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Photoperiodic regulation of mating behaviour in the linden bug, *Pyrrhocoris apterus*, is mediated by a brain inhibitory factor

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Abstract. Active inhibition of mating behaviour in a male insect is reported here for the first time. In *Pyrrhocoris apterus* L. (Heteroptera), the most important inhibitory pathway runs from the pars intercerebralis (PI) of the brain and does not pass through the corpora allata. The inhibitory activity of the PI is promoted by short day conditions and suppressed by long days. As the effect of photoperiod is delayed, transfer procedures enabled us to record daily rhythms in mating behaviour during short days. While the extirpation of the PI results in a discrete phase shift of the long day rhythm, there is a much less significant phase shift after this operation during short days. Thus the PI has been shown to mediate the effect of photoperiod on both the inhibition and the rhythm of mating behaviour.
Key words. *Pyrrhocoris apterus*; photoperiod; rhythms in mating behaviour; brain inhibitory factor; pars intercerebralis; corpora allata.

Adult diapause of *Pyrrhocoris apterus* L. (Heteroptera) is regulated by photoperiod. Reproductive activity is stimulated by long day photoperiod, whereas short days are inhibitory. In females, the arrest of ovarian maturation by short days is mediated by an active inhibition of the corpora allata (CA) from the pars intercerebralis (PI) of the brain[1,2]. The most conspicuous feature of diapause in insect males is the absence of mating behaviour and a reduction in size and activity of the accessory glands. While a stimulatory effect of the neuroendocrine system on mating behaviour has been demonstrated[3–6], active inhibition has not been reported. During long days, the intensity of mating behaviour of *P. apterus* males is not decreased by the absence of the CA and corpora cardiaca[7], so it is not likely that the regulatory pathway passes through the CA. Therefore, the aim of the present study has been to determine the role of the PI in the photoperiodic regulation of mating activity in males of *P. apterus*.

Material and methods

Males of *P. apterus* were reared from the egg stage onwards under long day (LD 18:6) or short day (LD 12:12) regimes at 26 ± 2 °C. Insects were given seeds of the lime tree and water ad libitum. Males for use in experiments were paired individually with long day females in Petri dishes. Mating activity was measured as the proportion that formed mating pairs. During the scotophase, observations were made in dim red light. Extirpation of the PI and the CA was performed through an incision in the neck membrane[8]. The neuroendocrine complex (brain + suboesophageal ganglion + corpora cardiaca + CA) was implanted through this incision immediately after extirpation of the PI. In the control males, an incision without implantation was made in the neck membrane. Long day males and short day males were operated on 1–2 weeks and 3–4 weeks after ecdysis, respectively.

Results and discussion

Males kept permanently under long day conditions exhibited a daily rhythm with a maximum in the night. After extirpation of the PI, the mean mating activity slightly increased and the phase of the daily rhythm was significantly shifted by 11.4 h (fig. 1A). This indicated that: 1) the mating activity is stimulated from outside the PI, 2) the PI has a slight inhibitory effect on the mating activity, and 3) the PI has an important effect on the phase of the activity rhythm under long day conditions. The rhythm itself was not disturbed by the extirpation of the PI. In contrast, even a partial destruction of the PI results in a considerable increase of locomotor activity and loss of rhythmicity in *Acheta domesticus*[9]. While mating behaviour is slightly stimulated by extirpation of the PI in *P. apterus*, it is not affected by cauterization of the PI in *Gomphocerus rufus*[10] and it is inhibited by electrocoagulation of the PI in *Locusta migratoria migratorioides*[11]. Pener et al.[11] suggest that the stimulatory effect of the PI is not mediated through the CA but that the CA exerts an additional stimulatory effect on mating behaviour.

Males kept permanently under short day conditions had very low mating activity. After extirpation of the PI, the mating activity increased considerably to a level similar to long day males (fig. 1B, table). This experiment showed that the inhibitory effect of short days is mediated by the PI. In contrast to long day conditions, the inhibitory effect of the PI in short days is very strong.

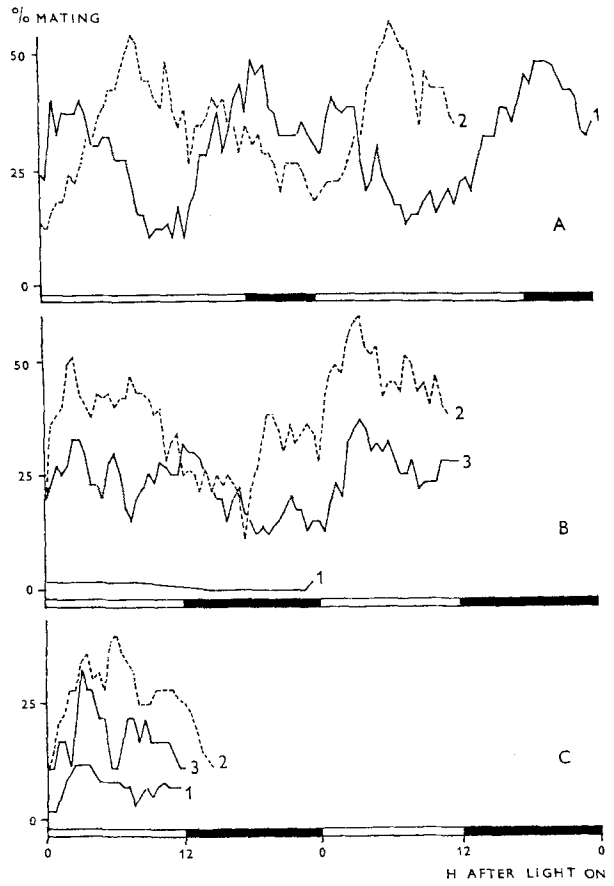

Figure 1. Daily changes in mating activity of *P. apterus*. The proportion of mating pairs in each group was recorded every 30 min.
A Long day males 14 days after operation; 1, controls (n = 60); 2, PI extirpated (n = 50).
B 1 and 2, short day males 14 days after operation; 1, controls (n = 60), 2, PI extirpated (n = 53); 3, males 14 days after transfer from long days to short days (n = 60).
C Short day males 8 days after operation; 1, controls (n = 60), 2, PI extirpated (n = 53); 3, PI and CA both extirpated (n = 19).
Lines B1, C1, and B2, C2 represent the same groups of males observed at different days after operation. Phase relationship of maximum mating activity to light on (Ph) and mean mating activity (I) were calculated as parameters of cosine regression. These parameters were compared by t-test.
A1 – Ph = 21.29 ± 0.26 h; I = 29.61 ± 0.62%. A2 – Ph = 9.88 ± 0.34 h; I = 32.90 ± 0.81%; values are significantly different at $p = 6.3 \times 10^{-13}$ (Ph A1–A2), $p = 6.4 \times 10^{-4}$ (I A1–A2).
B1 – I = 0.60 ± 0.06%. B2 – Ph = 4.80 ± 0.31 h, I = 35.17 ± 0.77%. B3 – Ph = 7.60 ± 0.50 h, I = 22.68 ± 0.55%; values are significantly different at $p = 4.7 \times 10^{-6}$ (Ph B2–B3), $p = 7.8 \times 10^{-14}$ (I B1–B2), $p = 3.2 \times 10^{-12}$ (I B2–B3).
C1 – I = 4.84 ± 1.22%. C2 – I = 19.45 ± 1.08%. C3 – I = 10.34 ± 2.88%; values are significantly different at $p = 4.9 \times 10^{-11}$ (C1–C2), p = 0.041 (C1–C3), $p = 9.96 \times 10^{-4}$ (C2–C3).

The inhibitory effect of the PI on mating behaviour has not yet been reported for other species. The phase of the rhythm in short day males with an intact PI could not be evaluated due to low mating activity. Therefore the males reared in long day conditions were transferred to short days, and mating activity was recorded 2 weeks after this transfer. These males with an intact PI showed a rhythm of mating activity with the phase differing from PI-ectomized males in short days by only 2.8 h (fig. 1B). Thus the phase of the rhythm was much less influenced by extirpation of the PI under short day than under long day conditions. The mating activity of long day/short day males was lower than that of males kept permanently under long day conditions. Mating stopped only about one month after the transfer from long to short day photoperiod[12].

Mating activity of short day males of *P. apterus* after various surgical treatments

Days after operation	% mating (n) A	B	C	D
2		18.8 (16)		7.9 (14)
3		12.5 (16)		23.1 (13)
7	10.0 (30)	50.0 (14)	42.1 (19)	27.3 (11)
9		64.3 (14)		45.4 (11)
14		85.7 (14)		72.7 (11)

A – controls, B – PI extirpated, C – PI and CA both extirpated, D – PI extirpated and neuroendocrine complex implanted. Males were allowed to copulate for 5 h on each of several days after the operation and the cumulative incidence of mating pairs was recorded. The values obtained on day 7 after operation were compared using 2 × 2 contingency tables. Values are significantly different at p = 0.005 (A–B), p = 0.01 (A–C).

The inhibitory effect of the PI on reproduction in females of *P. apterus* is due to the inhibition of the activity of the CA[1]. The following experiment was carried out to see whether the CA has an analogous role in males. In short day conditions, males of *P. apterus* with both the CA and PI extirpated showed an intermediate mating activity which was lower than that of the controls (fig. 1C). This quantitative difference indicated that an inhibitory factor from the PI acts through two pathways: via the CA and via a rhythmic stimulatory centre outside the PI. However, the pathway via the CA seems to be less important; the implantation of active CA into short day males of *P. apterus* evoked an increase of their sexual behaviour[7] after a much longer delay than did extirpation of the PI. Because the extirpation of the CA from males in long day conditions does not diminish mating behaviour, Zdarek[7] suggests that the CA stimulates mating behaviour via the central nervous system.

Inhibition from the PI may be due to a neurosecretory factor or factors, or to the activity of neurons adjacent to neurosecretory cells. The present data do not provide evidence that inhibition from the PI is transmitted via hemolymph. Nevertheless, involvement of neurosecretory factors may be indicated by two findings: 1) the increase of mating activity after extirpation of the PI was gradual (table), i.e. the time lag between the extirpation and maximum mating activity may be the result of a release of inhibitory material stored in neurosecretory axons; 2) an increase in the mating activity was slightly delayed by implantation of the neuroendocrine complex with the PI intact into PI-ectomized males (table).

Neuropeptides that inhibit mating behaviour in insect males have not yet been identified. Several allatostatins have been isolated and sequenced from *Diploptera punctata*[13]. In *P. apterus*, the inhibition of mating activity transmitted via the CA may be due to allatostatins. Recent analysis of the distribution of allatostatins suggest that they may have functions other than the regulation of juvenile hormone synthesis, e.g. neuromodulation[14]. We can speculate that, in *P. apterus*, the inhibition of mating activity, which is not transmitted via the CA, may also be caused by allatostatins within the nervous system. There are indications that hormones may modulate behavioural rhythms in insects[15,16]. The present results suggest that a factor from the PI modifies the rhythm in mating activity under the effect of photoperiod. It has been shown that phase-shifts produced by exogenous agents (e.g. serotonin) applied to the *Aplysia* eye depend on the rhythm phase when the agents are applied[17]. It is possible that a phase relationship between the rhythm of a stimulatory centre and timing of the release of an inhibitory factor from the PI determines the phase-shifting effect of the PI on the rhythm of mating activity in *P. apterus*. These phase relationships may be different under long day and short day conditions. It has been suggested that phase relations of circadian oscillations could be used as a 'clock' to recognize long days and short days during induction of diapause[18], but the physical basis of these oscillations is not known.

It can be concluded that regulation of mating activity in *P. apterus* involves at least 3 discrete effects: 1) a rhythmic stimulatory effect from outside the PI, 2) a strong inhibitory effect from the PI during short days, and 3) a significant phase-shifting effect from the PI under long day conditions (fig. 2). Inhibition from the PI during long days and the phase-shifting effect of the PI during short days are relatively weak. Under short day conditions, an inhibitory centre within the PI inhibits the activity of the CA and that of a stimulatory centre outside the PI. Mating activity is then very low. Extirpation of the PI results in high mating activity, because both these inhibitory effects are removed. Extirpation of both the CA and the PI results in an intermediate level of mating activity, because there is then no stimulatory effect from the CA. Based on Zdarek's[7] results, it is suggested that the CA stimulate mating activity indirectly via a stimulatory centre outside the PI. Long day conditions maintain high activity of this stimulatory centre. Neither the CA[7] nor the PI are necessary to produce high mating activity.

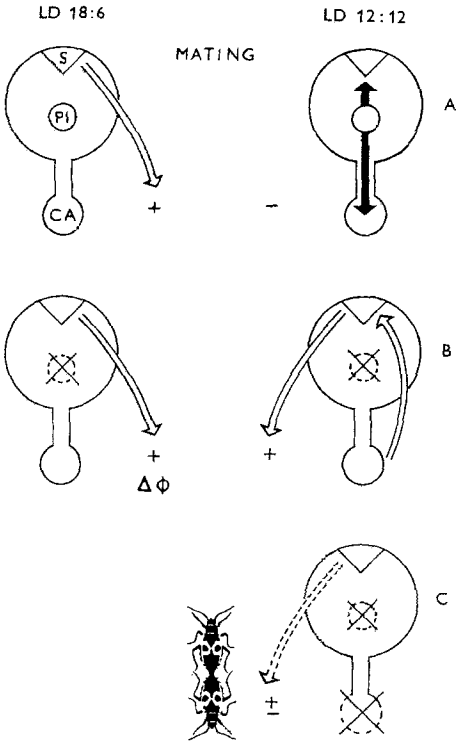

Figure 2. Hypothetical scheme of regulation of mating activity in *P. apterus*.
A controls, *B* PI extirpated, *C* PI and CA both extirpated. Large upper circle = nervous and neuroendocrine systems, S = stimulatory centre outside PI, PI = pars intercerebralis, CA = corpora allata, solid arrows = inhibitory effects, open arrows = stimulatory effects; + high mating activity, ± intermediate mating activity, − low mating activity, $\Delta\phi$ = phase shift.

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