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Revealing hidden species distribution with pheromones: the case of *Synanthedon vespiformis* (Lepidoptera: Sesiidae) in Sweden.

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MCL, LW, PM, NR & KOB conceived and designed the study. JB, MCL, IW, SO and FN collected most of the data. LW, JB, PM, SO & MCL analyzed the data. JB, MCL, NR, FN & IW contributed reagents/materials/analysis tools. JB, PM & MCL wrote the paper with assistance from all other coauthors.

Abstract

Synanthedon vespiformis L. (Lepidoptera: Sesiidae) is considered a rare insect in Sweden, discovered in 1860, with only a few observations recorded until a sex pheromone attractant became available recently. This study details a national survey conducted using pheromones as a sampling method for this species. Through pheromone trapping we captured 439 specimens in Southern Sweden at 77 sites, almost tripling the number of previously reported records for this species. The results suggest that *S. vespiformis* is truly a rare species with a genuinely scattered distribution, but can be locally abundant. Habitat analyses were conducted in order to test the relationship between habitat quality and the number of individuals caught. In Sweden, *S. vespiformis* is thought to be associated with oak hosts, but our attempts to predict its occurrence by the abundance of oaks yielded no significant relationships. We therefore suggest that sampling bias and limited knowledge on distribution may have led to the assumption that this species is primarily reliant on oaks in the northern part of its range, whereas it may in fact be polyphagous, similar to *S. vespiformis* found as an agricultural pest in Central and Southern Europe. We conclude that pheromones can massively enhance sampling potential for this and other rare Lepidopteran species. Large-scale pheromone-based surveys provide a snapshot of true presences and absences across a considerable part of a species national distribution range, and thus for the first time provide a viable means of systematically assessing changes in distribution over time with high spatiotemporal resolution.

Keywords: Ecology, saproxylic, moth, indicator of species richness, conservation, monitoring.

Introduction

Woodland habitats have undergone significant anthropogenic change in recent centuries, giving way to land use focused on agriculture and housing development (Eliasson & Nilsson, 2002). As well as habitat fragmentation, habitat alteration has also been an issue; whereby open, sunny woodland habitats have been transformed into shady, overgrown habitats less suitable for species dependent on sunlight (Kirby *et al.*, 2005). One such light-dependent species associated with oak woodland habitat and the focus of this paper is the clearwing moth *Synanthedon vespiformis* (L.) (Lepidoptera: Sesiidae), first recorded in Sweden in 1860 (Eliasson, 2007). This species is classified as Vulnerable on the Swedish Red List (Gärdenfors, 2010) and considered 'Nationally Scarce' in the United Kingdom (Greatorex-Davies *et al.*, 2003). The moth could potentially serve as a good indicator for the increasingly rare open woodland habitats. The location and distribution of clearwing moth populations are however difficult to assess, as the species of this family are relatively inconspicuous and frequently mistaken for members of the hymenoptera due to their mimicry (Duckworth & Eichlin, 1974). As a result, knowledge of their true distribution and ecology remains relatively limited.

S. vespiformis is a particularly interesting example of a saproxylic clearwing, as its habitat preferences seems to vary significantly across its geographical range in Europe. In the southern part of its range it is thought to be fairly polyphagous and is in fact considered a pest, attacking numerous tree species including beech (*Fagus silvatica* L.), oak (*Quercus* spp.), poplar (*Populus* spp.), willow (*Salix* spp.) and fruit crops of blackberry (*Rubus fruticosus* L.), raspberry (*Rubus idaeus* L.) and peach (*Prunus persica* L.) (Szántóné-Veszelka *et al.*, 2010). However, in the northern part of its range where the species is considered threatened and does not occur as an agricultural pest, the primary host of the larvae is assumed to be *Quercus robur* L. (pedunculate oak) with some suggestions of members of the Rosaceae and *Betula* spp. as secondary hosts (Eliasson, 2002; Waring & Townsend, 2003). However, the evidence is predominantly anecdotal or based on a small number of observations. Thus a better understanding of its ecology will aid in conservation of this species, particularly in places where it is considered to be in decline. These conservation efforts first require effective and accurate sampling and monitoring methods of the target species, something which has been identified as lacking in modern biodiversity management (Rademaekers *et al.*, 2010).

Identifying, protecting and monitoring key areas or habitat types that support a high number of rare or threatened species is essential in conservation (Henle *et al.*, 2013). However, broad systematic surveys are expensive, time-consuming and often reliant on a small number of taxonomic experts (e.g. Horak & Pavlicek 2013). Saproxylic habitats in particular are significantly more difficult and expensive to monitor using standard methods, with the number of site visits for establishing species assemblage being at least twice that of equivalent grassland habitats (pers. comm. David Heaver, Natural England). Thus for determining species distribution, systematic surveys of this nature risk generating expensive data of relatively low accuracy and precision.

As a result, bioindicator species are often used as a proxy in place of complete surveys in order to locate sites with high species richness and/or conservation value (Fleishman & Murphy, 2009). The most commonly used indicators of terrestrial biodiversity are butterflies, grasshoppers and wild bees, mainly for open environments like grasslands (Rosenberg *et al.*, 1986; Nilsson *et al.*, 1995; Bazelet & Samways, 2011, 2012; Bommarco *et al.*, 2012; Gerlach *et al.*, 2013). Bioindicator selection for assessing the quality of woodland or forest habitats is however heavily skewed towards saproxylic beetles due to their prevalence (Grove, 2002), meanwhile Lepidoptera are under-represented in these habitats despite their potential for assessing human impacts on biodiversity (Fiedler & Schultze, 2004). Some woodland moths are rather inconspicuous and are often under-reported in survey data despite being sampled regularly (Quinto *et al.*, 2013; Jonason *et al.*, 2013, 2014), possibly due to their predominantly nocturnal behavior, and sampling bias from recorders (Dennis & Thomas, 2000). Thus an effective method of standardized recording for these potential bioindicators is highly desirable.

In order to provide greater levels of accuracy and sampling power at a lower cost, insect pheromones have been suggested as a supplement to existing sampling methods for insects (Larsson *et al.*, 2003; Tolasch *et al.*, 2007; Larsson *et al.*, 2009; Harvey *et al.*, 2010; Millar *et al.*, 2010; Musa *et al.*, 2013; Andersson *et al.*, 2014). Given their widespread availability already in pest management for Lepidoptera and particularly clearwing moths (Braxton & Raupp, 1995), these tools could be redirected to provide great benefits to biodiversity monitoring of saproxylics. Pheromone monitoring systems in insects are generally species-specific once optimized, although cross-attraction exists, e.g. in some other Lepidoptera (Löfstedt *et al.*, 1991) and some groups of saproxylic beetle species (Hanks *et al.*, 2012). Regardless, this selectivity would be advantageous for a focus on defined indicator species, whilst ultimately a guild of bioindicators would be desirable in order to reflect a wider range of microhabitats within a system. In the present study, we carried out pheromone monitoring of one potential indicator, the clearwing moth *S. vespiformis*, whose pheromone system has recently been characterized (Levi-Zada *et al.*, 2011).

The first general aim of the present study was to establish whether pheromone monitoring could provide more accurate information about the species' distribution than standard monitoring practices. Secondly, we wanted to establish whether the apparent rarity of the species in Sweden was a real phenomenon, or simply a result of poor detectability. Additionally, to conserve threatened species, knowledge of the species' habitat requirements is essential. The type of habitat a species uses can be found by relating particular habitat elements to species abundances or occurrences, with quantity of habitat required being assessed at multiple scales (Bergman *et al.*, 2012; Musa *et al.*, 2013). Once the required type and quantity of that resource has been ascertained, an assessment of areas in the landscape suitable for a species is possible. The third aim of this study was therefore to establish the preferred habitat for *S. vespiformis* by correlating its abundance with habitat characteristics and, by extension, to establish whether it could be used as an indicator for the biodiversity potential of oak-dominated open woodlands with which it has traditionally been associated.

Materials & methods

Site selection

The study was conducted in the counties of Östergötland, Skåne, Blekinge and Kalmar where a number of sites with high density of old and/or hollow oaks were selected (Fig.1). These sites generally have high species richness (Nilsson *et al.*, 1995) but have suffered from severe decline and fragmentation over the last 200 years in Sweden, mainly due to the change in ownership of the oaks and shifts in farming and forestry practices (Eliasson & Nilsson, 2002). Included in the selection were a number of sites with lower proportions of oaks, and a higher number of other hardwood species and hollow trees for comparison, covering a total of 251 sites (numbers of traps per county are shown in table 1). Sites selection was also based on a minimum separation distance of 500m between traps, the closest two traps being separated by 512m in this study. In this survey, a total of eight traps had another trap within a 1000m radius, with the majority being separated by many kilometres. Our observations on the attractive range of similar moth pheromones in mark release recapture studies suggest that males may be able to detect lures at a maximum distance of 150-200m (unpublished data). Therefore these distances were maintained in order to rule out the effect of inter-trap competition.

Historical records of *S. vespiformis*

Pre-existing records were taken from Sweden's nationwide repository for animal, fungi and plant distribution data, the "Swedish Species Gateway" (www.artportalen.se). In order to compare historical records with our own more recent survey efforts, we used data dating back to 1976 when the first database entry for this species occurred from a site in Stockholm. These sightings (often recorded using standard observational methods such as larval/pupal searches) (Fig.1a, b) were then compared to recent inventories we carried out using pheromone lures in 2011-2012 (Fig.1c, d). Artportalen also included a small number of historical pheromone-based sightings between 2005 and 2013 which were excluded from our analyses, but are shown in Figure 1.

Biology of *S. vespiformis*

Very little is known about the biology of *S. vespiformis*, although it is considered to be saproxylic; feeding on the cortex of its various suggested host plants (Levi-Zada *et al.*, 2011). The adult moth is considered to have a 'moderate' flight range (Van der Meulen & Groenendijk, 2005) taking flight in the afternoon through into the early evening when male moths can be caught by pheromone lure (Levi-Zada *et al.*, 2011). In Sweden the flight period begins in the last week of June and can last until the first week of August, with peak activity taking place in the second week of June in both northern and southern counties (Artportalen, 2015). This species is also known to have an association with the gall-inducing bacterium *Agrobacterium tumefaciens*, which likely facilitates larval feeding in some host plants (Audemard & Vigouroux, 1982).

Pheromone lures

We used a modified version of the pheromone blend for *S. vespiformis*, that has been found to be more attractive to Swedish populations than the blend from Levi-Zada *et al.*, (2011) (Ryrholm *unpublished*). Pheromone lures were produced using a pre-prepared blend of four pheromone components dissolved in hexane. The blend was prepared so that 200µl hexane solution contained 300µg E3Z13-18:Ac, 90µg E3Z13-18:OH, 30µg Z3Z13-18:Ac and 3µg E2Z13-18:OH. A blend volume of 200µl was then pipetted onto the surface of a 20mm diameter grey rubber septum (PheroNet, Sweden) and left in a fume cupboard to allow the solvent to fully evaporate. Septa were subsequently stored in the freezer until further use in order to preserve the attractiveness of the lure.

Trap methodology

The whole rationale of the present study was to use a viable and consistent sampling effort to obtain the first semi-quantitative data on presence and abundance across a large number of sites over a wide geographical range, in contrast to the scattered records previously available. For this purpose, we considered sticky traps as the best available option. Lures were hung 2 cm from the roof of a clear plastic delta trap sourced from CSalomon pheromone traps, Budapest, Hungary. Instead of the original sticky bottom inserts, we used cardboard sticky inserts from Oecos ltd, UK, which preserved the morphology of the wings more effectively for subsequent species identification. Studies on destructive sampling have shown that insect populations are robust against lethal sampling methods even when multiple killing traps are used per hectare (Haniotakis & Koutroubas, 1999; Yamanaka *et al.*, 2001; Gezon *et al.*, 2015). Although we did not expect our sampling to affect the populations sampled significantly, we used a reduced sticky area in our traps (about 50% compared to their original size, or 80cm^2) to reduce catches. This was a precaution considering that we were working with a rare species and a pheromone of unknown attractiveness. At each site, one trap was placed 1 - 2 m above ground from a nearby tree. Tree selection was based on availability of trees and not individual tree species. Traps were placed throughout the ten day period which commenced on 1st July 2012, and were brought down in a ten day period after 6th August 2012 ensuring that all traps had been placed in the field for a minimum of four weeks. Traps were also left for four weeks during July of 2011 (for a small pilot study in the Västervik region), with the majority of the survey work being carried out in 2012. Subsequent catches were identified and recorded after traps and sticky inserts were removed at the end of this sampling period. Only data from 2012 were used for subsequent habitat analyses.

Statistical analyses

In order to test the relative efficacy of pheromone lures compared to standard historical methods logged on Artportalen, a Kruskal-Wallis test was performed on the catch abundance data. The test was carried out in order to determine whether the median number of individuals caught by standard methods from historical survey was significantly different from the median number of catches made through the use of a pheromone lure.

The study of habitat characteristics was carried out only in Östergötland, which had the largest proportion of occupied sites. This county also had the largest and most consistent set of tree data in Sweden whilst tree data were partially incomplete in other counties. Thus the 102 sites in this region were deemed most suitable for study of habitat characteristics preferred by *S. vespiformis*. Tree data used for this study were derived from the most recent survey, which was a 10 year old survey of the region commissioned by the County Administration Board of Östergötland. From these data, tree groups were categorized as oaks or non-oaks in order to test the hypothesis that *S. vespiformis* is associated more strongly with *Quercus spp.* in this northern part of its range. European aspen (*Populus tremula* L.) and silver birch (*Betula pendula* Roth.) were excluded from the study due to incomplete survey data as well as the following coniferous trees: Norway spruce (*Picea abies* (L.) H.Karst), larch (*Larix sp.*), common juniper (*Juniperus communis* L.), and scots pine (*Pinus sylvestris* L.).

Additionally, trees were classed by a further six groupings as follows; (i) all trees, (ii) trees > 450 cm circumference, (iii) hollow stage > 3 (where stages 1 - 3 have no significant hollows and 4 - 7 have hollows of increasing size categories larger than 10cm in diameter) (Claesson & Ek 2009), (iv) trees located in open areas, (v) trees > 450 cm circumference located in grasslands, (vi) hollow stage > 3 located in grasslands. These groupings would allow us to further determine preferences for different types of oak woodland/semi pasture. A national database on semi-natural grasslands (TUVA) was used to locate targeted trees situated in semi-natural grasslands. The "open areas" category included targeted trees with open canopy cover and open surrounding vegetation. Further explanations of the canopy cover and surrounding vegetation categories can be found in Claesson & Ek (2009).

Since saproxylic insect populations can respond to a wide range of geographical scales (Bergman *et al.* 2012), 28 radii, ranging from 30 m to 6,000 m (Fig.2), were used to calculate tree densities around each site (Quantum GIS 1.8.0-Lisboa 2013). The reasoning behind selecting 30 m as the minimum scale was that it needed to be small while still maintaining variation in tree densities among sites. The maximum scale of 6,000 m was selected based on the maximum dispersal range of other moths as well as to retain enough sites without the largest radii overlapping.

The scale(s) at which the species responds most strongly to the habitat variables (characteristic scale of response) was estimated for each tree group separately. At each scale, a negative binomial general linear model was run with abundance of *S. vespiformis* as response and tree counts as predictor variable. The computer program Focus (Geomatics and Landscape Ecology Research Lab, Canada) was used to extract 500 combinations of non-overlapping buffers at each radius: no radii overlapped below 500m. The median of the regression results were used to condense the results for one scale and tree group. The characteristic scale of response for a tree group was defined as the scale with the largest absolute Z-value. It is possible to use other variables, and rings instead of circle as buffers, to determine the characteristic scale of response. Tree content in ring buffers are less correlated between scales, and regression coefficients on standardized explanatory variables make comparisons of effect size easier. However, the connection between response to circle buffers and ecological processes is easier to understand. Furthermore, the interpretation of standardized coefficients need a measure of variance which the Z-value already provides. Results from ring buffers were also similar to circle buffers and standardized coefficients scaled with Z-value, thus the alternatives did not change the interpretation. There was generally a weak correlation between oak and non-oak trees which was negative (r = -0.1) for small radii and positive for larger radii (r = 0.1) while correlations within tree-groups of oak or non-oak were higher and positive. Running multiple correlations reduces the pseudoreplication of data points because each iteration uses spatially independent sites (Holland *et al.* 2004). However, the repeated analysis uses the same data so pseudoreplication may not be entirely avoided. The Focus program therefore allows for optimization of the data available and an increase in the power of the analysis. All analysis and data management was done in the statistical software R (R core team, 2014).

Results

In total 439 individual specimens of *S. vespiformis* were caught across the four counties, accounting for 77 newly identified localities (Fig.1c, d). We also recorded a total of 174 sites where the species was not found. The breakdown of records per county is shown in Table 1. The sampling effort from this study almost tripled the number of reported localities for this species during only two field seasons (47 previously reported sites found in Artportalen were increased to 124 occupied sites in total as a result of sites located by pheromone lure), after decades of reports by classical methods of surveying (Fig.3). The result is a slight apparent expansion of *S. vespiformis* range due to increased sampling effort/accuracy, and a much higher density of occupied sites noted within the existing range (Fig.1).

The new inventory of the species revealed a scattered pattern of distribution amongst woodland habitats sampled in southern Sweden. In some cases moths were locally abundant, but their absence is notable across significant areas of most counties despite the extensive and systematic sampling effort and high efficacy of the traps. Comparison of the median number of individuals caught by standard vs. pheromone methods however showed significantly higher numbers to be observed via pheromone trapping overall (H = 18.58 d.f. = 1 $p < 0.001$). The median catch using standard method was one individual, compared to a median of two individuals for pheromone method.

Habitat analyses in Östergötland (Fig.4), showed no significant relationship between the numbers of individuals caught in traps, with any of the habitat variables included at any scale. No absolute median Z-values were larger than 1.96 which is the 5% significance level. Focusing on effect size there was a relatively larger positive response to hollow oaks close to the trap (30-200m), while other tree species in general, and hollow trees in particular, seemed to have a negative effect on species presence. The reversed relationship between oak and non-oak was not a result from explanatory variables being highly correlated at lower scales (r was approximately -0.1 between 30 and 200 m).

In addition to catches of *S. vespiformis,* some traps in Skåne and Östergötland also caught another clearwing species, *Pennisetia hylaeiformis* Laspeyres. This species was present at fifteen sites in Skåne and nine in Östergötland, with the number of individuals totaling 128 and 23 across all traps in these regions respectively.

Discussion

S. vespiformis is considered a rare insect in Sweden, first recorded in 1860, and with just a few observations logged until the sex pheromone was identified and its synthetic constituents became available more recently. To our knowledge, our approach to addressing this situation represents the first systematic application of pheromones for any regional survey of a lepidopteran conservation target species, although large-scale pheromone surveys have previously targeted pests among the Lepidoptera (e.g. Tobin *et al.*, 2007) and other orders, and also the threatened click beetle *Elater ferrugineus* (Kadej *et al.*, 2015). This study shows that pheromone-based monitoring of woodland moths can provide a significant increase in accuracy and sampling effort of surveys targeting saproxylic insects. By use of sex pheromones an additional 77 sites with *S. vespiformis* were found; thereby increasing the known localities from 47 to 124. This represents a near tripling of the number of reported sites in just two field seasons. In addition, the number of individuals caught represents a significant increase in sampling efficiency. Previous site visits often report single insects, whereas we uncovered a total of 439 individuals across our survey sites, with the highest number of individuals at one site being 41 (a site in Västervik municipality, Kalmar county). This increased efficiency is further supported by a significant difference noted in the median number of insects caught when comparing recording based on observation to recording using a pheromone lure.

A minor limitation which was noted during this study was the tendency for this pheromone blend to cause cross-attraction to *Pennisetia hylaeiformis*; a similar looking sesiid which could potentially lead to misidentification of *S. vespiformis* sites. It was also noted that some potential predation had occurred in traps left for a period of time longer than a few weeks, which may have even led to an under-sampling at certain sites. Thus is suggested that for the inventory of this species, voucher specimens should be retained for taxonomic determination, and traps checked at regular intervals where possible.

We have not yet studied the specific accuracy of our pheromone lures in detecting populations of *S. vespiformis*, e.g. through recapture experiments or repeated sampling of known populations, but we would argue that empty pheromone traps in our study usually represent truly unoccupied sites, lacking reproducing populations. The generally high efficiency of these types of classical female-produced sex pheromones in detecting their target species has been well established in lepidopteran pest species (Zhang & Schlyter 1996) and in many other insect groups (Östrand & Anderbrant, 2003), including the threatened click beetle *E. ferrugineus* (Svensson *et al.*, 2011; Zauli *et al.*, 2014). In most cases it appears highly unlikely that a monitoring trap would fail to detect any males over a full flight season at a site with a local reproducing population. Absence data generated from sex pheromone lures have been shown to be much more reliable than stochastic methods such as unbaited pitfall or window traps in establishing presence or absence (Andersson *et al.*, 2014; Zauli *et al.*, 2014). The data generated by this methodology can therefore allow for better resolution in ecological studies, which rely strongly on accurate presence and absence data to characterize not only the species' habitat, but also its 'non-habitat'. Future studies of the use of pheromones would benefit from a full assessment of their monitoring accuracy in order to assess exactly how precise moth pheromone systems are for determining absence, similar to studies on emerging eDNA technology for species assessment of great crested newt (Biggs *et al.*, 2015).

Under-reporting of occupancy is a significant issue in biodiversity monitoring where sampling methods show low detection probability (Pellet & Schmidt, 2005), but this could be easily resolved with the high levels of detectability that pheromones provide (Andersson *et al.*, 2014). The previous lack of occupancy records are likely contributors to our view of *S. vespiformis* as a species with a very sparse distribution in Sweden. Our findings suggest this species is perhaps a little less rare than previously thought, and occasionally abundant locally despite notable long-term declines in the type of Swedish oak forest considered to be its primary habitat. The slight expansion of the species range, and higher density of known sites within its range seen in Figure 1 is likely to be reflective of increased sampling efficacy rather than actual recent expansion of the species itself. However, the distribution of *S. vespiformis* is still relatively sparse, with a large proportion of sites where the species was absent despite an apparent abundance of saproxylic resources, suggesting the species may still be under threat or that very little is known about its ecological requirements.

During the course of the study, *S. vespiformis* was found at a number of sites outside of its 'expected' habitat type. Singleton catches outside of the expected habitat may be a result of chance migrants caught nearby a 'true' locality. However, sampling bias has also been highlighted as a limiting factor in insect surveying particularly with Lepidoptera (Dennis *et al.*, 1999), and this may contribute towards the oak-biased recordings seen in previous survey data. Resource-limited county administrative boards and field entomologists can tend to focus their efforts on known sites, or sites similar to those where the species has been found already. This circular approach may obscure hidden biodiversity and niches that might otherwise be uncovered by a less biased search effort in the field. Our analyses of *S. vespiformis*' habitat further highlight this sampling bias when considering *S. vespiformis*' preferences in northern Europe. In Sweden, the species is considered to be reliant on oaks (Waring & Townsend, 2003; Eliasson, 2007), but our results showed only weak relationships with old oaks and oaks with holes for number of moths caught and oak and non-oak tree abundance (Fig.4). This is in stark contrast to results from the click beetle *E. ferrugineus* using similar methodology in relation to similar veteran tree habitats, which demonstrated a very strong association between the beetle and its nominal habitat (Musa *et al.* 2013). This casts some doubt on the previous assumptions made regarding host specificity and preference in *S. vespiformis*. Our findings were also echoed in a recent Swedish inventory, where it was noted that "Pheromone lures placed on several premises in Skåne and Öland and in eastern Småland have shown that the species is not as tied to large veteran trees as previously assumed, but is reliant on this habitat when others are not available." (Palmqvist 2014).

In the south of its range *S. vespiformis* is found to be rather polyphagous, yet its visibility in these regions is undoubtedly much higher due to its high population numbers in agricultural crops (Levi-Zada *et al.*, 2011), and thus the likelihood of observing the species on alternative hosts is higher. Based on these findings we suggest that Swedish *S. vespiformis* could be more polyphagous than previously expected, possibly in line with its southern siblings. This hypothesis could be further tested by using pheromones to survey in regions significantly outside of its known range (for example Småland), in habitats which are not typical oak woodland and contain higher numbers of "secondary" host species. Incidentally, a recent study on saproxylic beetles also showed that most were polyphagous (Milberg *et al.*, 2014), and the presumed oak-specificity was most likely due to sampling bias. It is therefore suggested that sesiids would benefit from a significant re-inventory in order to assess their true host relationships and conservation status.

In principle, the methods applied here could encompass all previous observations and attempts at surveying *S. vespiformis* in Sweden and surpass them several times over, in a single season, with comparably little effort. The high quality and potentially low bias of data generated by pheromone monitoring is also important, as knowledge on species distribution needs to inform decision making at both a local, national and international level (Pereira & Cooper, 2006). This increased sampling efficacy has the potential to solve a number of issues highlighted by the European Commission in their efforts to mitigate biodiversity loss by 2020. Firstly, it has been suggested that one of the major challenges in meeting this target is development of effective and standardized methods of monitoring for national or international biodiversity (Pereira & Cooper, 2006; Henry *et al.*, 2008). Additionally such international monitoring efforts must be viable within a restricted pool of resources (Bates *et al.*, 2007), since cost effectiveness is a significant consideration in any environmental monitoring system (Hauser *et al.*, 2006; Lovett *et al.*, 2006). In light of the reduced or unstable funding for conservation/biodiversity monitoring in many member states (Lindenmayer *et al.*, 2012), pheromone attractants could provide an essential tool for insect inventories across Europe. Not only are the pheromones relatively cost effective (costs are relatively low for these simple compounds where synthesis method is already established), but the relative sampling effort in terms of working hours for survey is also greatly reduced (Burman & Thackery, unpublished data). This is particularly promising, as time is also a limiting resource in biodiversity monitoring (Yoccoz *et al.*, 2001). Pheromones could allow those with limited resources the opportunity to spread their efforts further afield, and thereby improve the quality of data obtained. We demonstrate in this study that national level insect species surveys are feasible using sex pheromones, covering large spatial and potentially temporal scales to provide data relevant to international monitoring (Pereira & Cooper, 2006). Large-scale pheromone-based surveys provide a snapshot of presences and absences across a considerable part of a species national distribution range, and thus for the first time provide a viable means of systematically assessing changes in distribution over time with high spatiotemporal resolution.

In the present study destructive monitoring of *S. vespiformis* was a prerequisite for obtaining comparable semi-quantitative occupancy data with high resolution over an extensive geographical range, and was carried out in agreement with conservation authorities although neither the species nor many of the sites are protected. The species is believed to generally have a two-year life cycle in Sweden (Eliasson, 2007), which would mitigate any risk to individual populations. Whilst destructive monitoring of the kind carried out in this study should be avoided year on year, it does provide a new snapshot of species distribution previously unavailable to conservationists. This includes a significant number of previously unreported sites for this species, many of which receive no statutory protection or management at the time of writing, but which could now be considered for their conservation value. Repeated future monitoring of these sites over extended time intervals perhaps combined with more regular use of pheromone live-trapping at a limited number of sites, could provide regular insights into how these habitats are faring in the longer term in response to different management regimes.

Conclusions

In this study we have shown that hidden biodiversity and species preferences can be substantial due to sampling bias and less effective sampling methodology. The vast majority of species sampled by standard observational methods are likely to be underreported, and as a result the drop in data quality can lead to problems in conducting ecological studies to uncover true habitat characteristics (Pellet & Schmidt, 2005). By comparison, pheromone-based methods can significantly increase the sampling accuracy and give a much more reliable idea of a species' distribution. *S. vespiformis* appears to be genuinely quite rare still, despite a significant number of new sites being located. In addition, the increased resolution of data, showed no particular association for large oaks, despite previous assumptions.

We believe as a result, that pheromone-baited traps bring much promise for conservation, both for surveying and monitoring targeted species, and could be used as a powerful tool to achieve the EU's optimistic goals of halting insect biodiversity loss by 2020, as well as an invaluable resource for carrying out landscape level ecological studies.

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Figure captions

[1]**Fig.1** a) Map showing pre-2010 records (www.artportalen.se) of *S. vespiformis* in Southern Sweden. b) Recordings of *S. vespiformis* as of 2013 without pheromone survey. c) Reported sites in 2013 after inclusion of pheromone survey data. d) Map showing the distribution of pheromone traps in southern Sweden in the Skåne, Blekinge, Kalmar and Östergötland counties (Swedish “Län” = county). Filled and empty circles indicate sites where *S. vespiformis.* was recorded and was not recorded, respectively

[1]**Fig.2** Map of *S. vespiformis* sampling area in Östergötland, Sweden. The black dots represent trees, the large grey circles represent the largest scale (6,000 m) used to determine tree abundance, and the small white circles with (occupied) and without (empty) black crosses represent trap locations

[2]**Fig.3** Frequency of site reports of *S. vespiformis* since 1976 (www.artportalen.se). The implementation of pheromone lures contributes significantly towards the greatly increased numbers of reported sites in 2011 and 2012

[3]**Fig.4** The relationship (median Z-value from 500 negative binomial regressions at each scale and tree group) between abundance of *S. vespiformis* and amount of a) oak trees with various characteristics at multiple scales and b) non-oak deciduous trees at multiple scales. Light and dark gray circles denote negative and positive relationships respectively, and ring size above 1.96 is significant at 5% level.

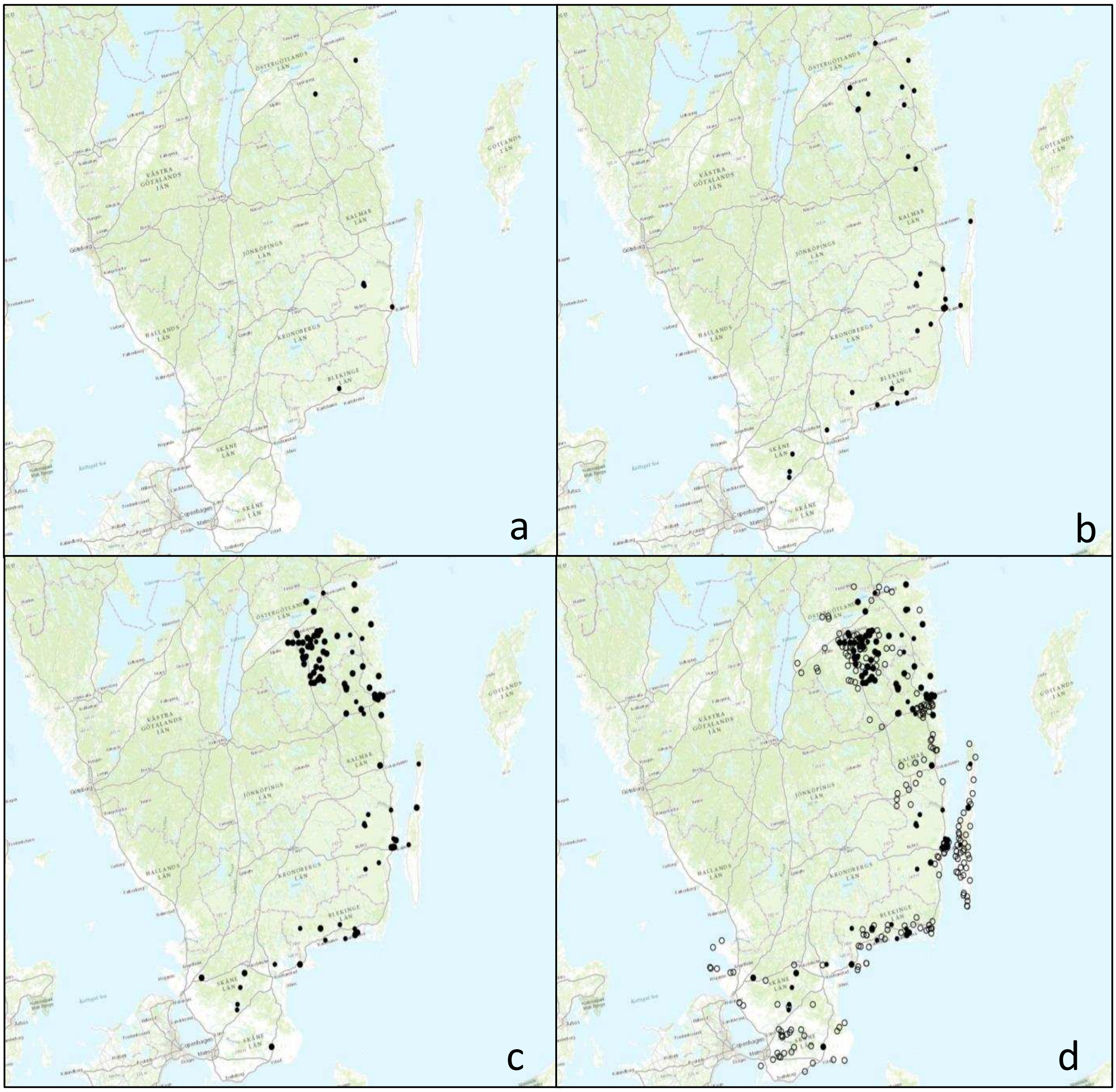
[1]Figure produced in ARCGIS

[2]Figure produced in Microsoft Excel

[3]Figure produced in R version 3.1.2

County	Number of moths caught	Number of sites with species present	Number of sites with species not present	Number of traps placed
Skåne	5	2	35	37
Blekinge	12	6	20	26
Kalmar	308	26	46	72
Östergötland	114	43	73	116
Total	*439*	*77*	*174*	*251*

Table 1. Pheromone based recordings of *S. vespiformis* across four counties in Southern Sweden.


a
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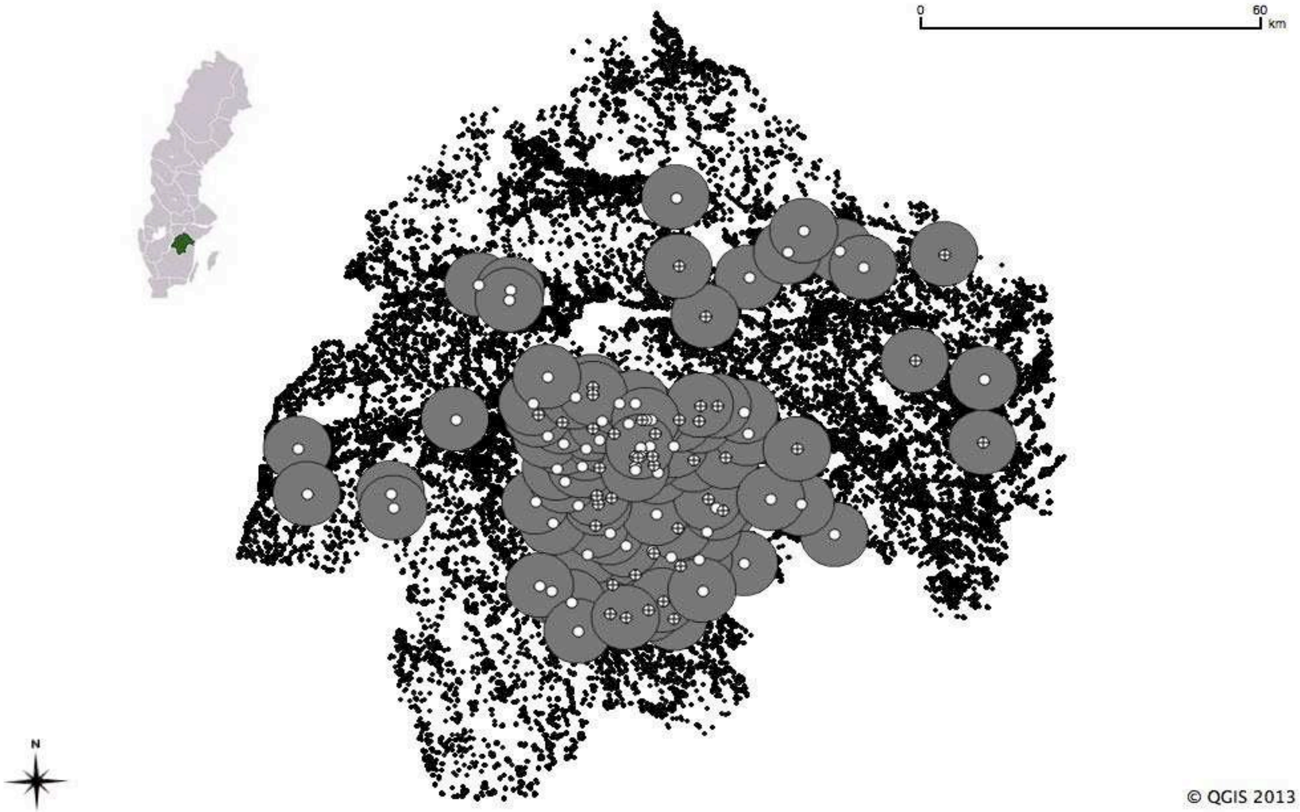

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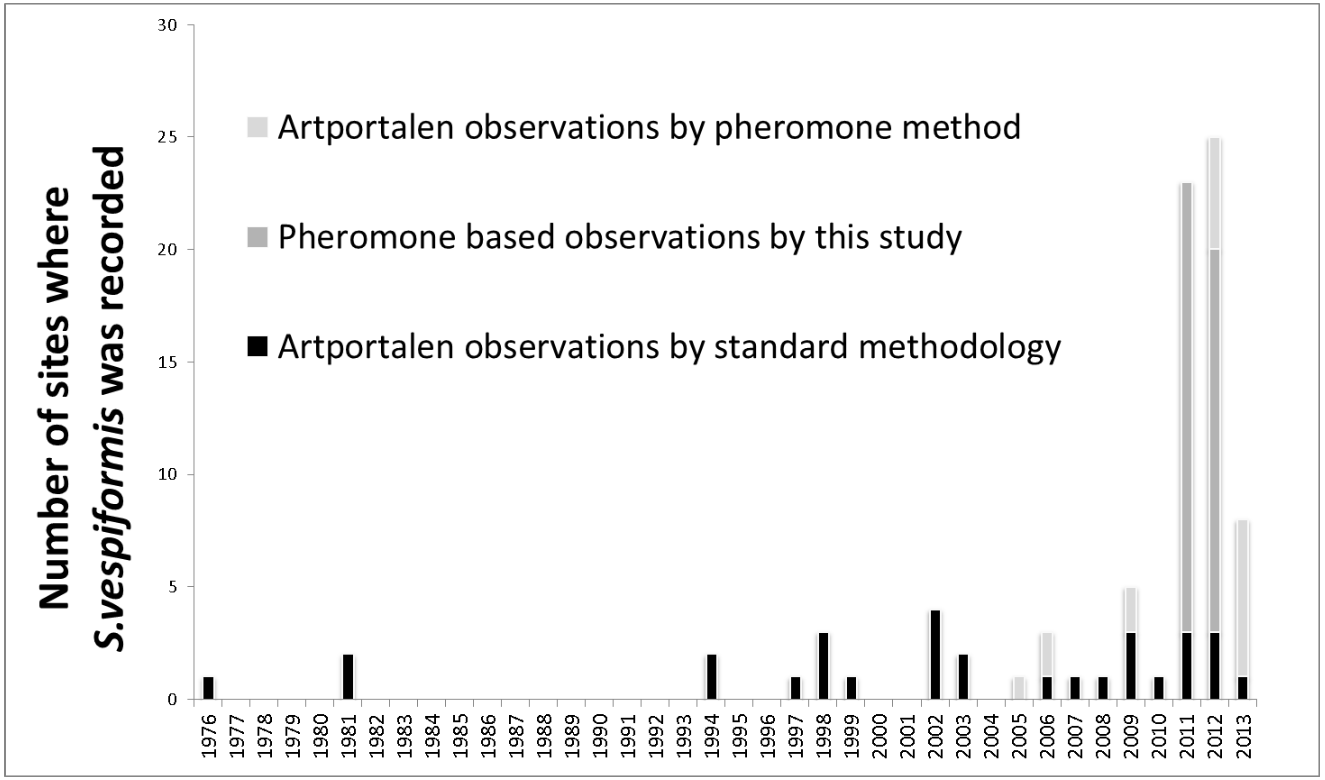

Number of sites where
S.vespiformis was recorded
Artportalen observations by pheromone method
Pheromone based observations by this study
Artportalen observations by standard methodology
0
5
10
15
20
25
30
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
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1989
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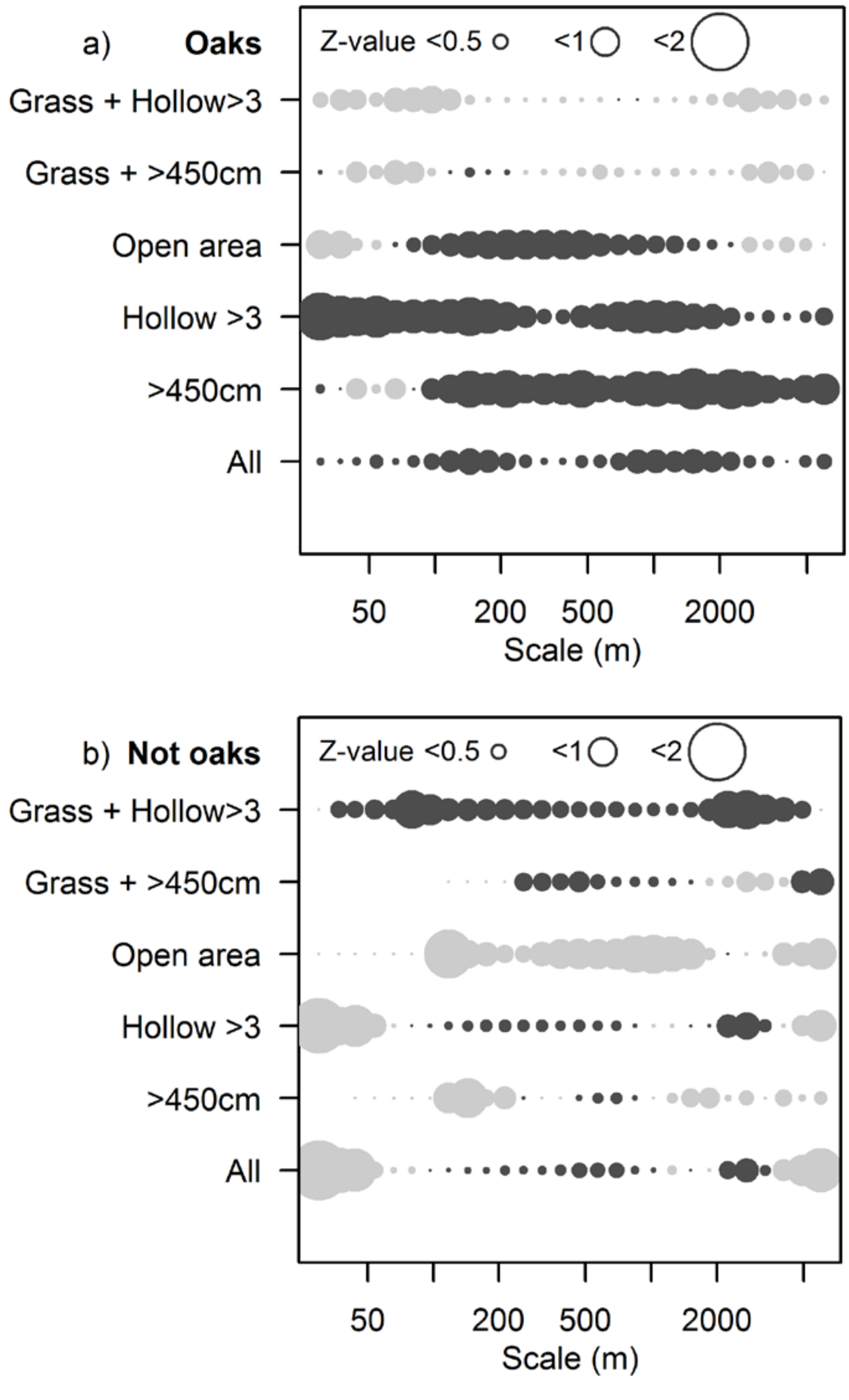
a) Oaks
Z-value <0.5 <1 <2
Grass + Hollow>3
Grass + >450cm
Open area
Hollow >3
>450cm
All
50
200
500
2000
Scale (m)
b) Not oaks
Z-value <0.5 <1 <2
Grass + Hollow>3
Grass + >450cm
Open area
Hollow >3
>450cm
All
50
200
500
2000
Scale (m)