

Pheromone release behaviour in females of *Phyllonorycter strigulatella* (Lien. & Z.) and *Ph. sorbi* (Frr.) (Lepidoptera, Gracillariidae) under daily cycling temperature regime

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The pheromone release postures of virgin *Phyllonorycter strigulatella* and *Ph. sorbi* females were very similar to those of other species of the same genus with antennae held close to the wings, which were slightly spread and lowered, the abdominal tip dorsally curved and the distal segments extended. In both species with equal sex ratios, one peak of signalling activity was registered 0.5 h after the light had been put on and when 84% of *Ph. strigulatella* and 90% of *Ph. sorbi* individuals were active. The high pheromone release behaviour with 50% active females lasted for two hours. The calling activity of the group of females was about 6 h/day for both species. The total period of calling activity of an individual female lasted for 131 ± 62 min (mean \pm SD) and 145 ± 73 min a day for *Ph. strigulatella* and *Ph. sorbi* respectively. The occurrence of one signalling peak per day in the species with equal sex ratios as found in *Ph. strigulatella* and *Ph. sorbi*, supported the hypothesis that pheromone release activity with a clear presence of two activity peaks during a photophase could be adaptive for species with a sex ratio strongly shifted towards females, as in *Ph. junoniella*.

Key words: leaf-miner, diurnal rhythm, calling, communication, behaviour

INTRODUCTION

Female moths of the species that use chemical communication for mate searching release their sex pheromones under favourable environmental conditions during a certain period of a day. According to Webster (1988), every species has a distinct time frame or a gate for pheromone release at each temperature within an appropriate range, and calling can occur at any combination of time and temperature within that calling window.

The strict timing of sex pheromone production is one of the mechanisms employed by insects to achieve species specificities of mate search signals. Inter- and intra-specific interactions by chemical means in the leaf-mining moth family *Gracillariidae* have been studied by our group for several years. These interactions can occur under certain conditions, one of which is an overlap of communication periods of interacting species. In addition, in order to obtain information about the chemical structure of the compounds used to transfer information among moths, these compounds should be collected during female calling periods. For such investigations, however, the diurnal rhythms of calling as well as

the environmental conditions favourable for those activities must be known. To our knowledge, such data are available for seven *Phyllonorycter* species.

The aim of the present study was to investigate the diurnal rhythms of pheromone release behaviour of virgin *Ph. strigulatella* and *Ph. sorbi* females with a common sex ratio and to compare the results with the ones obtained with *Ph. junoniella* (Z.) species with a distorted sex ratio under the same cycling temperature regime.

MATERIALS AND METHODS

Insects. Leaves with *Phyllonorycter strigulatella* (Lien. and Z.) and *Ph. sorbi* (Frr.) (Lepidoptera, Gracillariidae) mines were collected from grey alders, *Alnus incana* (L.) and rowans, *Sorbus aucuparia* L., respectively, at the end of October, just before the shedding of leaves. The mined leaves collected in the autumn were kept until the next spring on moistened peat moss in an outdoor container under conditions very similar to the natural ones. In spring, the leaves with diapausing pupae were placed in 28 ml glass vials (three mines per vial). The vials were closed with sponge stoppers which

were moistened regularly. Reactivation was carried out at a laboratory under the photoperiod and temperature conditions described below. After emergence, adult moths were collected daily just after the onset of the photophase, then sexed and placed into individual holding vials (28 ml volume), provided with a solution of 5% of sucrose in water.

Light/dark regime. The reactivation of the pupae and registration of the calling activities of females were conducted under a 16:8 h light/dark regime. A 1000 W daylight lamp (type DRF, for use in greenhouses) was used as the source of light. There was a 5-minute transition period of changing the light intensity at both dawn and dusk, arranged by a 300 W incandescent lamp and an automatic voltage changing device. The transition periods were considered as parts of the photophase. The photophase started at 7.00 a. m. and terminated at 11.00 p. m. (local summer time), which reflected natural conditions, as during the experiment the sun rose at c. 6.30 a. m. and set at c. 10.00 p.m.

Temperature conditions. The temperature was maintained nearly constant during the scotophase, with irregular fluctuations within the range 14 ± 1 °C. From the start of the photophase, it was gradually raised to 20 ± 1 °C. This temperature was reached in two hours, then kept constant until two hours before the end of the photophase, and then gradually decreased, reaching 14 °C when the next scotophase began.

Registration of the calling activity of females. The females of both species were found not to call during the first day after emergence. The behaviour of 2- to 4-day-old virgin females of *Ph. strigulatella* ($n = 18$) and *Ph. sorbi* ($n = 14$) was recorded basing on pheromone release postures. The calling behaviour during the scotophase was observed using a red 40 W incandescent lamp placed 2.7 m above the vials carrying the insects. During the periods of calling behaviour, observations were made every half an hour. In the non-calling periods, registrations were made at 1 h intervals. Each female was considered to start calling half an observation interval before her first calling observed and to finish calling half an observation interval after the last calling observed. The total period of calling activity of an individual female was calculated from results obtained during the third day after emergence.

RESULTS

Calling, intermediate and resting postures. The virgin calling females of the *Ph. strigulatella* and *Ph. sorbi* species remained immobile on the underside of the host plant leaves with the antennae held close to the wings which were slightly spread and lowered; the abdominal tip was curved dorsally and the distal segments were extended. An intermediate posture between the calling and the resting ones was noted. It differed from the calling posture in that the abdominal tip was less curved and the distal segments were not extended.

Thus, the pheromone gland was not exposed and the pheromone was not released. In the resting posture, the abdomen was kept straight and remained under wings.

Calling behaviour under the cycling temperature. Females of the *Ph. strigulatella* species started calling about one hour before the onset of the photophase, while the pheromone release activity of *Ph. sorbi* females commenced at the beginning of the photophase. In both species, one peak of signalling activity was registered 0.5 h after the light had been turned on, when 84% of the *Ph. strigulatella* and 90% of the *Ph. sorbi* individuals were active. The high pheromone release behaviour with 50% active females lasted for two hours. The calling activity of the group of females was about 6 h/day for both species (Figure). The total period of calling activity of each individual female lasted for 131 ± 62 min (mean \pm SD) and 145 ± 73 min a day for *Ph. strigulatella* and *Ph. sorbi*, respectively.

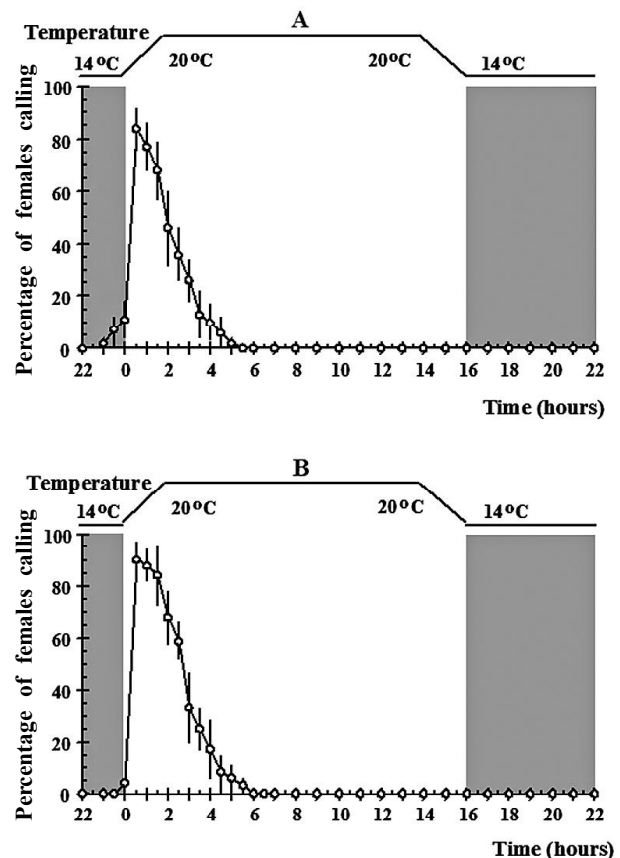


Figure. Diurnal rhythm of calling activity in virgin females of *Phyllonorycter strigulatella* (A) and *Ph. sorbi* (B) under cycling temperature conditions

Vertical bars indicate standard deviations (SD), dashed areas indicate scotophases, white area represents photophase. The females were not found calling on the first day after emergence. One-day results are counted as single replication starting from the second day after emergence. (A) $n = 18$, (B) $n = 14$ females in each of three replications.

DISCUSSION

The calling (or pheromone release) postures of *Ph. strigulatella* and *Ph. sorbi* females were very similar to those of other species of the same genus, *Ph. ulmifoliella* (Hbn.) (Mozūraitis et al., 1997), *Ph. blancardella* (F.) (Mozūraitis et al., 1999) *Ph. acerifoliella* (Z.) and *Ph. heegerella* (Z.) (Mozūraitis et al., 2000), *Ph. emberizaepenella* (Bouché) (Mozūraitis et al., 2002), *Ph. junoniella* (Z.) (Mozūraitis, Būda, 2006) and to a number of pyralids, for example *Ephestia kuhniella* (Z.) (= *Anagasta kuhniella* Z.) (Traynier, 1968) and *Plodia interpunctella* (Hb.) (Brady, Smithwich, 1968). The most pronounced differences were noted in the positions of the antennae. The antennae of calling pyralids were raised (Traynier, 1968; Barrer, Hill, 1977), but not those of *Ph. strigulatella* and *Ph. sorbi*. In these species they were placed along the wings as in other phyllonoryctids.

As regards the time of sexual activities in moths, it is known that a great majority of the females release sex pheromones and copulate during the dark period of a day (see reviews: Dreisig, 1986; McNeil, 1991; and papers: Kou, 1992; Kamimura, Tatsuki, 1993; West, Bowers, 1994; Monti et al., 1995; del Socorro, Gregg P., 1997; Kinjo, Arakaki, 1997; Kawazu, Tatsuki, 2002; Batista-Pereira et al., 2004; del Mazo-Cancino et al., 2004; Kessler, Baldwin, 2004; Nagayama et al., 2004; Taki, Nomura, 2004; Mazor, Dunkelblum, 2005). However, some species call during the light period, soon after dawn (Dreisig, 1986; McNeil, 1991; Tóth et al., 1995; Svatoš et al., 1999; Löfstedt et al., 2004) or late in the afternoon (Buda, Karalius, 1985; Dreisig, 1986, and McNeil, 1991), with one species, *Lymantria dispar* (L.), being active during the entire light period (Charlton, Cardé, 1982).

Our data indicate that *Ph. strigulatella* and *Ph. sorbi* belong to the scanty moth group calling at daytime. It is known that the early morning is a typical pheromone release period for moths of the genus *Phyllonorycter* (Mozūraitis et al., 1997; Boo, Jung, 1998; Mozūraitis et al., 1999; Mozūraitis et al., 2000; Mozūraitis et al., 2002). In only one species, *Ph. junoniella*, the calling pattern differs from the ones of other phyllonoryctids by the clear presence of two activity peaks occurring at the beginning and end of a photophase under cycling temperature regime (Mozūraitis, Būda, 2006).

It has been assumed that the occurrence of the second peak of calling activity might be adaptive for species with a sex ratio strongly shifted towards females, e. g., 8 females to 1 male as in *Ph. junoniella* (Mozūraitis, 2000). If the male refractory period between two copulations is shorter than 8 h (the duration of the dark non-calling period), the virgin females releasing sex pheromones may be inseminated during both the morning and evening periods of their activities. This might compensate significantly for small proportions of males in populations (Mozūraitis, Būda, 2006).

The sex ratios of the *Ph. strigulatella* and *Ph. sorbi* species were both found to be close to 1:1, which is

common for most Lepidoptera species (Gillott, 2005). Our results that females of these two species demonstrated only one peak of calling activity, at the beginning of the photophase when they were exposed to the same cycling temperature regime as *Ph. junoniella*, as well as previously published data for *Ph. acerifoliella* and *Ph. heegerella* with the male/female ratio close to 1:1 (Mozūraitis et al., 2000) supported the hypothesis that the unusual calling pattern of *Ph. junoniella* females could be explained by a sex ratio shifted towards females.

In addition, our data showed that females of the *Ph. strigulatella* and *Ph. sorbi* species started to call during the transition period from darkness to light, indicating that light might be an important cue to initiate calling. However, additional experiments are needed to show whether or not this cue induces calling or even sets the biological clock.

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**PHYLLONORYCTER STRIGULATELLA (LIEN. & Z.)
IR PH. SORBI (FRR.) (LEPIDOPTERA,
GRACILLARIIDAE) PATELIŲ FEROMONO
SKLEIDIMO ELGESYS TEMPERATŪRAI KINTANT
PAROS METU**

Santrauka

Neapvaisintos *Phyllonorycter strigulatella* ir *Ph. sorbi* patelės, skleisdamos feromoną, antenas laikė greta sparnų, kurie buvo praskleisti ir nuleisti žemyn, pilvelis išlenktas dorzaliai, distaliniai pilvelio segmentai išstumti. Tokia feromono skleidimo poza būdinga ir kitoms *Phyllonorycter* genties rūšims. Praėjus pusei valandos nuo fotofazės pradžios, stebėtas vienas signalinio

aktyvumo pikas, kurio metu feromoną skleidė 84% *Ph. strigulatella* ir 90% *Ph. sorbi* patelių. Intensyvus feromono skleidimo periodas, kai buvo aktyvios daugiau kaip 50% patelių, truko apie dvi valandas. Abiejų rūšių patelių grupių signalinio aktyvumo trukmės siekė 6 h per dieną. Individualios patelės signalinio aktyvumo trukmė per parą siekė 131 ± 62 min (vidurkis \pm SD) *Ph. strigulatella* ir 145 ± 73 min *Ph. sorbi* rūšiai. Vienas signalinio aktyvumo pikas per parą, kuris buvo registruotas *Ph. strigulatella* ir *Ph. sorbi* rūšims, turinčioms patinų ir patelių santykį, artimą 1:1, patvirtino hipotezę, jog du signalinio aktyvumo pikai per parą gali būti adaptyvūs rūšims, kurių populiacijose vyrauja patelės, kaip yra nustatyta *Ph. junoniella* rūšiai.

Raktažodžiai: minuotojai, paros ritmas, elgsena, plačia-sparnis kertosios kandelės, komunikacija