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Enantiomeric Specificity in a Pheromone–Kairomone System of Two Threatened Saproxylic Beetles, *Osmoderma eremita* and *Elater ferrugineus*

Glenn P. Svensson · Mattias C. Larsson

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Abstract The scarab beetle *Osmoderma eremita* and its larval predator, the click beetle *Elater ferrugineus*, are threatened saproxylic beetles regarded as indicators of the species-richness of insect fauna of hollow deciduous trees. Male *O. eremita* produce the pheromone (*R*)-(+)-γ-decalactone to attract conspecific females, and this compound is also utilized by *E. ferrugineus* as a kairomone, presumably for detection of tree hollows containing prey. We have investigated enantiomeric specificity to γ-decalactone in this pheromone–kairomone system by electrophysiological and field trapping experiments. In single-sensillum recordings from male and female *O. eremita*, which used the (*R*)-enantiomer and the racemic mixture of γ-decalactone as odor stimuli, numerous olfactory receptor neurons (ORNs) responding to both stimuli were found. No neurons responded preferentially to the racemic mixture, showing that these beetles seem to lack receptors specific for the (*S*)-enantiomer. The enantiomeric specificity of ORNs was confirmed by gas chromatography-linked single-sensillum recordings where the two enantiomers in a racemic mixture were separated on a chiral column. Furthermore, in field experiments that used the (*R*)-enantiomer and the racemic mixture as lures, the attraction of *O. eremita* females corresponded to the amount of (*R*)-enantiomer released from lures with the (*S*)-enantiomer displaying no antagonistic effects. Trap catch data also suggested that the (*S*)-enantiomer is not a behavioral antagonist for *E. ferrugineus*. The odor-based system can be highly efficient in attracting the larval predator where trap catch in 1 yr almost equaled the total number of specimens collected in Sweden until 1993. Our study shows that racemic γ-decalactone could be used for cost-effective monitoring of both beetles.

Keywords *Osmoderma eremita* · *Elater ferrugineus* · Scarabaeidae · Elateridae · γ-Decalactone · Sex pheromone · Kairomone · Predator–prey interaction · Single-sensillum recording · Olfactory receptor neuron · Conservation

G. P. Svensson (✉)
Department of Ecology, Ecology Building, Lund University,
SE-223 62 Lund, Sweden
e-mail: glenn.svensson@ekol.lu.se

M. C. Larsson
Department of Crop Protection Biology,
Swedish University of Agricultural Sciences,
P.O. Box 44, SE-230 53 Alnarp, Sweden

Introduction

Ever since the first insect pheromone was identified half a century ago (Butenandt et al. 1959), the ultimate practical goal of research on insect chemical ecology has been the development of semiochemical-based strategies to control populations of agricultural and forest pests (e.g., Cardé and Minks 1995; Borden 1997; Foster and Harris 1997). In contrast, surprisingly little effort has gone into the exploration of the chemical ecology of threatened insects and the potential to use infochemicals in conservation biology. Monitoring with volatile attractants could be more efficient than traditional survey methods in gathering ecological data on focal species for conservation and in assessing species diversity of threatened insect communities. Priority should be given to assemblages that are difficult to detect and monitor with currently available methods. One such group is saproxylic beetles that live in hollow deciduous trees. The severe fragmentation and isolation of this habitat in recent centuries (Hannah et al. 1995) has resulted in the associated insect fauna being endangered all over Europe. Surveys of beetles that live inside tree trunks are often both

labor-intensive and time-consuming, and in many cases, only a small fraction of suitable trees in an area can be surveyed.

We recently initiated a research program to integrate chemical ecology and conservation biology of saproxylic beetles by using *Osmoderma eremita* Scopoli (Coleoptera: Scarabaeidae) as a model. This beetle is strongly associated with hollow deciduous trees (Luce 1996; Ranius and Nilsson 1997) and it has become a major model for ecological research on insects associated with this habitat in Europe (e.g., Ranius 2001; Ranius and Hedin 2001). Because of its role as an indicator for the species-rich fauna of saproxylic insects, *O. eremita* has high conservation priority according to the European Union's Habitat Directive (Anonymous 1992), and it has recently been intensively surveyed in Europe (Ranius et al. 2005). The most well-known feature of *O. eremita* is its fruity, plum-like or peach-like odor, which is emitted exclusively by males. We identified the odor as (*R*)-(+)-γ-decalactone and demonstrated its function as a sex pheromone used to attract conspecific females (Larsson et al. 2003). We have also shown that the likewise-threatened click beetle *Elater ferrugineus* L. (Coleoptera: Elateridae), whose larvae prey upon the larvae of *O. eremita*, utilizes (*R*)-(+)-γ-decalactone as a kairomone to locate its prey (Svensson et al. 2004). The *Osmoderma* pheromone is produced in exceptionally large amounts and can even be detected inside tree hollows by using traditional headspace sampling techniques (Svensson et al. 2003).

Sexual communication in beetles is often achieved by chiral pheromone compounds (e.g., Birch et al. 1980; Mori et al. 1986; Leal 1991). When one enantiomer of a chiral molecule is used for intraspecific communication, its antipode may function as a behavioral antagonist to avoid cross-species attraction, if the antipode is used by a closely related species. For example, the sympatric scarabs *Popillia japonica* Newman and *Anomala osakana* Sawada utilize the (*R*)- and (*S*)-configuration of japonilure, respectively, as sex pheromones, and strong reciprocal behavioral antagonism to the opposite enantiomer has been observed (Tumlinson et al. 1977; Leal 1996). Similar stereochemical discrimination has also been documented in insect predators that eavesdrop on the pheromone communication systems of their prey (Aldrich 1999), such as the clerid beetle *Thanasimus dubius* F., which only responds to the (*S*)-enantiomer of frontalin, the major enantiomer of the pheromone of its prey *Dendroctonus frontalis* Zimmerman (Payne et al. 1982, 1984).

However, behavioral effects of nonpheromonal enantiomers vary considerably among different insect species (Mori 1998; Leal 1999), and more studies are needed to understand the adaptive significance of these effects, including mechanisms of enantiomeric perception and behavioral data. From an applied perspective, evaluating the enantiomeric specificity in insect pheromone or kairomone systems is important for efficient semiochemical-based monitoring. Because pure enantiomers are usually more expensive than racemic mixtures (in the case of commercially available (*R*)-(+)-γ-decalactone, currently about 15 times as expensive, see the "Methods and Materials" section), use of the latter could significantly reduce the cost for monitoring. We, therefore, conducted electrophysiological analyses and field experiments to evaluate the effects of the (*S*)-enantiomer of γ-decalactone on the behaviors of *O. eremita* and *E. ferrugineus*.

Methods and Materials

Insects *O. eremita* is a large (25–35 mm) scarab beetle living exclusively in hollow deciduous trees, mainly oaks (Luce 1996). It is dependent on hollows with large amounts of wood mold, i.e., a mixture of loose, rotten wood, fragments of insects, fungi, and old bird nests. The development time is normally 3 yrs (Ranius et al. 2005). The species is distributed in most parts of Europe, but a recent survey that included 33 countries indicated that it has decreased in all areas (Ranius et al. 2005). Today, it has a relict distribution and only occurs in isolated populations in, e.g., woodland pastures. In Sweden, it is currently known from only 130 sites (Antonsson et al. 2003), and many populations occupy single isolated trees with few or no suitable trees nearby, and may, therefore, be doomed to extinction. *E. ferrugineus* is a large (15–25 mm) click beetle and larval predator on several saproxylic beetles associated with hollow trees, including *O. eremita* (Hansen 1966; Dajoz 2000). In Sweden, it is currently known from only 25+ sites (Nilsson and Baranowski 1994). Ranius (2002) found a strong correlation of occupancy between *E. ferrugineus* and *O. eremita* with fragments of the predator found almost exclusively in trees with fragments of its prey. The development time is several years (Palm 1959). According to Hansen (1966) and T. Tolasch (personal communication) this species swarms during evenings and nights, and its female-produced sex pheromone was recently identified (Tolasch et al. 2007). The species is rare in Sweden with only 147 adult specimens collected from about 25 localities until 1993 (Nilsson and Baranowski 1994). It is considered endangered in the Swedish Red List of threatened species (Gärdenfors 2005).

Chemicals and Dispensers (*R*)-(+)-γ-Decalactone (97% enantiomeric purity) was purchased from Sigma-Aldrich, Sweden (catalog no.: W236012). Because the (*S*)-enantiomer of the compound is not commercially available, we used a racemic mixture of γ-decalactone (catalog no.:

W236004) to study the electrophysiological and behavioral effects of the (*S*)-enantiomer. Dispensers for traps were made from 2 ml glass vials loaded with 600 µl of neat (*R*)-(+)-γ-decalactone or the neat racemic mixture. Cut strings of cotton dental rolls (Celluron, Paul Hartmann, S.A., France) were inserted as wicks into the glass vials, and dispensers were attached to traps via a metal hook.

Electrophysiology Single-sensillum recordings were performed on both female and male *O. eremita*, but not on *E. ferrugineus*, which is rare in Sweden. To restrain a beetle, it was wrapped with parafilm, placed on a microscope slide, and fixed with dental wax. To get access to olfactory receptor neurons (ORNs), the three lamellae on the antenna were held apart with thin metal pins. A tungsten microelectrode or a thin silver wire was inserted into the abdomen of the insect, serving as a ground electrode, and a second tungsten microelectrode (electrolytically sharpened in KNO_2 solution) was inserted into an olfactory sensillum to establish contact with ORNs. The recording electrode was connected to a ×2 gain probe (Syntech, Hilversum, The Netherlands). A microscope with up to ×500 magnification and a DC-3K micromanipulator with a Piezo translator (PM10) (Märzhauser, Wetzlar-Steindorf, Germany) were used to position the electrode. Charcoal-filtered and humified air passed over the antenna from a glass tube outlet at 10 mm distance from the preparation. Odor stimulation of an ORN was achieved by inserting the tip of a Pasteur pipette, containing defined amounts of a stimulus, into a hole in the glass tube 10 cm before the outlet. The pipette was linked to an air control system (Syntech, Hilversum, The Netherlands), which generated 0.5 sec air puffs through the pipette into the air stream of the glass tube.

Odor Stimuli Neat (*R*)-(+)-γ-decalactone and the racemic mixture were diluted in hexane in decadic steps down to concentrations to be used in electrophysiological experiments. A test stimulus was then applied to a small piece of filter paper inserted into the test pipette, and the solvent was allowed to evaporate. Each pipette was loaded with 10 µl of a stimulus solution, and the amount of compound loaded into a pipette ranged from 100 ng to 100 µg. Pipettes loaded with 10 µl of hexane served as controls. After establishing contact with an ORN, it was stimulated with both test odorants at the highest dose (100 µg) and the control. If responses to the test odorants were not greater than to the control, the neuron was classified as non-responding. If the neuron responded to any of the test odorants, a dose–response trial was often performed by using the test stimuli at increasing concentrations. To enable the receptor neuron to recover after odor stimulation, at least 20 sec passed between each stimulation event. Responses from ORNs were calculated as the total number of spikes during 0.5 sec after the onset of stimulation minus the number of spikes during 0.5 sec before the onset of stimulation. The net response to an odor stimulus was calculated by subtracting the response generated by the control stimulation. In dose–response trials, net responses for females and males were compared for stimulations with the (*R*)-enantiomer and the racemic mixture at each dose using paired *t* tests, and net responses for stimulations with (*R*)-enantiomer were compared between the sexes at each dose using unpaired *t* tests.

Coupled Gas Chromatography and Single-sensillum Recordings To further test for the enantiomeric specificity of ORNs to γ-decalactone in *O. eremita*, gas chromatography linked to single-sensillum recordings (GC–SSR) was performed with a chiral column to resolve the enantiomers in the racemic mixture. A Hewlett-Packard 5890 Series II Plus gas chromatograph was used, equipped with a Cyclosil-B chiral column (30 m×0.25 mm i.d., 0.25 µm film; J&W Scientific, USA). Hydrogen was used as carrier gas (40 cm/sec), injector temperature was 220°C, and the following temperature program was used: 60°C for 2 min, 10°C/min to 170°C, followed by 2°C/min to 200°C, and then 10°C/min increase to 225°C. The transfer line temperature was maintained at 225°C. As stimulus, 100 ng of the racemic mixture were injected into the GC.

Field Trapping Field bioassays were conducted in Bjärka-Säby (58°16′N, 15°46°E) and Brokind (58°12′N, 15°40′E), southeast Sweden, during July–August, 2006 and 2007. The study area included five large stands of old hollow oaks housing some of the largest populations of *O. eremita* and *E. ferrugineus* in Sweden (Nilsson and Baranowski 1994; Ranius 2001; Antonsson et al. 2003). In 2006, two different nondestructive trapping systems were used, allowing beetles to be released alive after examination: Lindgren funnel traps (Phero Tech, Delta, BC, Canada), and custom-built traps consisting of two black plastic sheets (25 cm height×30 cm width, 3 mm thickness) arranged in a cross and attached perpendicular to a black plastic funnel (upper diameter, 27 cm) leading down to a 5-l white plastic container [for a picture of a trap with the same general design, see Ruther et al. (2000)]. In 2006, 20 replicates of 4 traps were used. Each replicate contained two traps of each type, baited with either 600 µl of the (*R*)-enantiomer or 600 µl of the racemic mixture. Custom-built traps were about 1.5 times more efficient than Lindgren traps in catching *O. eremita* (191 vs. 141 catches, $\chi^2=7.53$; $df=1$; $P<0.01$) and *E. ferrugineus* (88 vs. 55 catches, $\chi^2=7.62$; $df=1$; $P<0.01$). Thus, only custom-built traps were used in 2007. Twelve replicates were used, each containing 2 traps baited with either 600 µl of (*R*)-enantiomer or 2×600 µl of racemic

mixture, and a control trap without odorants. Traps were suspended from oak branches at 2–4 m height and at least 10 m apart, and they were checked for the presence of beetles every second day. The relative positions of traps within replicates were changed every eighth day.

In 2006 (but not in 2007), captured *O. eremita* were marked with shallow marks drilled in the elytra (Ranius 2001), and *E. ferrugineus* were marked on the elytra with a permanent marker pen to estimate the proportion of recaptures, which comprised <10% of the total number of captures in *O. eremita* and <4% in *E. ferrugineus*. Statistical analyses on trapping data were based on the total number of captures, including recaptures. For male and female *O. eremita* and *E. ferrugineus*, χ^2 analyses were performed to check for the difference in trap catch among treatments. We found no published information allowing the sex of *E. ferrugineus* individuals to be distinguished easily without genital examination, and to avoid killing them, we did not determine the sex of most captured individuals in 2006, except for a subset of 23 individuals, mostly consisting of individuals found dead in the traps.

Results

Electrophysiology Recordings were performed only from sensilla on the inner lamella of the *O. eremita* antenna. High-quality recordings were obtained from 234 sensilla of 30 females and 101 sensilla of 21 males. Based on their spike amplitude, most sensilla appeared to contain two ORNs, but odor stimulation always affected only one of these neurons, whereas the other was nonresponsive to both stimuli. In 88% of the recordings from both females and males, the ORNs in the sensillum did not respond to the (*R*)-enantiomer or racemic mixture of γ-decalactone. The remaining sensilla (29 in females and 10 in males)

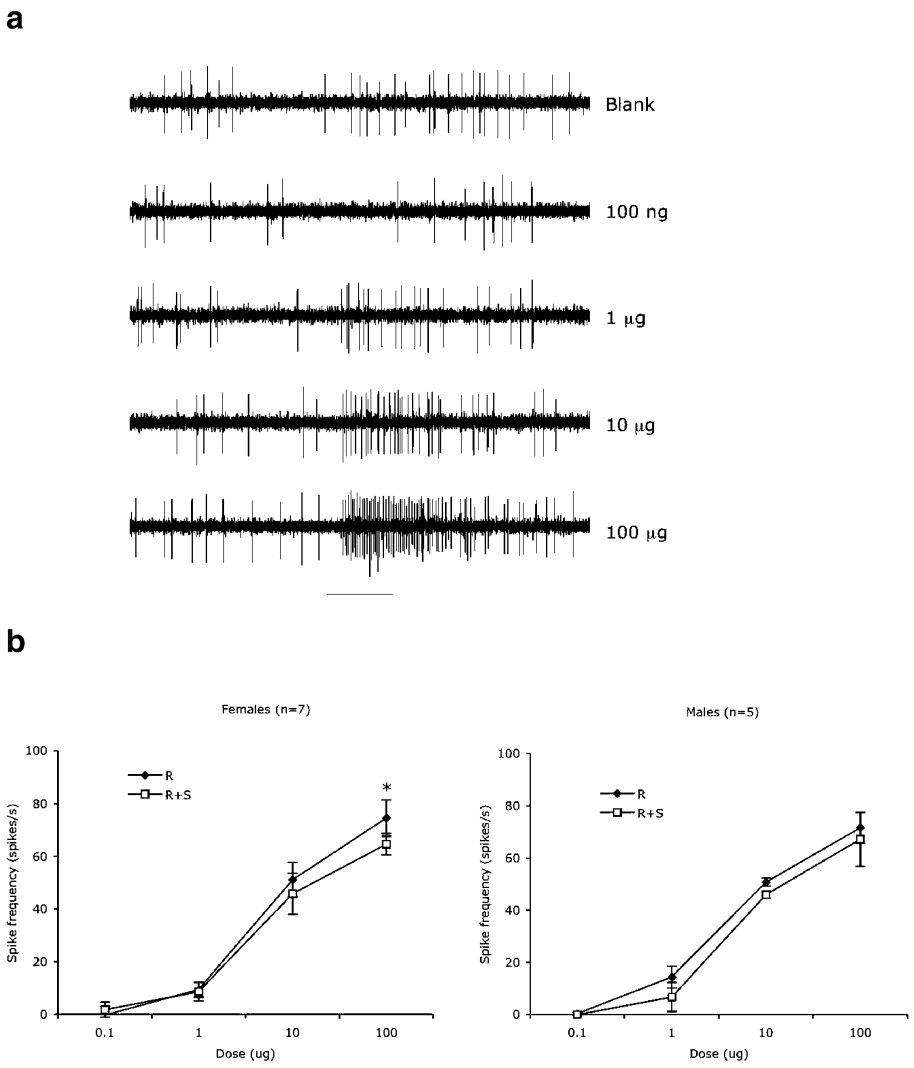

Fig. 1 **a** Single-sensillum recordings from the antenna of a female *O. eremita* showing the response of an ORN to the blank control and to increased doses of (*R*)-(+)-γ-decalactone. The *bar below* the recordings indicates the stimulus duration (0.5 sec). **b** Dose–response relationships of ORNs of female and male *O. eremita* stimulated with the (*R*)-enantiomer (*R*) or a racemic mixture (*R*+*S*) of γ-decalactone. For each dose, the same amount of (*R*)-enantiomer was used in both test stimuli. The response to a stimulus is quantified as the net number of spikes (number of spikes during 0.5 sec after the stimulation minus the number of spikes during 0.5 sec before the stimulation) minus the net blank response. *Error bars* show the standard error of the mean. The *asterisk* indicates a significant difference in spike activity between stimuli within the dose (paired *t* test, $P<0.05$)

contained a receptor neuron that responded to both test odorants (Fig. 1a). No neurons were found that responded primarily to the racemate, which would have indicated specificity to the (*S*)-enantiomer. The electrophysiological recordings revealed that γ-decalactone-sensitive ORNs were sparsely distributed over the whole inner antennal lamella of both sexes, but mainly found in the smooth area close to the ventral edge of the lamella (cf. Larsson et al. 2001, 2003). In dose–response trials, ORNs of females and males showed equal sensitivity to (*R*)-(+)-γ-decalactone (for all doses, $P>0.05$; Fig. 1b). However, a small but significant difference in spike frequency was observed for ORNs of females when stimulated with the (*R*)-enantiomer compared to the racemic mixture at the highest dose ($t=2.96$, $df=6$, $P<0.05$; Fig. 1b). Single-sensillum recordings coupled with gas chromatography confirmed that the γ-decalactone-sensitive ORNs indeed only responded to the (*R*)-enantiomer (Fig. 2). There was a clear separation of the two compounds on the chiral column with the (*R*)-enantiomer eluting 8.5 sec earlier than its antipode, which allowed the γ-decalactone-sensitive receptors to recover between stimulation events.

Trap Catch O. eremita: In 2006, 332 females were caught in odor-baited traps with significantly more caught in traps baited with the (*R*)-enantiomer compared to those baited with the racemic mixture (with half the amount of (*R*)-enantiomer) (200 vs. 132; $\chi^2=13.93$; $df=1$; $P<0.001$; Fig. 3a). Also, 87 captures of males were observed, but no difference was observed in the number of captures between odor treatments (51 vs. 36 for *R* and *R*+*S*, respectively; $\chi^2=2.59$; $df=1$; $P>0.05$; Fig. 3a). In 2007, 122 captures of females in odor-baited traps were observed (Fig. 3b). No significant difference in trap catch was observed between traps baited with the (*R*)-enantiomer and those baited with the racemic mixture (with the same amount of (*R*)-enantiomer) (56 vs. 66; $\chi^2=0.82$; $df=1$; $P>0.05$; Fig. 3b). Seven and three males were captured in attractant-baited traps with (*R*)-enantiomer and the racemic mixture, respectively, and three females were found in control traps.

E. ferrugineus: In 2006, 143 captures of 139 individuals were observed (Fig. 3a). The majority of trapping events (60%) included single individuals, whereas up to six beetles were found in a trap on other occasions. Similar to *O. eremita* females, significantly more insects were caught in traps baited with the (*R*)-enantiomer compared to those baited with the racemic mixture with half the amount of (*R*)-enantiomer (88 vs. 55; $\chi^2=7.62$; $df=1$; $P<0.01$). The subset of 23 beetles used for sex determination contained 22 females and only 1 male. In 2007, only six females were trapped (three for each treatment, data not shown in Fig. 3b).

Discussion

In this study, we show both electrophysiological and behavioral evidence for enantiomeric anosmia in the pheromone communication system of *O. eremita*. Recordings from single sensilla showed that both sexes have

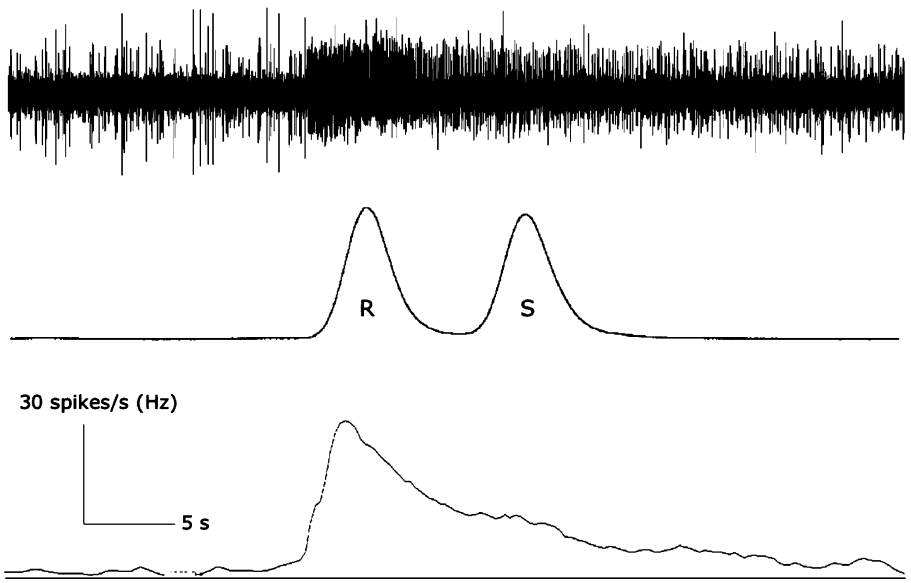

Fig. 2 Example of enantiomer-specific response to γ-decalactone in the ORN of a female *O. eremita* using GC–SSR. The *upper* trace shows the spike activity of the ORN, the *middle* trace shows the FID response to the two enantiomers separated on a chiral column, and the *lower* trace shows the spike activity of the ORN calculated as the number of spikes per second. After responding to the (*R*)-enantiomer, the ORN recovers almost completely and no second activity peak is observed during stimulation with the (*S*)-enantiomer

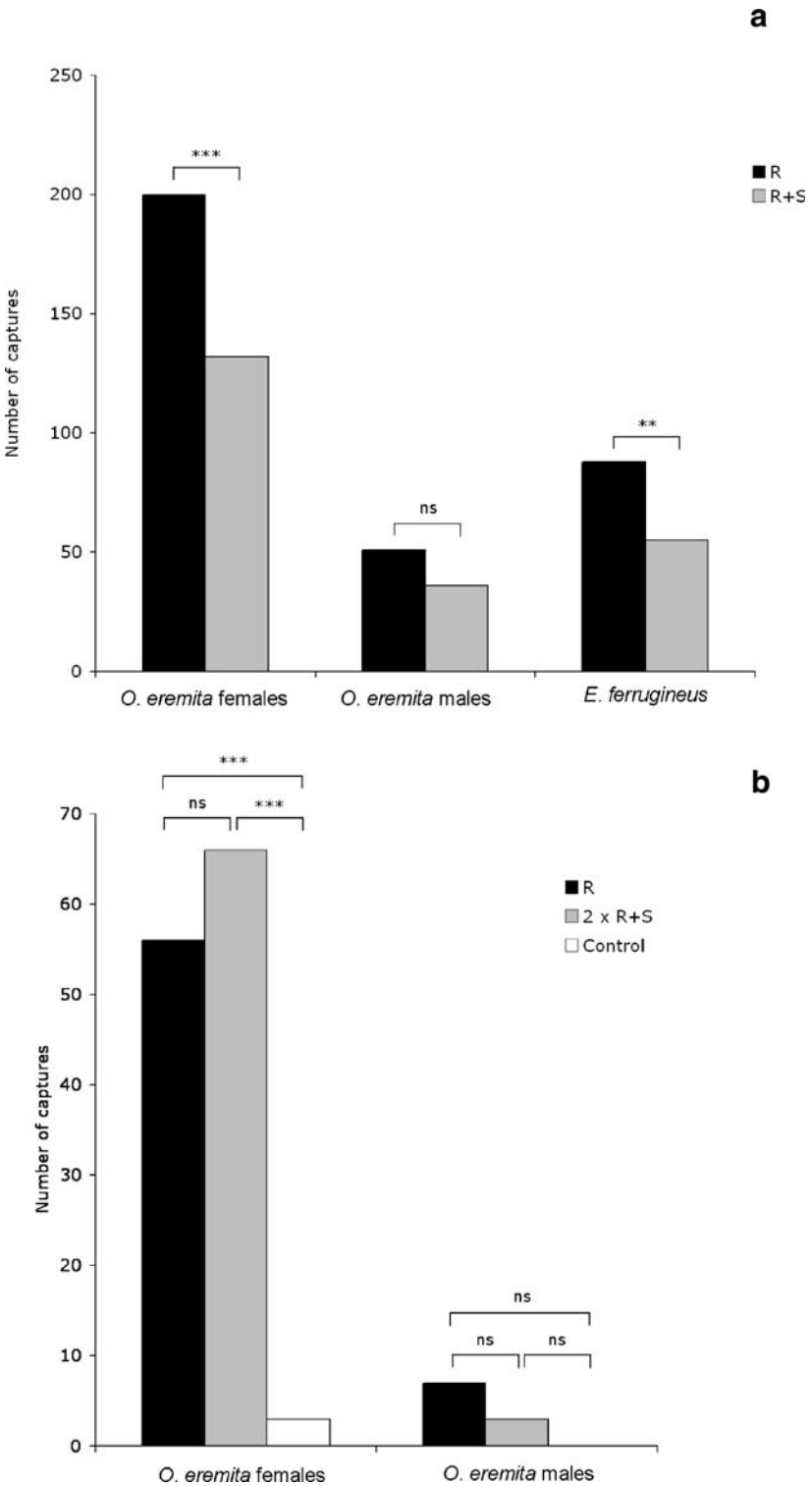

Fig. 3 **a** Trap catch of *O. eremita* and *E. ferrugineus* in 2006 using traps baited with 600 µl of (*R*)-enantiomer (*R*) or 600 µl of the racemic mixture (*R*+*S*) of γ-decalactone (χ^2 test: ***P*<0.01, ****P*<0.001). **b** Trap catch of *O. eremita* in 2007 using traps baited with 600 µl of (*R*)-enantiomer (*R*) or 2×600 µl of the racemic mixture (2×*R*+*S*) of γ-decalactone, and control traps (χ^2 test: ****P*<0.001)

ORNs specific to the (*R*)-enantiomer of γ-decalactone, the compound produced by conspecific males. No neurons tuned to the opposite enantiomer were found in the sensilla investigated. In dose–response trials that used the (*R*)-enantiomer and the racemic mixture as stimuli, the spike frequency of female ORNs was significantly higher for the pure (*R*)-enantiomer than for the mixture (with half the amount of (*R*)-enantiomer) at the highest dose (100 µg), also indicating that those neurons do not respond to the (*S*)-enantiomer (Fig. 1b). These observations were confirmed by using coupled gas chromatography and single-sensillum recordings where the two enantiomers were resolved on a

chiral column: the neurons always responded to the first-eluting (*R*)-enantiomer and never to the later-eluting (*S*)-enantiomer (Fig. 2).

Field trapping data were in concordance with electrophysiological data, and the attraction of both *O. eremita* females and its predator *E. ferrugineus* to attractant-baited traps was correlated with the amount of (*R*)-enantiomer released from lures. In 2006, significantly more captures of *O. eremita* females and *E. ferrugineus* were observed in traps with pure (*R*)-enantiomer compared to the racemic mixture, releasing half the amount of (*R*)-enantiomer. In 2007, no difference in trap catch of *O. eremita* females was observed when both treatments released the same amount of (*R*)-enantiomer. Thus, there is no inhibitory effect of adding the (*S*)-enantiomer to the *O. eremita* pheromone. From a practical point of view, the choice of lure is not critical, and the much cheaper racemic mixture can be used if the lure constitutes a significant part of the trap costs.

Effects of nonpheromonal enantiomers on insect pheromone attraction range from attractive to neutral to strong antagonistic effects (Mori 1998). The lack of inhibitory effects on the attraction of *O. eremita* and *E. ferrugineus* by the antipode of their pheromone/kairomone is in stark contrast to many other cases where the presence of the wrong enantiomer, sometimes at concentrations of only a few percent, results in strong inhibition of response to the pheromone. This phenomenon is common in many insect groups that use chiral pheromones, including scarab (Tumlinson et al. 1977; Leal 1996; Tolasch et al. 2003) and other beetles (Birch et al. 1980; Levinson and Levinson 1999; Lacey et al. 2004), gall midges (Hillbur et al. 2001), and moths (Szöcs et al. 1993; Larsson et al. 2002). The generally accepted explanation for this strong antagonistic effect is that it prevents cross-attraction of sympatric, usually congeneric, species that use the antipode as a pheromone component. Electrophysiological investigations of insect olfactory receptor neurons have demonstrated special sensory adaptations for avoiding enantiomers produced by heterospecifics. Species that use chiral pheromones often possess dedicated olfactory receptor neurons that respond selectively to each enantiomer, also when only one of these constitutes a pheromone component (Okada et al. 1992; Larsson and Hansson 1998; Larsson et al. 2002; Wojtasek et al. 1998).

In several scarab species, racemic mixtures do attract significant numbers of beetles, indicating that these species are indifferent to the presence of nonpheromonal enantiomers (Leal 1999). Absence of biological activity to nonpheromonal enantiomers has been demonstrated in the scarabs *Anomala octiescostata* Burmeister and *A. cuprea* Hope, for which the racemic mixture and pure enantiomer are equally attractive (Leal 1999). Electrophysiological investigations have shown that both species appear to be anosmic to the nonpheromonal enantiomers, entirely lacking receptors for their detection (Larsson et al. 1999, 2001; Leal 1999), which seems to be the case also for *O. eremita*. When the nonpheromonal enantiomer in a racemic mixture cannot be detected, the expected outcome might be a slightly lower attraction to the racemate than to the pure pheromone, considering that the release rate of the attractant is only 50% as high in the racemic mixture.

The high numbers of *E. ferrugineus* observed in attractant-baited traps in 2006 suggest that this click beetle may have higher populations than previously recognized. Scientists used to dealing with pest insects may not consider 139 beetles impressive, but it is close to the total of 147 specimens documented in Swedish collections until 1993 (Nilsson and Baranowski 1994) and is certainly many times more than entomologists have previously encountered during a lifetime. Earlier investigations of only eight *E. ferrugineus* individuals found in attractant-baited traps indicated that both males and females might be attracted to the *O. eremita* pheromone (Svensson et al. 2004). This study, with a larger sample analyzed, showed that the pheromonal signal is exploited almost exclusively by females, presumably to find trees containing *O. eremita*. The much lower number of *E. ferrugineus* captured per trap in 2007 suggests that populations of this predator fluctuate much more than populations of its prey.

Many trees suitable for saproxylic beetles cannot be analyzed with traditional methods, such as wood mold sampling or pitfall trapping, because of the characteristics of their hollows. For example, only about 25% of the oaks in the current study areas that potentially harbor *O. eremita* and *E. ferrugineus* have been investigated because other hollows have been too far up in a tree to be reached by a ladder or the cavity has been too deep to place a trap in the wood mold (Hedin and Mellbrand 2003). With a majority of suitable habitat patches not investigated, estimates of population sizes of focal species may be severely biased, which may in turn affect conservation strategies. With efficient nondestructive trapping systems available, we can now gather detailed ecological data about these species that have not been possible with previously available methods. Attractant-baited traps placed outside tree hollows will only catch those individuals that have left their natal tree and should be used as a complementary method to pitfall traps and other methods to increase the accuracy of population size estimates and analyses of dispersal rates and dispersal distances. Odor traps may also be used for targeted efforts to rediscover species at localities where they have not been observed for sometime. Our data show that attractant-based monitoring could be a powerful tool for conservation purposes, and more research should be focused on the chemical ecology of threatened insects to develop new strategies for their preservation.

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