sweden with pheromone traps. The sites were selected in places where either the species has been observed during the last century, areas known for high wood-living beetle biodiversity, or in completely randomly selected oak environments. Contrary to my expectations, the species was found on the majority of all sites and appeared to have been overlooked rather than being rare. The same cannot be said of the pine forest dependent species, *Tragosoma depsarium*, which was surveyed in the third study. It is also red-listed as vulnerable and estimated to be declining with the present forestry regimes. *T. depsarium* has relatively high substrate requirements, with larval development in thick pine logs, which often must have been dead for at least a decade before being suitable for this species. The species has previously been common in Sweden, but is now rarely seen in production forests. Instead, it seems to occur mainly in set-aside areas where sparse and sunlit older pine stands still can be found. In Östergötland and Småland, a survey was carried out in 2014 where the species was only found on a fraction of the surveyed premises. Even when the species was found, only few individuals were caught, indicating very weak populations in South-Eastern Sweden. An exception was the semi-protected Hornsö Ekopark where a more detailed study was done. Pheromone traps were there spread across the park, and all captured beetles were marked with pens on the elytras and released. By then comparing the number of new individuals and recaptured beetles in the traps, the population size could be estimated to about 330 individuals. In 2019, I carried out another survey of *T. depsarium* in Norra Ny in the province of Värmland, where the species was previously regularly inventoried by counting emergence holes in pine logs. By systematically placing 251 traps in a grid pattern, I investigated the link between the number of traps and environmental factors, such as the amount of dead wood or vegetation types. Just as in Hornsö Ekopark, captured individuals were marked and released, which gave an estimated population size of about 2040 individuals. The number of captured individuals greatly exceeded the number of hatching holes in the pine logs of the area, which indicates a major effect of dispersal within the surrounding landscape. As North-Western Värmland contains significantly larger belts of continuous pine forest than South-Eastern Sweden, it is possible that the species has an easier time to maintain stable populations. Even if a pine stand is felled, the species may be able to find refuge in nearby areas, to later return and recolonize the stand. In Småland and Östergötland, this seems to be more difficult. Potentially, too large proportions of the forest have been felled simultaneously, resulting in no permanent populations left to later recolonize the areas. Instead, only small isolated populations are remaining which require active conservation measures to be maintained.

Overall, my thesis demonstrates some of the difficulties in conserving dead wood dependent forest biodiversity. Apparently similar species might have very different responses to modern forestry regimes, and we still have much to learn of their substrate and habitat requirements. To conduct relevant and cost efficient conservation strategies, systematic and accurate monitoring schemes are of utmost importance. As many wood-dwelling species seem elusive with more general trapping methodology, pheromone-based methods could potentially play an important role in future conservation work. By identifying pheromones of important indicator species, whose presence could give us information about larger species assemblages and environmental factors, large-scale studies could be set up to systematically compare whole regions at a time. In the future, monitoring programs could potentially be set up to continuously survey a portfolio of such species, to rapidly detect threats and environmental changes at both national and international levels.

Populärvetenskaplig sammanfattning

Sveriges skogar har under det senaste århundradet genomgått stora förändringar. Efter andra världskriget skedde en snabb mekanisering av skogsbruket och ett allt mer rationell och produktionsanpassad skogsindustri tog fart. De naturligt varierade skogarna ersattes i allt snabbare takt med täta likåldriga bestånd av framförallt gran. De nya skötselregimerna ledde till en väldigt effektiv virkesproduktion, men tyvärr även till problem för stora delar av den skogliga biologiska mångfalden. Under miljoner år har tusentals arter anpassat sig till specifika ekosystem och funnit sig olika nischer i alla typer miljöer. När skogarna började homogeniseras innebar det samtidigt att en mängd olika tidigare vanliga habitat och substrat minskar. Under det gångna seklet har ett ständigt ökande antal arter hamnat på den svenska rödlistan och ett femtiotal arter bedöms helt dött ut och försvunnit från Sverige. Sedan 1993 har ett allt större fokus lagts på bevarandet av skoglig biodiversitet, vilket i dagens nationella riktlinjer är satt som ett mål jämställt med virkesproduktion. Sedan dess har generell hänsyn i produktionsbestånd kombinerats med nyckelbiotoper och formellt skyddade naturreservat för att utforma ett hållbart svenskt skogsbruk. En viktig del för detta är bevarandet och återförandet av död ved, då svenska skogar innehåller cirka 7 000 vedlevande arter. En av de största grupperna bland dessa är de vedlevande skalbaggarna med omkring 1 200 arter direkt beroende av död ved, vanligtvis framförallt för sin larvutveckling. De olika arterna är ofta specialiserade på olika typer av ved (t.ex. trädslag, grovlek, ålder, typ av svampangrepp) och kan ha ett larvstadium på allt från ett år till ett decennium. I veden utför skalbaggarna flera viktiga ekosystemtjänster, framförallt som nedbrytare av ved och som basföda för många andra arter, såsom hackspettar.

Trots långsamma förbättringar i biologisk hänsyn så är många vedlevande skalbaggar fortfarande hotade och ovanliga. För många arter går trenden till och med fortfarande åt fel håll och allt fler skalbaggar tas upp på den svenska rödlistan. För att vända trenden genomförs idag allt fler naturvårdsinsatser för att återskapa habitat och substrat i svenska skogar. Ett stort problem är dock att vi fortfarande saknar mycket kunskap om vedlevande insekters habitatkrav. Då många arter är sällsynta eller lever undangömt är de ofta svåra att systematisk inventera. Pålitliga studier har ofta varit dyra och tidskrävande men under senaste år har nya feromonbaserade metoder tagits fram.Feromoner är sexuella lockdofter som insekter använder för att kommunicera med artfränder. Oftast används de av antingen honor eller hanar för att locka till sig det andra könet för parning. Genom att beta insektsfällor med sådana lockdofter är det möjligt att enkelt genomföra extremt pålitliga inventeringar av både närvaro och abundans av specifika arter. Hittills har feromoninventeringar framförallt använts för att inventera skadeinsekter, men på senare år har nya feromonidentifieringar gjorts för bevarandebiologiskt intressanta arter.

I den här avhandlingen har jag utvecklat och applicerat feromonbaserade inventeringsmetoder för vedlevande skalbaggar. I den första delen identifierade jag feromoner från tre skalbaggsarter, de bägge långhorningarna prydnadsbock (*Anaglyptus mysticus*) och ekgetingbock (*Xylotrechus antilope*) samt rödvingad kapuschongbagge (*Bostrichus capucinus*). Alla tre arterna är upptagna på den svenska rödlistan och anses hotade i det svenska skogsbruket. Arbetet påbörjades med att samla in död ved som misstänktes vara koloniserad av skalbaggarnas larver. Veden placerades i plastlådor i ett växthus på campus Alnarp, där de tilläts förpuppa sig så att vuxna individer kunde kläcka ut under kontrollerade former. Därefter separerade jag hanar och honor för att genomföra doftuppsamlingar. De gick ut på att skalbaggarna placerades i olika glasbehållare, varigenom en pump kontinuerligt sög igenom luft. Vid utloppet till varje behållare satt ett filter som kemiskt var kapabelt att fånga upp potentiella doftmolekyler som följde med luftströmmen. Genom att med kemiska analysinstrument jämföra vilka molekyler som fastnat i filtret för hanar respektive honor kunde jag sedan identifiera potentiella feromonkomponenter. Oftast var det enskilda ämnen som fanns i väldigt stora mängder hos hanarna, men saknades helt hos honorna. De potentiella feromonerna kunder därefter köpas in eller framställas i laboratorier för att testas i fält. Under arternas flygperioder satte jag upp insektsfällor betade med arternas feromoner i östra Småland och på Öland. Genom att låta skalbaggarna välja på fällor betade med de olika molekylerna eller obetade kontrollfällor kunde jag bedöma hur attraktiva och användbara feromonerna var. För alla tre arterna visade de sig vara väldigt effektiva och kan med hög säkerhet användas för att bedöma huruvida arterna finns på en plats eller ej.

I avhandlingens andra del använde jag nyligen identifierade feromoner för tre studier av förmodat hotade vedskalbaggars utbredning och habitatkrav. I den första studien inventerades sex långhorningsarter i Småland och Blekinge under tre somrar (2016-2018). Arterna har överlappande habitatkrav med larver som lever i tunna, nyligen döda kvistar och grenar av ek. Trots att arterna är populära hos insektssamlare så dyker de sällan upp i generella insektsinventeringar anses därför lokala och sporadiskt förekommande. För att undersöka effekterna av olika skötselregimer i eklandskap sattes fällor upp i produktionsskogar och avsatta eller skyddade ekmiljöer. I hälften av produktionsskogarna hade det skett avverkningar under vintern 2015-2016, och jag ville följa hur arternas populationsstorlekar förändrades under de första åren efter skogsåtgärder. De resterande områdena användes som kontroller att jämföra med. Där placerades även fällor både i utkanten och långt inne i bestånden för att undersöka effekten av öppenhet och ljusinsläpp. Studien visade att alla sex arter ökade i antal efter skogliga åtgärder, antagligen för att kolonisera grenar och kvistar som lämnats kvar på platsen. Medan de vuxna individerna kläckte ut (1-3 år efter avverkning, beroende på art) fortsatte populationsstorlekarna vara höga, varefter de åter närmade sig kontrollokalernas. Det tycktes även finnas positiva effekter av ljusinsläpp för fem av arterna. I jämförelsen mellan produktionsskog och skyddade områden varierade arternas preferenser. Alla arter kunde dock återfinnas på samtliga inventeringslokaler inom respektive utbredningsområde, och de tycks klara sig bra i produktionslandskapet. Antagligen finns det tillräckligt med ek i landskapet för att det kontinuerligt ska dö nog med grenar och kvistar som kan upprätthålla skalbaggspopulationerna.

I den andra studien undersökte jag den hotade (sårbar på den svenska rödlistan 2020) skalbaggen rödvingad kapuschongbagges utbredning i Blekinge, Småland och Östergötland. Arten lever i hård död och solbelyst ekved och har ansetts som väldigt sällsynt i Sverige. Under det senaste seklet har förhållandevis få observationer gjorts dess aktiva utbredningsområde misstänkts ha tryckts tillbaka från stora delar av Småland och Östergötland, till bara Kalmartrakten. Under artens aktivitetsperiod i maj-juni 2019 inventerade jag arten på 135 lokaler i sydöstra Sverige. Lokalerna valdes ut på platser där arten setts de senaste 100 åren, områden kända för hög artrikedom av vedlevande skalbaggar och på helt slumpmässigt utvalda ekmiljöer. Tvärt emot mina förväntningar hittades arten på majoriteten av alla lokaler och tycks snarare vara förbisedd än ovanlig. Det samma går inte att säga om raggbock (*Tragosoma depsarium*), som inventerades i den tredje studien. Även den är rödlistad som sårbar och bedöms minska i dagens skogsbruk. Raggbock har relativt höga substratkrav, med en larvutveckling i tjocka tallågor som minst ska ha varit döda i cirka ett decennium. Arten har tidigare varit allmänt förekommande i Sverige men ses nu sällan i produktionsskogar. Istället tycks den framförallt förekomma i avsatta områden med glesa och solbelysta tallbestånd. I Östergötland och Småland genomfördes en inventering under 2014 där arten bara återfanns på en bråkdel av de undersökta lokalerna. När arten väl hittades fångades endast enstaka individer vilket tyder på väldigt svaga populationer i sydöstra Sverige. Ett undantag var Hornsö Ekopark där en mer detaljerad studie gjordes. Feromonfällor spreds ut över parken och alla fångade raggbockar märktes på täckvingarna och släpptes fria. Genom att sedan jämföra antalet nya individer och återfångster i fällorna kunde populationsstorleken uppskattas till cirka 330 individer. Under 2019 genomförde jag ytterligare en inventering av raggbock, denna gång i Norra Ny i Värmland, där arten tidigare regelbundet inventerats genom att räkna kläckhål i tallågor. Genom att regelbundet placera 251 fällor i ett rutnät ville jag undersöka kopplingen mellan antalet fällfångster och miljöfaktorer, såsom mängden död ved eller vegetationstyper. Precis som i Hornsö Ekopark märktes och släpptes infångade individer, vilket gav en uppskattad populationsstorlek på cirka 2040 individer. Antalet infångade individer översteg kraftigt mängden utkläckningshål i områdets tallågor vilket tyder på en kraftig migration inom det omkringliggande landskapet. Då nordvästra Värmland innehåller betydligt större sammanhängande tallskogsbälten än sydöstra Sverige, så är det möjligt att arten enklare kan hålla sig kvar. Även om ett tallbestånd blir avverkat kanske arten kan återkolonisera lokalen igen. I Småland och Östergötland tycks detta vara svårare. Potentiell har för mycket skog avverkats samtidigt med följden att det inte fanns några beständiga populationer kvar att senare återkolonisera lämpliga områden igen. Istället återfinns bara små isolerade populationer som kräver aktiva bevarandeåtgärder för att överleva.

Min avhandling demonstrerar några av svårigheterna i arbetet med att bevara den biologiska mångfalden av vedlevande insekter. Till synes liknande arter kan egentligen skilja sig åt mycket i sina förutsättningar för att överleva under moderna skogsbruksregimer. Trots att mycket forskning har gjorts, så har vi fortfarande mycket kvar att lära oss om habitat- och substratkrav hos olika typer av vedlevande skalbaggar. För detta krävs effektiva och pålitliga inventeringsmetoder. Då många arter är ovanliga eller svårfångade med mer generell fångstmetodik, kan feromonfällor ha en viktig framtida roll. Genom att identifiera feromoner för viktiga indikatorarter, vars närvaro kan användas som information om det omkringliggande landskapets övriga insektsfauna och miljöfaktorer, kan storskaliga studier sättas upp för att jämföra hela regioner. Sådana studier skulle vara oöverträffade, både för att bedöma arters nuvarande situation, men också för att utvärdera olika naturvårdsinsatser. I framtiden skulle övervakningsprogram kunna inrättas för att kontinuerligt följa en portfölj av sådana arter på både inom Sverige och internationellt. På så sätt skulle hot och miljöförändringar snabbt kunna upptäckas för ett effektivt bevarande av den skogliga biologiska mångfalden.

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ORIGINAL ARTICLE

The aggregation-sex pheromones of the cerambycid beetles *Anaglyptus mysticus* and *Xylotrechus antilope* ssp. *antilope*: new model species for insect conservation through pheromone-based monitoring

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Abstract

We studied the pheromone chemistry of the cerambycids *Anaglyptus mysticus* and *Xylotrechus antilope* ssp. *antilope* with the goal of identifying attractants that could be used as tools for pheromone-based monitoring of these two species, which are rare and red-listed in parts of northern Europe. Beetles were reared from naturally colonized branches of hazel (*Corylus avellana*) or oak (*Quercus robur*), respectively, and used for headspace sampling. The extracts of volatiles were analyzed by gas chromatography–mass spectrometry. Males of *A. mysticus* consistently produced large quantities of (*R*)-3-hydroxy-2-hexanone and 2-nonanone, minor amounts of 2,3-hexanedione, and trace amounts of six other compounds. The average proportion of 2-nonanone to (*R*)-3-hydroxy-2-hexanone was 70:100. Males of *X. antilope* produced large quantities of (*S*)-2-hydroxy-3-octanone, and minor quantities of 2,3-octanedione. None of these compounds were present in the corresponding extracts of females from either species. The attractiveness of the dominant compounds produced by each species was determined in field bioassays. Lures with racemic 3-hydroxy-2-hexanone and 2-nonanone as single compounds were not attractive to *A. mysticus*. However, the compounds acted synergistically in blends of 100:100 and 160:100 of 2-nonanone to the hydroxyketone's (*R*)-enantiomer, but not in a 40:100 blend. Similarly (*S*)-2-hydroxy-3-octanone and its racemate attracted significantly more *X. antilope* than controls. Males and females of both species were captured in approximately equal numbers. The aggregation-sex pheromones of *A. mysticus* and *X.antilope* have high potential to serve as sensitive and efficient tools for detection and monitoring of local populations, and in studies of the species' ecology and conservation requirements.

Keywords Cerambycidae · (*R*)-3-Hydroxy-2-hexanone · 2-Nonanone · (*S*)-2-Hydroxy-3-octanone · Semiochemical · Monitoring

Introduction

The benefits of exploiting pheromones to detect and monitor a wide variety of pest insect species have long been recognized (Baker and Heath 2005; Witzgall et al. 2010), but pheromones have rarely been used for monitoring and study of rare species of conservation concern (Larsson et al. 2009; Tolasch et al. 2013; Oleander et al. 2015; Larsson 2016; Leal 2017). However, over the past decade, several studies have shown that pheromone-based sampling can be highly efficient for detecting and monitoring populations of rare and threatened saproxylic insect species that are difficult to observe with traditional survey methods (Larsson and Svensson 2009; Kadej et al. 2015; Burman et al. 2016; Harvey et al. 2017). In addition to mapping and monitoring populations, data collected from pheromone-based trapping can be used to study more complex questions associated with the

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optimal management of habitats, and the resource requirements of threatened insect species at landscape or local scales (Musa et al. 2013). To date, most research employing pheromone-based sampling methods has focused on saproxylic beetles dependent on hollow tree cavities (Larsson and Svensson 2009; Andersson et al. 2014), in large part because pheromones or related attractants have not been identified for other possible species of interest. However, the studies cited above have clearly demonstrated the value of pheromone-based methods as sensitive and highly efficient tools for sampling rare and cryptic species, providing substantial incentive to identify the pheromones of other rare or threatened species with different ecological demands (Larsson 2016; Larsson and Molander 2016). Pheromone-based monitoring could aid significantly in overcoming several key impediments to insect conservation, particularly in addressing the urgent need for more accurate information on the distribution and population changes of threatened species (Cardoso et al. 2011).

Longhorn beetles (Cerambycidae) are a useful group of model species for ecological and conservation research in forest ecosystems. In particular, the large numbers of species that have adapted to a variety of ecological niches provide a multitude of interesting model species that indirectly deliver information on the availability and status of specific habitats and wood substrates within an area. Owing to the group's popularity with natural historians and collectors, the habitat and substrate requirements (in a broad sense) are relatively well-known for many species (Linsley 1959; Ehnström and Holmer 2007; Klausnitzer et al. 2016), and historic population trends are comparatively well documented in certain regions for at least some species. These data show that many species have declined as a consequence of rapid, widespread environmental changes (McCorquodale et al. 2007; Jeppsson et al. 2010; Cálix et al. 2018).

Rapidly accumulating data have shown that longhorn beetles make extensive use of long-range attractant pheromones (Millar et al. 2009; Hanks and Millar 2016). The compounds are often shared by multiple species, and are usually fairly simple structures (Millar and Hanks 2017). Traps baited with cerambycid pheromones have proven to be efficient for sampling, and can easily be designed for catch and release purposes, depending on the status of the species being studied. Thus, longhorn beetles constitute excellent candidates for pheromone-based field studies of threatened forest insects. However, to date, although pheromones or likely pheromones have been identified from several hundred cerambycid species (Hanks and Millar 2016), pheromones have only been reported for a small number of rare or threatened species, such as a few species of the *Prionus* genus (Barbour et al. 2011), *Desmocerus californicus* ssp. *dimorphus* (Ray et al. 2014), *Dryobius sexnotatus* (Diesel et al. 2017), *Rosalia alpina* (Žunič Kosi et al. 2017), *Phymatodes pusillus* ssp. *pusillus* (Molander and Larsson 2018), and *Plagionotus detritus* ssp. *detritus* (Molander et al. 2019).

Anaglyptus mysticus (Linnaeus, 1758) and *Xylotrechus antilope* ssp. *antilope* (Schönherr, 1817) (henceforth, this subspecies will be referred to as *X. antilope*) are two cerambycid species (Cerambycinae; Clytini) of interest to conservation biology, especially in Northern Europe where the species are considered rare and occur in local populations (Ehnström and Axelsson 2002; Ehnström and Holmer 2007). *Anaglyptus mysticus* is present in southern Sweden, Denmark, the Baltic states, and in a small area of southern Norway (Laugsand et al. 2008; Danilevsky 2018). *Xylotrechus antilope* is only present in two small, isolated, and disjoint areas, one in southeastern Sweden, and one in southern Norway (Ehnström and Holmer 2007). Both species have been assessed as near threatened (NT) on the Swedish national Red List (ArtDatabanken 2015), and *A. mysticus* as critically endangered (CR) in Norway (Odegaard et al. 2015). Habitat fragmentation and loss of suitable substrates are the main (known) threats to their continued survival in these areas (ArtDatabanken 2015; Odegaard et al. 2015). The two species mainly inhabit deciduous forests and woodlands, but have different substrate preferences, with *A. mysticus* mainly developing in dead branches of hazel (*Corylus avellana*), in addition to a number of other deciduous trees, whereas *X. antilope* is dependent on fresh, recently dead oak branches (mainly *Quercus robur*) (Ehnström and Axelsson 2002). Both species are difficult to observe unless substantial amounts of fresh wood are present, that can be inspected visually on days with favorable weather. Thus, identification of the pheromones of these species could greatly facilitate detection and population monitoring, as well as a variety of ecological field studies on these species. The pheromone chemistry of *A. mysticus* has not been studied previously, but Schröder (1996) identified a number of compounds from extracts of volatiles from male *X. antilope* (ssp. *antilope*), some of which may function as pheromone components. However, to our knowledge, behavioral assays to confirm the identity and composition of a pheromone for the latter species have not been reported.

In this study, we demonstrate that males of *A. mysticus* produce a two-component blend of (*R*)-3-hydroxy-2-hexanone and 2-nonanone as an aggregation-sex pheromone that attracts conspecific females and males. Further, we confirm that males of *X. antilope* produce (*S*)-2-hydroxy-3-octanone, and show that this single component is an aggregation-sex pheromone which attracted both female and male beetles in field trials.

Methods and materials

Study sites

For field work (collection of substrates with larvae and bioassays), two different study areas were used, one for each species (for details on the individual sites, see Online Resource 1, Tables S1 and S2). *Anaglyptus mysticus* was studied on the Swedish island of Öland in the Baltic Sea, Kalmar County. Several sites were used for collection of infested wood and insect trapping in the southern part of the Mittland forest (approximate center coordinates: DD 56.6800N, 16.6027E). The area consists of a diverse mixture of dense forest stands managed for production of timber and forest biofuel, interspersed with grazed woodlands, semi-natural grasslands, and agricultural fields. Hazel (*C. avellana*), pedunculate oak (*Q. robur*), European ash (*Fraxinus excelsior*), and elm (*Ulmus* spp.) dominate the tree cover. *Anaglyptus mysticus* occurs throughout the Mittland forest and is fairly abundant; no other area in Sweden has a higher number of recent observations of this species (see Lindhe et al. 2010; Swedish Species Observation System 2018a). The species' comparatively high abundance in the Mittland forest may be explained by the large quantities of old hazel bushes present, because decaying hazel wood appears to be the preferred substrate of the larvae in Sweden (Ehnström and Holmer 2007). Therefore, we also focused on this tree species for collecting wood to rear out *A. mysticus* (see below).

Xylotrechus antilope was studied at Ecopark Hornsö (approx. center coordinates: DD 57.0120N, 16.0897E) on the Swedish mainland, Kalmar County. The Ecopark comprises a mixed coniferous–deciduous forest of ~ 9200 hectares that is one of the most important areas for rare and red-listed saproxylic insects in Northern Europe (Nilsson 2001; Anonymous 2008). The forest is predominantly managed for timber production, but several nature reserves have been established and management practices are tailored to support biodiversity throughout the area (Anonymous 2008). Small, open grasslands, rocky areas, and lakes and streams of different sizes increase the landscape's heterogeneity. Young and middle-aged pedunculate oak trees are abundant and *X. antilope* is a common and widespread species within the Ecopark (Nilsson and Huggert 2001; MAM, pers. obs.), which may harbor the largest population of this species in Northern Europe (Nilsson and Huggert 2001).

Collection of beetles

Hazel wood with larvae of *Anaglyptus mysticus* was collected from three different locations in the Mittland forest in early December 2015. At each location, we sought to collect different types of dead hazel wood because we did not know which logs might contain developing larvae, or the preferred stage of decay. Thus, substrates in different decay stages were collected, from relatively fresh, hard wood to decaying wood that could easily be broken up with a knife. Diameters varied from ~ 3 to 12 cm. We collected standing and fallen hazel wood stems, from both sun-exposed and shady positions. In all, ~ 1 cubic meter of hazel wood was gathered. The collected wood samples represented only a small proportion of the entire amount of dead hazel wood present at each of the three surveyed sites.

The collected wood was cut into ~ 0.5-m sections and placed in transparent plastic containers (L × W × H: 56 × 39 × 42 cm). From late December, the containers were kept in a greenhouse with an average daily temperature of about 15 °C and examined twice daily for emerging beetles. Occasionally, a spray bottle was used to dampen the wood with water to reduce desiccation. The first individuals of *A. mysticus* emerged from the wood after about 4 weeks (in early February). Adults continued to emerge for about a week until a total of 29 individuals had emerged. The beetles were continuously removed from the boxes, separated by sex, and stored in two plastic containers with fresh pieces of hazel branches (*C. avellana*), in an effort to stimulate pheromone release. For nourishment, we added pieces of paper saturated with honey water, and exchanged them for new ones every second day. The beetles were sexed by comparing the length of the antennae to the length of the body (from the mouthparts to the tip of the abdomen); males of *A. mysticus* have antennae of a greater relative length than females (Hansen 1966). The containers were stored in a refrigerator (8 °C) before (and sometimes in between) collections of headspace odors.

Xylotrechus antilope was reared from wood substrates collected on two occasions in Ecopark Hornsö. In the early spring of 2011, freshly cut branches of pedunculate oak (*Q. robur*) collected from various sources in the southern Swedish counties of Skåne, Blekinge, and Kalmar were transported to Lindsudd near Barnebo Lake (Barnebo N, Online Resource 1, Table S2), held outdoors through the summer, and collected again in the autumn, after natural colonization by the insects. The wood was stored outdoors and brought into a greenhouse in early 2012. In 2015, substrates were collected from a large heap of dead twigs and branches (up to ~ 20 cm diameter) of pedunculate oak at Ecopark Hornsö (Barnebo NE, Online Resource 1, Table S2). The substrates were left-over wood residues from logging activities in an adjacent mature oak forest in the winter of 2013–2014. A large number of saproxylic insects had colonized the wood in the summer of 2014, when it had been left to dry along a sunny south-facing forest edge. Once dry, the material would eventually be shredded as biofuel, which occurred in late winter 2015. Thus, the heap of substrates formed a so-called ecological trap, as described by Hedin et al. (2008). In February 2015, before several species with short larval developmental times were about to emerge from the wood in the following summer season, we collected ~ 1.5 cubic meters of wood substrates of mixed dimensions from the top and sides of the heap, which were brought back to the SLU campus and held in a greenhouse. In both 2011 and 2015, the wood material was processed as described for *A. mysticus* (see above).

In both 2012 and 2015, the first individuals of *X. antilope* emerged after about 3 weeks in the greenhouse, and large numbers of adults continued to emerge over about 1 week. The numbers of emerging beetles were quantified only in 2015, when a total of 527 *X. antilope* emerged, of which a subset of about 25 individuals (selected at random from multiple containers with wood, and initially stored singly in tubes) was used for headspace sampling (see below). The roughly 25 individuals that were selected to be used for aerations (the technique used for collecting volatile compounds emitted by the insects, see below) were separated by sex and stored in two plastic containers with pieces of fresh oak branches and slips of paper soaked in honey water. Since females and males of *X. antilope* have nearly identical external morphology, to sex the beetles, we put two individuals (from the group of ~ 25 individuals) together in a small container and observed if the animals attempted to copulate. In most cases, when a male and female were put together, copulation was initiated immediately after the male first touched the female with his antennae. If the beetles did not make physical contact after ~ 0.5 min, or if they made physical contact but did not start to copulate, we excluded the animals and continued with a new pair of individuals. The containers with males and females (about ten individuals of each sex) were stored in a refrigerator before headspace collections (see below).

Collection of volatiles

We performed headspace sampling using the same approach and equipment for both study species. The beetles, in their containers with males and females separated, were transferred from the refrigerator to a climate-controlled chamber (25 °C constant temperature, light 8:30 AM–9 PM, 65% relative humidity) in the afternoon of the day before aerations were to commence. The next morning, 5–8 individuals of each sex were picked randomly and placed in two 1 L gas washing bottles (males and females in separate bottles) (Lenz Laborglas Gmbh, Wertheim, Germany). An identical, but empty bottle was used as a blank control. Strips of a fine metal mesh (previously rinsed with ethanol and acetone) were inserted into the bottles to increase the surface area over which the beetles were distributed to reduce the risk of aggression. The bottles were then transferred to a second climate chamber (but with the same settings), and headspace sampling was initiated.

Detailed specifications of the materials and techniques used for the headspace sampling can be found in Molander and Larsson (2018). Briefly, collectors consisted of Porapak™ Q adsorbent, enclosed in Teflon® tubing, and attached with Teflon® thread-sealing tape to one of the two top openings of each glass bottle. An identical collector was attached to the second opening of each bottle to remove volatiles from the incoming air. Polyvinyl chloride (PVC) tubing, plastic connectors, and Teflon® tape were used to connect the three collectors to a single air pump. A constant flow rate of 0.25 L min^{-1} through each collector was established using a triple air flow meter and plastic valves on the PVC tubing. Two sets of glass bottles and several sets of collectors were alternated between different aerations, to avoid systematic errors. In between aerations, the bottles were rinsed with ethanol followed by acetone, and occasionally placed in an oven (400 °C) overnight.

Aerations lasted for about 5–6 h (~ 10 AM to 4 PM) because both species are diurnal with maximal activity in the late morning to early afternoon under field conditions (MAM, pers. obs.). In between aerations, the beetles were released back into the containers with fresh wood where they mixed with other same-sex individuals. Thus, most collections were carried out with mixtures of new and previously aerated individuals. After headspace collections had been initiated, the beetles were stored in the original climate chamber in between aerations (or in the refrigerator if aerations were not performed on consecutive days). A total of 4 headspace collection sessions were conducted with *A. mysticus* and 7 with *X. antilope*, with each session consisting of a collection from males, females, and a blank control sample.

Immediately after aeration, collectors were extracted with 300 µl of hexane, then washed with an additional 3 × 300 µl of hexane followed by 3 × 300 µl of acetone, before reuse. The extracts, in vials with closed butyl/PTFE seal screw caps (articles 11,090,210 and 08,151,653, Skandinaviska Genetec AB, Stockholm, Sweden), were kept in a freezer (− 18 °C) until analysis by gas chromatography–mass spectrometry (GC–MS; see below).

Identification of compounds

Extracts of volatiles from both species were analyzed with the same methods and GC–MS instruments. At the Alnarp campus, a 6890N model GC interfaced to a 5975 mass selective detector (Agilent Technologies, Palo Alto, CA, USA) was used, with the transfer line temperature set at 150 °C. The GC was fitted with an HP-5ms capillary column (95% polydimethylsiloxane/5% phenylmethylsiloxane, 60 m × 0.25 mm inner diam., 0.25 µm film thickness, Agilent Technologies). Injections of 2 µl of each aeration sample were made manually in splitless mode (split vent opened after 0.5 min, injector temperature 225 °C). Control injections of 2 µl of hexane were performed before and in between analyses of headspace samples. The carrier gas was helium with a constant flow rate of 1.8 ml min^{-1} (inlet pressure 172 kPa). The oven program started at 30 °C, with a 3 min hold, thereafter rising by 8 °C min^{-1} to 260 °C, with a 10 min hold. We also analyzed the extracts on a second GC–MS (GC model 7890B and MS model 5977Agilent Technologies) equipped with a DB-WAX capillary column (polyethylene glycol, 60 m × 0.25 mm inner diam., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Injections, carrier gas, and settings were identical to those used with the HP-5ms column, except that the flow rate was 1.9 ml min^{-1}, and the final temperature was 230 °C. The mass spectrometers were set with a solvent delay of 7 min. Spectra were taken in electron impact ionization (EI) mode at 70 eV, with a scanning range of 29–400 *m/z*.

Calculations of the relative proportions of potential pheromone compounds (see "Results") were based on the results from the DB-WAX column for *A. mysticus* because the trace components separated better on this column, and the HP-5ms column for *X. antilope*. Due to the temperatures used during analyses, we observed that one of the main pheromone components, 3-hydroxy-2-hexanone, was partially rearranged into 2-hydroxy-3-hexanone on both columns, with peaks partially separated on the DB-WAX column. We therefore pooled the peak areas of the two isomers from the DB-WAX chromatogram to achieve a combined estimate of the relative quantities of pheromone compounds produced by *A. mysticus*. When extracts were analyzed at lower temperatures on a chiral stationary phase GC column (see below), essentially a single peak corresponding to 3-hydroxy-2-hexanone was observed.

Extracts of volatiles were also analyzed at UC Riverside by GC–MS using an Agilent 7820A GC interfaced to a 5977E mass selective detector, and an autosampler. The GC was equipped with a DB-5 column (95% polydimethylsiloxane/5% phenylmethylsiloxane, 30 m × 0.25 mm inner diam., 0.25 µm film thickness; J&W Scientific). One µl injections were made in splitless mode (injector 250 °C, transfer line 280 °C, split vent opened after 0.5 min), with helium carrier gas (inlet pressure 89.6 kPa, linear velocity 34 cm/sec). The oven was programmed from 40 °C for 1 min, 10 °C min^{-1} to 280 °C, hold 20 min. Mass spectra were taken in EI mode at 70 eV, scanning a mass range from 40–450 *m/z*, with a 3 min solvent delay.

The chromatograms from extracts of males, females, and controls were compared visually with Agilent ChemStation software (version E.02.02.1431) using the overlay function. The observed sex-specific peaks were first tentatively identified by matching their mass spectra to reference spectra in the Wiley (10th edition) and NIST (NIST 14) commercial mass spectral databases where possible, and/or to reference spectra from previous work with cerambycid semiochemicals. The identities were then confirmed by comparing mass spectra and retention times to those of authentic standards.

The absolute configurations of the insect-produced compounds were determined by analyses of aliquots of the extracts at UC Riverside using a GC equipped with a chiral stationary phase Cyclodex B GC column (10.5% β-cyclodextrin in DB-1701, 30 m × 0.25 mm inner diam., 0.25 µm film thickness, J&W Scientific), with an injector temperature of 150 °C to minimize isomerization of the thermally labile hydroxyketones. Injections were made in split mode (split ratio ~ 20:1) at 172 kPa inlet pressure, with a temperature program of 50 °C/1 min, 3 °C/min to 220 °C, hold 20 min. The GC was equipped with a flame ionization detector (detector temperature 250 °C), and chromatograms were processed with Peak Simple software (SRI Instruments, Torrance, CA, USA). Authentic standards were analyzed using identical parameters, and the identification of the insect-produced enantiomer of each hydroxyketone was verified by coinjection of a blend of the insect extracts with the racemic standards, determining which of the two peaks in each racemate was enhanced.

Sources of chemicals

Racemic 3-hydroxy-2-hexanone (CAS number: 54123-75-0) was purchased from Bedoukian Research (Danbury, CT, USA). Standards of 2-nonanone (99% purity, CAS: 821-55-6), 2-heptanone (99%, CAS: 110-43-0), 2-undecanone (99%, CAS: 112-12-9) and 2-nonanol (99%, CAS: 628-99-9) were obtained from Sigma-Aldrich (Hamburg, Germany), and 2,3-hexanedione (90%, CAS: 3848-24-6) from Aldrich Chemical Co. (Milwaukee, WI, USA). Racemic 2-hydroxy-3-octanone was synthesized from racemic lactic acid methyl ester (TCI America, Portland, OR, USA), and (*S*)-2-hydroxy-3-octanone was synthesized from (*S*)-lactic acid methyl ester, using methods previously described by Hall et al. (2006). An authentic standard of 2,3-octanedione was produced by oxidation of a few mg of (*S*)-2-hydroxy-3-octanone with pyridinium dichromate in dichloromethane.

Field bioassays

The biological activity of the identified compounds was examined in three separate field bioassays. For *A. mysticus*, we used racemic 3-hydroxy-2-hexanone and 2-nonanone because pure (*R*)-3-hydroxy-2-hexanone was not available when the bioassays were conducted. For *X. antilope*, racemic 2-hydroxy-3-octanone was used in 2015, whereas (*S*)-2-hydroxy-3-octanone was used in 2016. We choose to exclude

2,3-hexanedione and 2,3-octanedione from our bioassays because these compounds are likely to be artefacts from degradation of the corresponding ketols (Millar and Hanks 2017). In addition, we did not test a number of trace components (see "Results") in the extracts of male *A. mysticus*.

Five treatments were used in the bioassay with *A. mysticus* in 2017, including racemic 3-hydroxy-2-hexanone and 2-nonanone as single components at 100 mg and 100 mg per ml in isopropanol, respectively, and three blends with variable quantities of 2-nonanone to that of the racemic hydroxyketone (20:100, 50:100, and 80:100 mg/ml respectively, in isopropanol). Thus, the resulting proportions of 2-nonanone to the insect-produced (*R*)-enantiomer of 3-hydroxy-2-hexanone were 40:100, 100:100, and 160:100. Polyethylene Grippie® zip-lock bags (6.5 cm × 5.5 × 40 µm, Grippie Light Nr-02, b.n.t. Scandinavia AB, Arlöv, Sweden) were used as dispensers. At the field sites, 0.5 ml of each treatment was loaded into each bag, and the bag was attached with wire to the south-facing side of the trap without puncturing the bag. A detailed description of the custom-built flight-intercept traps that were used in all bioassays can be found in Molander et al. (2019). The traps had two cross-vane panels mounted atop a funnel that emptied into a collecting jar, and a top cover for rainwater protection. The panels and the inside of the funnel were coated with Fluon® (polytetrafluoroethylene dispersion, 60 wt% in H_2O, Sigma-Aldrich, St. Louis, Missouri, USA, further diluted 1:1 with water) to increase trap efficiency (see, e.g., Graham and Poland 2012). Each trap contained ~ 0.25 L propylene glycol as a preservative and killing agent.

Six replicates (sites) were used in the bioassay with *A. mysticus*, each consisting of one trap with each of the five treatments and a control trap (with a lure of 0.5 ml of isopropanol alone). The traps were deployed in linear transects at six sites in the Mittland forest on April 29 and positioned in glades or along sunny forest edges with large amounts of hazel in the immediate vicinity. Replicates were separated by a minimum of 300 m and posts of reinforcement steel were used to hang the traps in a row with 10 m between traps. The center of each trap, and the location of the pheromone lure, was ~ 1.5 m above ground. All replicates were checked three times (May 21, June 10, and July 2) resulting in three sampling periods which covered the main activity period of the species in southeastern Sweden (Lindhe et al. 2010).

For *X. antilope*, racemic 2-hydroxy-3-octanone was tested at 100 mg/ml of isopropanol (2015), whereas (*S*)-2-hydroxy-3-octanone was tested at 50 mg/ml (2016). Consistent with *A. mysticus*, Grippie® zip-lock bags were loaded with 0.5 ml of test solutions and control lures were loaded with 0.5 ml of isopropanol alone. In 2015, four replicates (sites) were deployed in Ecopark Hornsö, separated by 600 m or more. The traps were suspended from protruding oak branches at a height of ~ 1.5–2 m and the traps within each replicate were separated by ~ 10 to 20 m. Two sampling periods were used. For the first sampling period, three replicates were deployed on May 29 and the fourth replicate on May 30. Three replicates were checked on June 24 and the fourth replicate on June 25. The second sampling period ended on July 26 when the traps were emptied a second time and taken down. Late July marks the end of the species' main activity period in Sweden (Lindhe et al. 2010). In 2016, when pure (*S*)-2-hydroxy-3-octanone was tested, five replicates were deployed in the Ecopark. Most sites were not the same as in 2015 and sites were separated by at least 1.1 km. In 2016, traps were hung from steel posts (see above) and spaced 10 m apart within each replicate. Only a single sampling period was used with all replicates deployed on June 5. Two replicates were checked on June 28 and three replicates on June 30.

In all three bioassays, the positions of the lures were randomized within the replicates at deployment, and each replicate for *A. mysticus* was re-randomized after the first and second sampling periods. For *X. antilope*, we switched the position of the treatment and the control after the first sampling period in 2015. Tea filters were used to separate the insects from the propylene glycol. The glycol was reused, while the filters with the insects were saved in plastic bags with details on site, date, lure, etc. recorded on each bag. After each sampling period, new pheromone lures were added to all traps by attaching a new Grippie® zip-lock bag.

Furthermore, in 2017 we undertook a large-scale landscape study (unpublished) of *X. antilope* and other longhorn beetles at 68 sites in southern Sweden that consisted of forests and woodlands dominated by mature oak trees (mainly *Q. robur*) (for locations see Fig. 5 and Online Resource 1, Table S3). At each site, we used three traps with (*S*)-2-hydroxy-3-octanone (50 mg/ml isopropanol) and three traps with a blend of heterospecific cerambycid pheromone compounds consisting of racemates of 3-hydroxy-2-hexanone (100 mg/ml isopropanol; from Bedoukian Research, see above), 3-hydroxy-2-decanone [20 mg/ml; synthesized as described in Allison et al. (2012)] and 3-hydroxy-2-octanone [10 mg/ml; made as described in Imrei et al. (2013)]. Traps (of the same type as used in the bioassays and hung from steel posts at all sites with polypropylene glycol as a preservative) were spaced ~ 10 m apart and lure treatments were assigned randomly. Lure solutions (0.5 ml/lure) were loaded into Grippie® zip-lock bags as above. All traps were deployed during the peak of the activity period of *X. antilope* (7–12 June), and emptied once (28 June–3 July) when trapping was terminated. The specimens of *X. antilope* that were captured in this study were used to compare the trapping efficiency of the species' own pheromone to that of the blend of heterospecific pheromone compounds.

The numbers of *A. mysticus* and *X. antilope* per sample were counted and the sex ratio determined in a subset of samples (see "Results") from the bioassays. The key by

Ehnström and Holmer (2007) was used to identify specimens. For *A. mysticus*, the sex was mainly determined by the relative length of the antennae (see above), but ~ 15 specimens were also dissected to confirm that the character was reliable. For *X. antilope*, every beetle in the subset was dissected and the genitalia were studied to confirm the sex. The trapped beetles are preserved in 70% ethanol and voucher specimens will be deposited in the public Lund entomological collections (Biological Museum, Lund University, Sweden).

Statistical analysis

To analyze trap catches of *A. mysticus*, the effects of the five different lure treatments were compared to the control in a Generalized Linear Mixed Model (GLMM) using a Poisson distribution and a $\log_e$ link function. The model compared how the number of captured *A. mysticus* (response variable) depended on lure treatment (fixed factor) and was set up with replicate (six sites) and sampling period (three trap counts) as random effects to control for dependencies. The model was checked for over- and underdispersion using Pearson residuals. The resulting dispersion statistic ($\varphi = 1.24$) was deemed acceptable (cf. Hilbe 2011), with no need for model corrections. The different treatments and the control were then compared by multiple pairwise post hoc tests (*Tukey's HSD test*), using least squares means with the package emmeans (Lenth 2018). Flight activity of *A. mysticus* was low during the first sampling period (early May) and four replicates where no beetles were captured during this sampling period were omitted from the analyses (and Fig. 3).

GLMMs with Poisson distributions were also used to compare the treatment with the control for each of the two bioassays with *X. antilope*. A $\log_e$ link function was used for the 2015 dataset, but exchanged for a square root link function in the 2016 dataset, because no beetles were caught in any control traps. For the 2015 dataset, replicate (four sites) and sampling period (two trap counts) were used as random effects, while only replicate (five sites) was used for the 2016 dataset. Dispersion statistics were calculated for both models using Pearson residuals and deemed negligible ($\varphi = 1.05$ for 2015, $\varphi = 1.17$ for 2016). Finally, a GLMM with Poisson distribution and $\log_e$ link function was used to compare captures of *X. antilope* baited with the species' own pheromone versus traps baited with the blend of heterospecific cerambycid compounds in the 2017 landscape study, with site as a random effect. Pearson residuals were used to calculate the models' dispersion statistic, which was satisfactory ($\varphi = 1.23$).

Statistical significance was defined as probability values (P), or adjusted probability values (P_a) for post hoc tests, of less than 0.05. All statistical analyses were performed in R version 3.5.1 for Windows (R Core Team 2018) with the lme4 package for GLMMs (Bates et al. 2015).

Results

Identification of insect-produced compounds

Analysis by gas chromatography–mass spectrometry showed that a total of nine insect-produced compounds were consistently present in, and specific to, all four extracts from male beetles of *A. mysticus* (Fig. 1). The two compounds that were present in the highest mean proportions were identified as 3-hydroxy-2-hexanone and 2-nonanone (Table 1), and the absolute configuration of the 3-hydroxy-2-hexanone was determined to be (*R*). In three extracts, 2-nonanone was present in lower quantities than (*R*)-3-hydroxy-2-hexanone, while one sample contained essentially equal quantities, indicating that (*R*)-3-hydroxy-2-hexanone was the major component. Further, 2,3-hexanedione was present in low amounts in all extracts from males. Only trace quantities of the remaining 5–6 male-specific compounds were observed (depending on which GC column was used). The three trace compounds with the highest mean proportions relative to the major component were identified as 2-heptanone, 2-undecanone, and 2-nonanol (Table 1). The remaining three trace compounds occurred in even lower amounts, and were not identified.

For *X. antilope*, two compounds were present in all seven extracts from male beetles, but missing in all corresponding extracts from females and blank controls (Fig. 2). The two compounds were identified as (*S*)-2-hydroxy-3-octanone and 2,3-octanedione, with (*S*)-2-hydroxy-3-octanone being the major component (Table 1). No other male-specific compounds were detected in extracts from either of the two species, no female-specific compounds were detected, and no compounds were consistently detected in extracts from both males and females of either species, but missing in the blank controls.

Field bioassays

A total of 121 individuals of *A. mysticus* were captured in the field bioassay. From the GLMM analysis, all three blends of racemic 3-hydroxy-2-hexanone and 2-nonanone attracted significantly more *A. mysticus* than the control, whereas the numbers of beetles attracted to the individual compounds were not significantly different from the control (Online Resource 1, Table S4). In the pairwise post hoc comparisons, the two blends with higher proportions of 2-nonanone to the (*R*)-enantiomer of the hydroxyketone (100:100 and 160:100, respectively), attracted significantly more *A. mysticus* than the control and the individual compounds (Fig. 3,

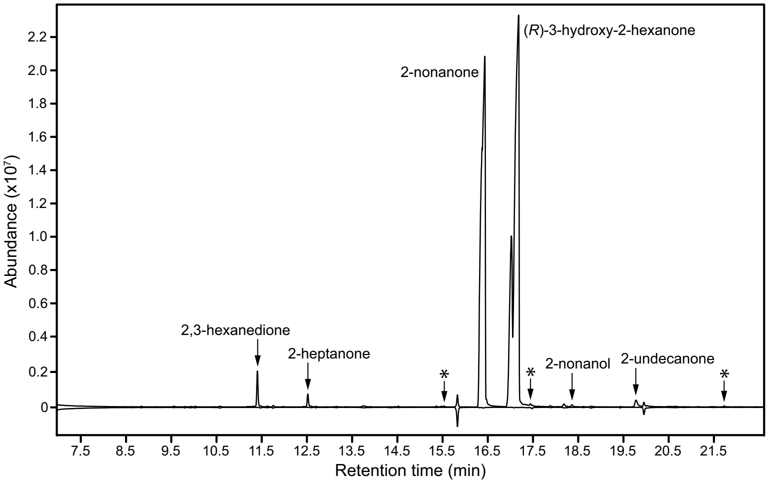


Fig. 1 Representative total ion chromatograms (DB-WAX column) of headspace volatiles collected from adult male (top trace) and female *A. mysticus* (bottom, inverted trace). Two compounds (*R*)-3-hydroxy-2-hexanone and 2-nonanone, were consistently present in large quantities in samples from males. In addition, minor quantities of 2,3-hexanedione and six trace compounds were also repeatedly found in samples from males. The three most abundant trace compounds were 2-heptanone, 2-nonanol, and 2-undecanone, whereas those present in even lower quantities were not identified (asterisks). Due to thermal rearrangement, the insect-produced (*R*)-3-hydroxy-2-hexanone was in part rearranged into 2-hydroxy-3-hexanone, with the latter compound forming a partially separate peak in front of (*R*)-3-hydroxy-2-hexanone

Table 1 Overview of compounds consistently present and specific to the extracts of volatiles from male beetles of *A. mysticus* and *X. antilope*

Species	Compound	Mean proportion (± SE)
A. mysticus	(*R*)-3-hydroxy-2-hexanone	100
	2-nonanone	70.0 (± 11.0)
	2,3-hexanedione	4.0 (± 1.0)
	2-heptanone	1.2 (±0.5)
	2-undecanone	0.7 (±0.1)
	2-nonanol	0.3 (±0.2)
X. antilope	(*S*)-2-hydroxy-3-octanone	100
	2,3-octanedione	4.0 (± 1.1)

The mean proportion of the less-abundant compounds is expressed relative to the main pheromone component of each species (100); (*R*)-3-hydroxy-2-hexanone and (*S*)-2-hydroxy-2-octanone, respectively. There were four extracts from *A. mysticus* males and seven from *X. antilope* males

Online Resource 1, Table S5). The sex ratio among individuals captured with the 160:100 blend was 40% males and 60% females (total of 45 individuals).

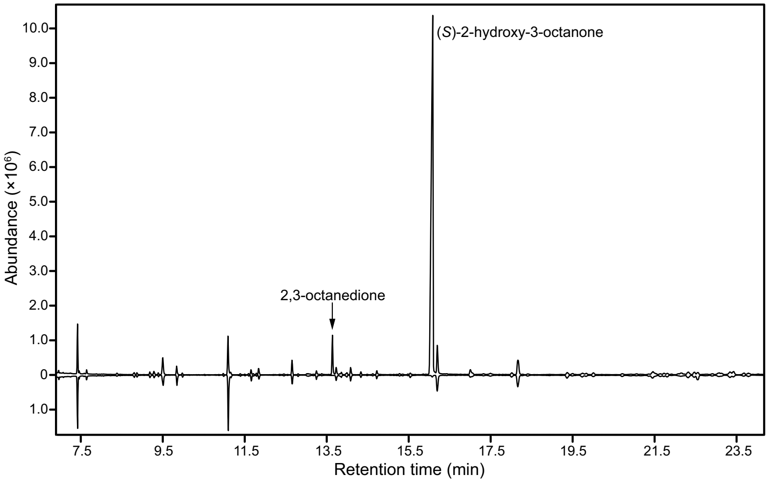
In 2015, racemic 2-hydroxy-3-octanone attracted significantly more *X. antilope* (total 43 individuals) than the control (total of 2 individuals) (Fig. 4a, Online Resource 1, Table S6), and pure (*S*)-2-hydroxy-3-octanone was significantly more attractive (total of 44 individuals) than the control (no captured beetles) in 2016 (Fig. 4b, Online Resource 1, Table S6). The sex ratio in 2016 was 52% males and 48% females.

Attraction of *X. antilope* also differed between the lures in the 2017 landscape study (Online Resource 1, Table S6). The traps with (*S*)-2-hydroxy-3-octanone captured a total of 1193 *X. antilope* (5.85 ± 0.44 beetles/trap, mean ± SE), which was significantly more than the total 162 individuals (0.79 ± 0.10 beetles/trap) in the traps with the blend of common heterospecific pheromone components. The traps with lures of the species' own pheromone detected *X. antilope* at 55 of the 68 surveyed sites, while traps with the blend of heterospecific compounds captured the species at 41 sites (Fig. 5).

Discussion

The consistent presence of high quantities of (*R*)-3-hydroxy-2-hexanone and 2-nonanone in the headspace extracts from male *A. mysticus*, and high quantities of (*S*)-2-hydroxy-3-octanone in the extracts from male *X. antilope*, paired with the significant attraction of females and males of both species to the synthetic compounds tested in bioassays, demonstrate that these compounds function as male-produced aggregation-sex pheromones in these species. In *A. mysticus*,

Fig. 2 Total ion chromatograms (HP-5ms column) of headspace volatiles collected from adult male (top trace) and female *X. antilope* (bottom inverted trace). Two sex-specific compounds (*S*)-2-hydroxy-3-octanone and 2,3-octanedione, were consistently present in extracts from male beetles



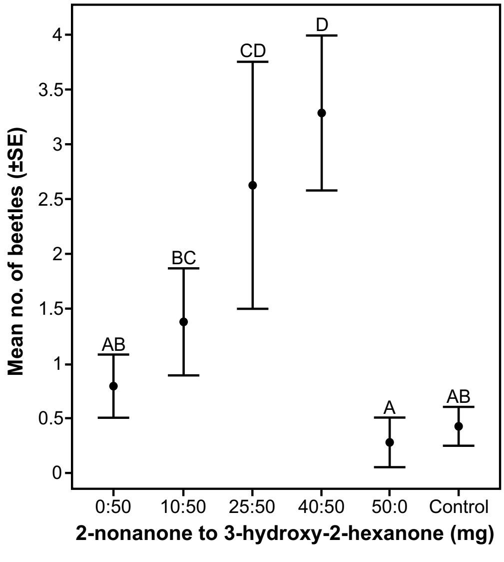


Fig. 3 Mean (± SE) numbers of *A. mysticus* captured per trap and sampling period with different quantities of 2-nonanone and racemic 3-hydroxy-2-hexanone formulated in 0.5 ml isopropanol ($n = 14$ samples). Two treatments tested the compounds as single components (50 mg racemic 3-hydroxy-2-hexanone and 50 mg 2-nonanone per lure, respectively), while three treatments consisted of blends with a variable quantity of 2-nonanone to the hydroxyketone's (*R*)-enantiomer (proportions 40:100, 100:100, and 160:100, respectively). The control was 0.5 ml isopropanol alone. Mean values that do not share a common letter are significantly different ($P < 0.05$)

the individual compounds were not attractive, but the two compounds acted synergistically when tested in blends with proportions of 100:100 or 160:100 of 2-nonanone to the naturally produced (*R*)-enantiomer of the hydroxyketone. In contrast, the blend with a lower proportion of 2-nonanone (40:100) appeared to be less attractive, indicating that both the presence of the two components and their relative proportions were important, analogous to what is seen in pheromone blends of other insects (e.g., moths; Löfstedt and Herrebout 1988).

Anaglyptus mysticus is the second cerambycid, and the first species of the *Anaglyptus* genus, reported to use 2-nonanone as a pheromone component, but the North American species *Cyrtophorus verrucosus* (Olivier, 1795) produces a blend of the same components as *A. mysticus* (Mitchell et al. 2013). Thus, these species represent another example of the general trend of pheromone compounds being conserved among related species of longhorn beetles, even when the species may have been spatially separated for millennia (Millar and Hanks 2017). The two genera belong to the same tribe (Anaglyptini), and the morphology of the two species is also similar; both species mimic the black and reddish brown coloration of several species of common wood ants (*Formica* spp. and *Camponotus* spp.).

Both racemic and pure (*S*)-2-hydroxy-3-octanone as a single compound proved attractive to *X. antilope*. However, the pheromone chemistry of *X. antilope* was first studied by Schröder (1996), who reported three male-specific compounds. As in our study (*S*)-2-hydroxy-3-octanone was the major component, but Schröder (1996) also reported lesser quantities of (*R*)- and (*S*)-3-hydroxy-2-octanone, as well as trace quantities of the diastereomers of 2,3-octanediol (Schröder 1996). We did not observe these other compounds

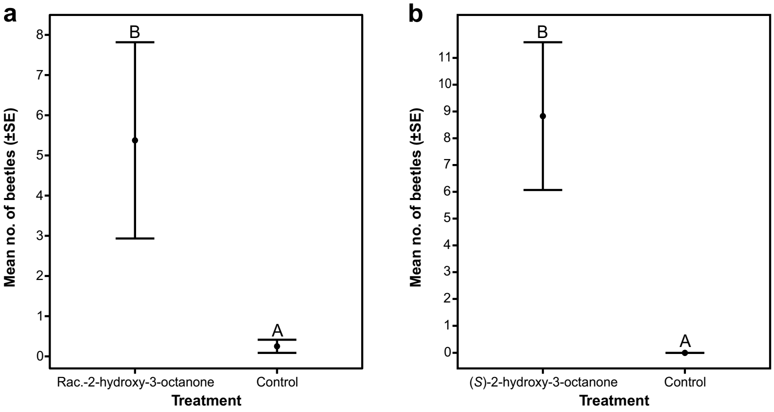


Fig. 4 **a** Mean (± SE) numbers of *X. antilope* captured per trap and sampling period with lures of racemic 2-hydroxy-3-octanone (50 mg per lure) in 0.5 ml isopropanol ($n = 8$ samples). Controls were baited with 0.5 ml isopropanol alone. Mean values that do not share a common letter are significantly different ($P < 0.05$); **b** Mean (± SE) numbers of *X. antilope* captured per trap and sampling period with (*S*)-2-hydroxy-3-octanone (25 mg per lure) in 0.5 ml isopropanol ($n = 5$ samples). Controls were baited with 0.5 ml isopropanol alone. Mean values that do not share a common letter are significantly different ($P < 0.05$)

in our extracts. It is possible that the (*R*)- and (*S*)-3-hydroxy-2-octanone were artefacts generated by thermally induced isomerization of (*S*)-2-hydroxy-3-octanone, which is known to occur during GC analysis (Leal et al. 1995). Nevertheless, our bioassays showed that (*S*)-2-hydroxy-3-octanone was attractive as a single component, suggesting that the other components reported by Schröder (1996) are not essential to elicit significant attraction.

Considerable numbers of *X. antilope* had been captured previously in two studies that utilized ''multi-lures'' consisting of a blend of common cerambycid pheromones including 3-hydroxy-2-hexanone, 3-hydroxy-2-octanone (both racemic) and *syn*-2,3-hexanediols (Flaherty et al. 2018; Rassati et al. 2018). A direct comparison to our study is not possible, but the blend used by Flaherty et al. (2018) and Rassati et al. (2018) was similar to the blend that we used in parallel to the specific pheromone of *X. antilope* in our 2017 landscape study. As in the studies by Flaherty et al. (2018) and Rassati et al. (2018), we also captured a large number of *X. antilope* with the blend of heterospecific pheromone compounds (total of 162 individuals), but the species-specific pheromone proved significantly more attractive, attracting 7.4 times more beetles (1193 individuals). More importantly, the heterospecific blend failed to detect *X. antilope* at 14 (26%) of the 55 sites where the species was captured with its own pheromone (Fig. 5). Cross-attraction to the pheromone components of heterospecifics is common in the subfamily Cerambycinae, and the pheromones of other species could function as cues signaling the location of suitable substrates for species with similar host requirements (Hanks et al. 2007; Molander and Larsson 2018). However, using the species' specific aggregation-sex pheromone, rather than more generic attractants, can clearly be important to maximize sensitivity during surveys, particularly at low population densities (cf. Svensson et al. 2012). Further, 11 of the 13 sites in the landscape study where no *X. antilope* were captured are situated well outside of the species' current known distribution in Sweden (Fig. 5) (Lindhe et al. 2010; Swedish Species Observation System 2018b). Consequently, traps baited with its own pheromone detected *X. antilope* at 97% of the surveyed sites that were located within its distribution, and our results extended the species' distribution further south than what was previously known.

With their pheromones identified, *A. mysticus* and *X. antilope* can be added to the small but expanding group of rare and threatened cerambycids that can be monitored with aggregation-sex pheromones. Our survey of *X. antilope* at a large number of sites in the landscape study demonstrated a high rate of detection, showing much promise for future studies. Further, the major components of the pheromones of *A. mysticus* and *X. antilope* are the same as the two-component aggregation-sex pheromone of *Plagionotus detritus* ssp. *detritus* (Linnaeus, 1758), a cerambycid dependent on fresh, coarse oak wood that is highly threatened in parts of Europe (Molander et al. 2019). The fact that compounds are partially shared by multiple rare species of cerambycids is a great advantage because it may be possible to sample multiple target species simultaneously with the same lures and traps, reducing labor and other monitoring costs. However, blends of compounds should first be tested for

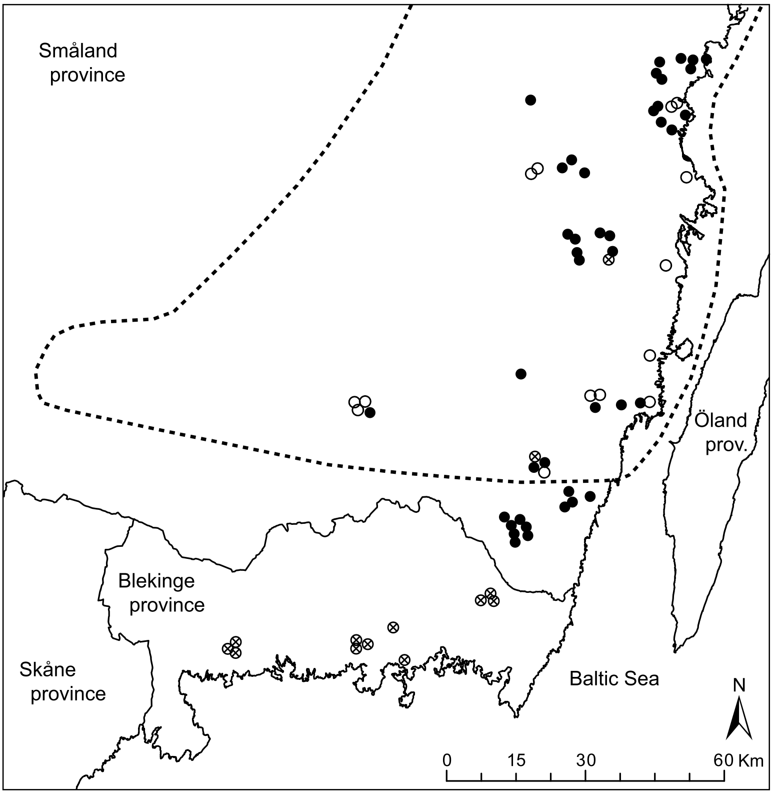


Fig. 5 Map of southeastern Sweden showing the location of 68 sites in a pheromone-based landscape survey of longhorn beetles in 2017, and the approximate southern extent of the distribution of *X. antilope* [hatched line, based on reported records 1700–2016 in Lindhe et al. (2010) and the Swedish Species Observation System (2018b)]. At each site, three traps with the species' specific pheromone (*S*)-2-hydroxy-3-octanone (25 mg per lure) were deployed, and three traps with a blend of common heterospecific cerambycid pheromone components as racemates (50 mg 3-hydroxy-2-hexanone, 10 mg 3-hydroxy-2-decanone, and 5 mg 3-hydroxy-2-octanone per lure). Filled circles denote sites where the species was detected by traps with both types of pheromone lures, unfilled circles denote sites where the species was detected only by traps with lures of the species' specific pheromone, but not in traps with the heterospecific blend. Circles with a cross denote sites where the species was not captured by any trap. Due to the large scale, the location of certain sites is shown with a minor offset (<2.0 km) to enhance visibility. Map: GSD-Översiktskartan, vector© Lantmäteriet, Gävle, Sweden

antagonistic effects among species, because the compounds of one species occasionally can inhibit responses of other species (Millar and Hanks 2017; Hanks et al. 2019). The minor component (2-nonanone) for *A. mysticus* might be a relatively species-specific compound, at least compared to (*R*)-3-hydroxy-2-hexanone and (*S*)-2-hydroxy-3-octanone, but this component is commercially available at low cost. A portfolio of species with different substrate requirements that can be monitored cost-efficiently with pheromones, could be highly beneficial in designing robust ecological studies that examine, for instance, the effects of different forest management practices on biodiversity. Such studies are needed given the rapid environmental changes that are taking place in European forests, such as the intensifying harvest of logging residues (wood substrates) as biofuel, a management practice that removes large quantities of suitable substrates for many longhorn beetles, including the focal species in this study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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In this thesis, I identified the aggregation-sex pheromones of three rare saproxylic beetles. In an effort to determine the effects of forest management regimes, they and other threatened beetles were then surveyed in large-scale pheromone-based monitoring schemes across their Swedish distribution ranges. My results reveal large variability among the studied species in their responses to modern forestry practices, seemingly dependent on differences in their spatiotemporal ecology in relation to dead-wood substrate dynamics.

Björn Eriksson completed his graduate education at the Department of Plant Protection Biology, SLU, Alnarp, and received his Master of Science in Ecology and the Environment from Linköping University, Sweden.

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