Journal of Insect Physiology 57 (2011) 529–535

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Novel roles for the corpus allatum hormone in the cost of sexual interactions in the linden bug *Pyrrhocoris apterus*

Hana Blazkova [1], Jan Provaznik [1], Magdalena Hodkova *

Institute of Entomology, Biological Centre, Branisovska 31, Academy of Sciences, Ceske Budejovice 37005, Czech Republic

ARTICLE INFO

Article history:
Received 13 October 2010
Received in revised form 4 February 2011
Accepted 4 February 2011
Available online 17 February 2011

Keywords:
Life-span
Mating activity
Allatectomy
Juvenile hormone
Ovary
Vitellogenin
Male accessory glands

ABSTRACT

The cost of sexual interactions, usually expressed as a reduction of life-span, is a fundamental but poorly understood aspect of life. According to a widely accepted view, a rise in the "pro-aging" juvenile hormone (JH) might contribute to the decrease of life span caused by sexual interactions. We tested this hypothesis using the linden bug *Pyrrhocoris apterus* by removing the corpus allatum (CA), the source of JH. If JH is causally involved in the cost of sexual interactions, then the absence of CA (JH) should decrease the negative effect of sexual interactions on survival. As expected, ablating the CA significantly prolonged life-span of both virgin females and virgin males. Mated insects of both sexes lived significantly shorter than virgins. However, contrary to prediction, the decrease of life span by sexual interactions was similar in control and CA-ablated males, and was even enhanced in CA-ablated females. Another unexpected finding was that males paired with CA-ablated females lived almost as long as virgin males and significantly longer than did males paired with control females, although ablating the female CA did not cause any decrease in mating activity. On the other hand, females paired with CA-ablated males lived only slightly longer than did females paired with control males. These results highlight several important points. (1) In both genders, the negative effect of sexual interactions on insect's survival is not mediated by the insect's own CA. (2) The male CA has only minor effect on female survival, while (3) the female CA (JH) is principally responsible for the sex-induced reduction in the male survival.

1. Introduction

Negative effects of mating on survival is of widespread occurrence, but the underlying physiological mechanisms are not well understood. In many species, male accessory gland proteins (Acps) transferred to females during mating reduce female survival, thus constituting a cost of reproduction (Arnqvist and Nilson, 2000; Gillot, 2003). In females of *Drosophila melanogaster*, Acp 70 (sex peptide) causes the mating cost (Wigby and Chapman, 2005). This peptide also stimulates biosynthesis of the principal reproductive hormone of insects, the juvenile hormone (JH) by the corpus allatum (CA) in vitro (Moshitzky et al., 1996). Mating was shown to enhance JH levels in many other species, for instance in females of the moth *Heliothis virescens* (Ramaswamy et al., 2000), males of the Carribean fruit fly *Anastrepha suspensa* (Teal et al., 2000), males, but not females of the burying beetle *Nicrophorus orbicollis* (Scott and Panaitof, 2004; Trumbo and Robinson, 2004) or males and females of the mealworm *Tenebrio molitor* (Rolff and Siva-Jothy, 2002). A negative effect of JH on life span was convincingly shown in grasshoppers (Pener, 1972), butterflies (Herman and Tatar, 2001) and in the linden bug *Pyrrhocoris apterus* (Hodkova, 2008), where surgical removal of the CA, the source of JH, considerably prolonged life span. Therefore, the enhanced production of JH is thought to be at the basis of elevated egg production and shorter life span after mating (Chapman et al., 1995; Rolff and Siva-Jothy, 2002; Kubli, 2003; Wigby and Chapman, 2005). However, a causal relationship between the mating-induced increase of JH levels and survival has never been experimentally tested.

Another question is whether insect's survival may be influenced by the JH of sexual partners. This question has not yet been addressed, although it arises from available experimental data. The negative effect of Acps on female survival (Chapman et al., 1995) might depend on male JH because JH stimulates growth and protein synthesis in the male accessory glands of many insects (e.g. Chen, 1984; Yamamoto et al., 1988; Wilson et al., 2003; Parthasarathy et al., 2009) including *P. apterus* (Socha et al., 2004). Furthermore, JH participates in the regulation of pheromone production (e.g. Cusson and McNeil, 1989; Smith and Schal, 1990; Trabalon et al., 1990; Fan et al., 1999; Sréng et al., 1999; Rafaeli and Bober, 2005) and a substance(s) called dauer inducing pheromone can extend the life span of adult *Caenorhabditis elegans* (Kawano et al., 2005).

* Corresponding author. Tel.: +420 38 777 5253; fax: +420 38 531 0354.
E-mail address: magda@entu.cas.cz (M. Hodkova).
[1] The first two authors contributed equally to this work.

0022-1910/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2011.02.007

The main aim of this study was to determine whether the CA is causally involved in the decrease of life-span caused by continuous exposure of insects to sexual partners. Hypothetically, the CA may have two principal roles in the survival cost of sexual interactions. First, the sex-induced decrease of life-span of an individual may be mediated by changes in the activity of its *own CA*. Second, survival may be reduced by the *sexual partner's CA* via its effect on the secretion of signalling substances (e.g. accessory gland peptides, pheromones). We tested these hypotheses by using males and females of *P. apterus* with surgically removed CA. If the individual's own CA mediates the cost, then the sex induced reduction of life-span should be more extensive in control (with intact CA) than in allatectomized insects. On the other hand, if the sexual partner's CA is responsible for the cost, then the reduction of life-span should be more extensive in insects exposed to allatectomized than to control partners.

2. Materials and methods

2.1. Insects

Colonies of *P. apterus* (L.) (Heteroptera) originated from insects collected in the field near Ceske Budejovice (south Bohemia). Insects were reared at $26 \pm 1\,^{\circ}C$ and a diapause-preventing photoperod of 18 h light/6 h darkness and supplied ad libitum with linden seeds and water. The mean duration of larval development was about 1 month.

2.1.1. Experiment 1: isolated virgins vs. isolated pairs

Males and females were selected separately from the colony within 24 h after adult ecdysis, and were deprived of food. Two days later, insects destined for operation were narcotized by submergence in water for 15 min and the CA was removed through the neck membrane incision under Ringer insect saline as described (Slama, 1964a,b). Control insects were either sham-operated (neck membrane was cut) or left intact. Insects were then kept in Petri dishes with linden seeds and water, either individually (virgins) or in pairs until death. Mortality, mating status and egg numbers were checked every two days. Dead sexual partners were replaced from stock insects (intact or allatectomized) or widows and widowers were combined.

Seven experimental groups were examined: virgin control males, virgin control females, virgin allatectomized males, virgin allatectomized females, control pairs (both partners with the CA), pairs with allatectomized males, pairs with allatectomized females.

2.1.2. Experiment 2: homosexual vs. heterosexual groups

Intact insects were selected from the colony within 24 h after adult ecdysis and kept in several 500 ml glass containers (cca 50 individuals per container) with continuous supply of food. Four experimental groups were examined: virgin males, virgin females, non-virgin males and females (equal numbers of males and females in container). Mortality was checked every two days.

Insect colonies used in experiments 1 and 2 were offspring of insects collected in the field in different years.

2.2. Mating activity

Earlier data based on short-term (30 min) tests indicate that the CA hormone plays no important role in the control of mating activity in *P. apterus* (Zdarek, 1966, 1968). To check whether ablating the CA influenced mating behaviour in males and females continuously exposed to one another, we recorded the mating status of individual pairs every 30 min throughout a 27 h period. Several parameters were measured: (1) temporal pattern of mating activity (changes in the number of copulating pairs), (2) number of copulation bouts (uninterrupted mating associations) and (3) duration of a copulation bout.

2.3. Statistics

Log-rank tests were used to assess similarity of longevity between the two groups. Differences between parameters of mating activity and fecundity were analyzed by two-way analysis of variance (temporal patterns of mating proportions) or one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test (numbers of copulation bouts, duration of a copulation bout, egg numbers, oviposition and post-oviposition periods). GraphPad Prism 4 software was used.

3. Results

3.1. Experiment 1 – isolated virgins vs. isolated pairs

3.1.1. Effect of the CA on survival of virgins

Ablating the CA prolonged the median life span by 70 days (103%) in virgin females (Fig. 1A). A less extensive increase of median life span by allatectomy was observed in virgin males (33 days, 37%) (Fig. 1B).

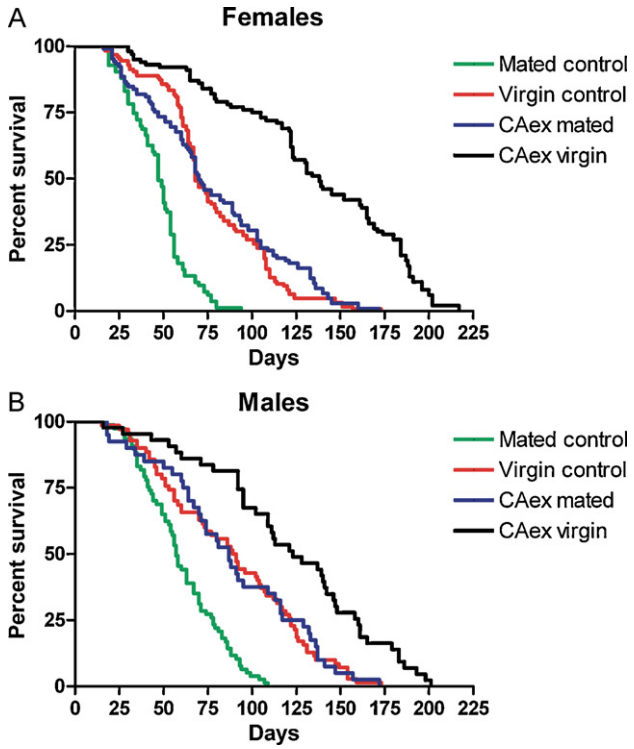

Fig. 1. Effect of the exposure to control (with the corpus allatum) sexual partners on the survival of allatectomized and control *P. apterus* females (A) and males (B). CAex – corpus allatum ablated. (A) Median life spans of females: mated control, 47 days ($n = 83$); virgin control, 68 days ($n = 126$); CAex mated, 70 days ($n = 105$); CAex virgin, 138 days ($n = 100$). Curve comparison: mated control vs. virgin control, $P < 0.0001$; mated control vs. CAex mated, $P < 0.0001$; mated control vs. CAex virgin, $P < 0.0001$; virgin control vs. CAex mated, $P = 0.4129$ (n.s.); virgin control vs. CAex virgin, $P < 0.0001$; CAex mated vs. CAex virgin, $P < 0.0001$. (B) Median life spans of males: mated control, 57 days ($n = 77$); virgin control, 90 days ($n = 70$); CAex mated, 87 days ($n = 40$); CAex virgin, 123 days ($n = 43$). Curve comparison: mated control vs. virgin control, $P < 0.0001$; mated control vs. CAex mated, $P < 0.0001$; mated control vs. CAex virgin, $P < 0.0001$; virgin control vs. CAex mated, $P = 0.9115$ (n.s.); virgin control vs. CAex virgin, $P < 0.0001$; CAex mated vs. CAex virgin, $P < 0.0001$. For all comparisons between survival curves (log-rank test), the statistical significance was confirmed by a Bonferroni correction. Insects were kept as isolated virgins or isolated pairs.

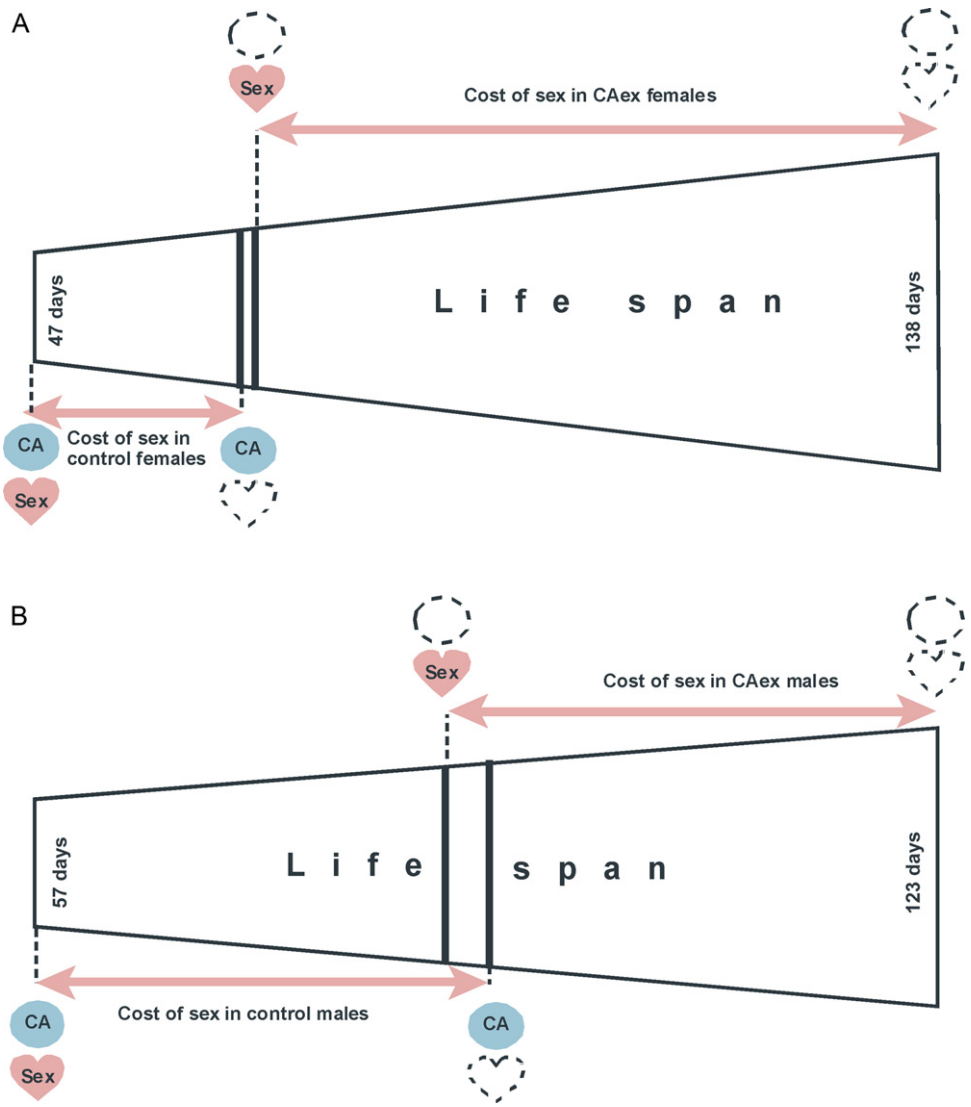

Fig. 2. Relative survival costs of sexual interactions in control and allatectomized *P. apterus* females (A) and males (B). CA – corpus allatum; CAex – CA removed. Median life spans are indicated by vertical lines. The costs of sexual interactions (horizontal lines) are expressed as proportions of the difference between maximum (both the CA and sexual partner absent) and minimum (both the CA and sexual partner present) median life spans. For other information see Fig. 1 and text.

3.1.2. Role of the insect's own CA (endogenous JH) in the survival cost of sexual interactions

Life spans of insects were compared among four groups: (a) paired insects, both sexes with the CA, (b) allatectomized insects exposed to sexual partners with the CA, (c) virgins with the CA and (d) allatectomized virgins.

If the cost of sexual interactions is independent of CA, then the increase of life span produced by both virginity and allatectomy [(d) minus (a), representing the cost of both sexual interactions and CA] should be the additive benefit on life span produced by only allatectomy [(b) minus (a), representing only the cost of CA] and only virginity [(c) minus (a), representing only the cost of sexual interactions]. Otherwise, the CA may influence the cost of sexual interactions in two ways. (1) If the cost of sexual interactions is enhanced by CA, then allatectomy should reduce (or eliminate) the cost of sexual interactions and the difference in life span between (d) and (a) should be lower than the sum of [(b) minus (a)] and [(c) minus (a)]. (2) If the cost of sexual interactions is reduced by the CA, then allatectomy should enhance the cost of sexual interactions and the difference in life span between (d) and (a) should be higher than the sum of [(b) minus (a)] and [(c) minus (a)].

In females (Fig. 1A), the increase of median life span produced by both the absence of males and allatectomy [(d) minus (a)] was 91 days (193.6%), which was, by 47 days (100%), a higher benefit on life span than the sum of effects of only virginity [(c) minus (a)] (21 days, 44.7%) and only ablating the CA [(b) minus (a)] (23 days, 48.9%). These results indicate that the longevity cost of sexual interactions (the benefit of virginity) is enhanced by the absence of the CA or, conversely, the negative effect of males on female life span is reduced by the female CA.

In males (Fig. 1B), the increase of median life span produced by both the absence of females and allatectomy [(d) minus (a)] was 66 days (115.8%), which was almost exactly the additive benefit on life span produced by only virginity [(c) minus (a)] (33 days, 57.9%) and only ablating the CA [(b) minus (a)] (30 days, 52.6%). These results indicate that sexual interactions shorten male life span independently of the male CA.

Effects of the CA and sexual interactions in the regulation of life span are summarized in Fig. 2.

3.1.3. Role of the sexual partner's CA (JH) on the survival cost of sexual interactions

The median life span of control females (with CA) exposed to allatectomized males was by 7 days (14.9%) longer relative to females exposed to control males, while the benefit on median life span produced by the absence of males was 21 days (44.7%)

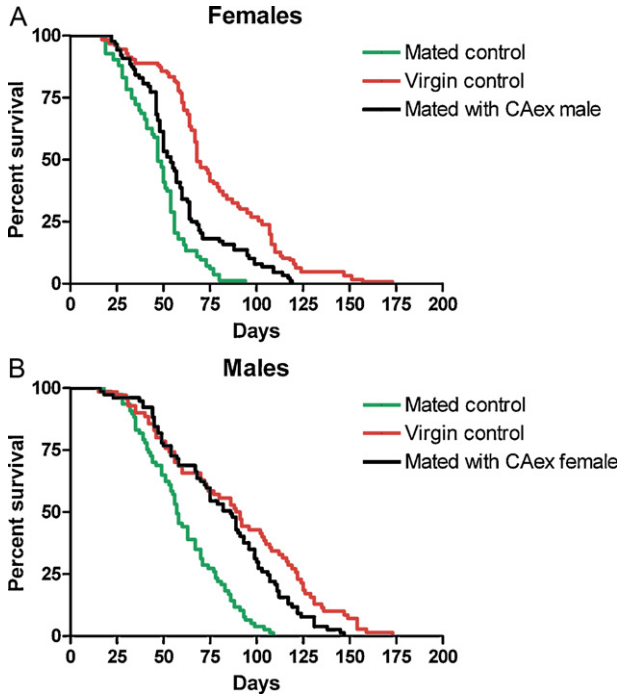

Fig. 3. Effect of the exposure to allatectomized sexual partners on the survival of control (with the corpus allatum) *P. apterus* females (A) and males (B). (A) Median life span of females mated with CAex males, 54 days ($n = 88$). Curve comparison: mated control vs. mated with CAex males, $P < 0.0003$; virgin control vs. mated with CAex males, $P < 0.0001$. (B) Median life span of males mated with CAex females, 86 days ($n = 77$). Curve comparison: mated control vs. mated with CAex males, $P < 0.0001$; virgin control vs. mated with CAex males, $P < 0.0358$. For other information see Fig. 1.

(Fig. 3A). Thus the male CA seems to be responsible for only one third of the negative effect of males on female life span.

The median life span of control males exposed to allatectomized females was by 29 days (50.9%) longer relative to males exposed to control females, which was only a slightly smaller benefit on median life span than that produced by the absence of females (33 days, 57.9%) (Fig. 3B). These results indicate that the female CA is responsible for most (87.9%) of the negative effect of females on male life span.

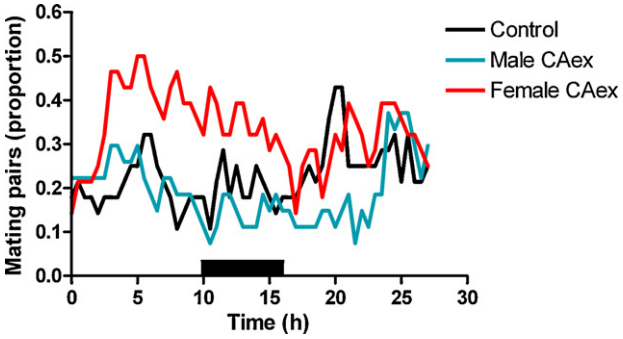

Fig. 4. Effect of allatectomy on the temporal pattern of mating proportions in *P. apterus*. Control – both partners with the corpus allatum (CA); Male CAex – male without CA; Female CAex – female without CA. Mating status was recorded every 30 min for 27 h. Time 0 = 8 h after light on. Solid column indicate the period of darkness. Two-way analysis of variance (ANOVA): The effect of time is considered not significant. The effect of allatectomy is considered extremely significant ($F = 59.94$, $DF_n = 2$, $DF_d = 108$, $P < 0.0001$); Control vs. Female CAex ($F = 56.58$, $DF_n = 1$, $DF_d = 54$, $P < 0.0001$); Control vs. Male CAex ($F = 6.85$, $DF_n = 1$, $DF_d = 54$, $P = 0.0115$). For other information see Table 1 and text.

Table 1
Effect of allatectomy on mating activity in *Pyrrhocoris apterus*.

Insect pairs	*n*	Copulating pairs (%)[a]	Number of copulation bouts[b]	Duration of copulation bout (h)[b]
Normal male Normal female	28	71.4	3.1 ± 0.4 [a]	2.7 ± 0.3 [a]
Normal male Allatectomized female	28	67.9	3.0 ± 0.3 [a]	4.5 ± 0.4 [b]
Allatectomized male Normal female	27	70.4	2.7 ± 0.3 [a]	2.8 ± 0.3 [a]

Two-week old insects were monitored every 30 min during 27 h.
[a] Pairs observed to mate at least once within 27 h.
[b] Means ± SEM are given. Values with different letters are significantly different at $P < 0.01$–0.001 (ANOVA, Tukey's Multiple Comparison Test).

3.1.4. Mating activity

Means of 54 recordings of proportions of mating pairs (every 30 min. during 27 h) were not significantly different between control pairs (both partners with CA, 22.6 ± 0.01%) and pairs of a control female and allatectomized male (19.2 ± 0.02%), but pairs of a control male and allatectomized female showed a significantly higher mean proportion of mating pairs (33.4 ± 0.01%) than pairs of the other two groups (one-way analysis of variance, $P < 0.001$) (Fig. 4). Two-way analysis of variance confirmed the significant increase of mating activity caused by ablating the female CA ($P < 0.0001$), but also indicated a slight, but significant decrease of mating activity caused by ablating the male CA ($P < 0.05$) (Fig. 4). Proportions of mating pairs (checked every second day at arbitrarily chosen hours within photophase) did not show any clear tendency to decrease or increase (data not shown). This observation is consistent with Zdarek's (1970) finding that the mating activity of *P. apterus* does not essentially change throughout the insect's life.

Proportions of pairs observed to mate at least once (67.9–71.4%) and numbers of copulation bouts (2.7–3.1) recorded within 27 h were similar in the three groups, but copulation duration was significantly longer in pairs with an allatectomized female (4.5 h) compared to other two groups (2.7–2.8 h) (Table 1). Differences in the temporal patterns of mating proportions between pairs with an allatectomized female and the other two groups were evidently attributable to differences in the copulation duration. The results indicate that absence of the CA (lack of JH) has no significant effect on the mating activity of males, but females with CA ablation show shorter re-mating intervals (higher receptivity) relative to control females.

3.1.5. Egg production in virgin and mated females

Egg production was monitored in a fraction of females from Fig. 1A and Fig. 3A. Three experimental groups were examined: virgin control, mated control (paired with control male), mated with allatectomized male (Table 2). There was no significant difference in the rate of egg production (eggs/oviposition period) between virgin females and females mated with control males or between females mated with control males and females mated with allatectomized males. Total number of eggs was significantly higher in virgins than in females mated with control males. This difference is attributable to a significantly longer oviposition period in virgin females. Allatectomized females did not produce eggs.

3.2. Experiment 2 – homosexual vs. heterosexual groups

To discriminate between effects of sex-dependent and sex-independent interactions on life span, survival was compared between insects kept in homosexual or heterosexual groups.

The median life span of females kept without males (114 days) was longer than the median life span of females kept with males

Table 2
Egg production in females with intact corpus allatum.

Females	*n*	Eggs (total)	Eggs/oviposition period	Oviposition period (days)	Post-oviposition period (days)	Life span (days)[a]
Virgin control	75	433.0 ± 18.0 [a]	7.4 ± 0.2 [a]	59.9 ± 2.4 [a]	20.7 ± 2.5 [a]	80.2 ± 3.5 [a] (70.0)
Mated control	56	312.0 ± 21.8 [b]	7.8 ± 0.3 [ac]	41.6 ± 2.1 [b]	8.2 ± 0.9 [b]	49.7 ± 2.2 [b] (50.0)
Mated with males CAex	58	396.7 ± 21.0 [a]	8.6 ± 0.3 [bc]	46.1 ± 2.5 [b]	13.4 ± 1.9 [b]	59.6 ± 3.4 [b] (54.0)

Means ± SEM are given. Values with different letters are significantly different at $P < 0.05$–0.001 (ANOVA, Tukey's Multiple Comparison Test).
[a] Numbers in parenthesis are median life spans.

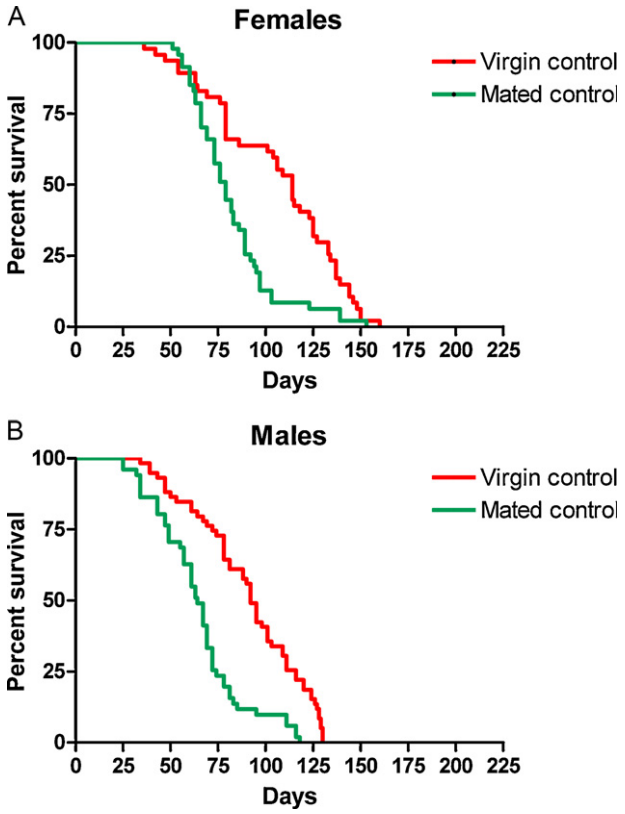

Fig. 5. Survival of heterosexual and homosexual groups of females (A) and males (B). Median life spans of females: homosexual group, 114 days ($n = 47$), heterosexual group, 79 days ($n = 47$); $P = 0.0001$. Median life spans of males: homosexual group, 92 days ($n = 59$); heterosexual group, 64 days ($n = 51$); $P < 0.0001$. Differences between survival curves were analyzed by log-rank test.

(68 days) (Fig. 5A). The absolute life spans of females originated from the colony used in this experiment were longer than life spans of females originated from the colony used in experiment 1 (Fig. 1A). However, the relative benefits of virginity on life spans were similar, 44.3% and 44.7%, respectively. These results indicate that female survival is not influenced by homosexual interactions.

The median life span of males kept without females (92 days) was by 43.8% longer than the median life span of males kept with females (64 days), suggesting an important negative effect of heterosexual interactions on male life span (Fig. 5B). In experiment 1, isolated virgin males lived 57.9% longer than males in heterosexual pairs (Fig. 1B). A slightly higher benefit of virginity on life span of isolated males compared to males kept in homosexual groups indicates a slight negative effect of homosexual interactions on male survival. In the absence of females, males were observed to court other males.

4. Discussion

We show that the CA (JH) as well as sexual interactions significantly reduce life span in both males and females of *P. apterus*. The most important outcome of our study is the disclosure of novel and unexpected roles of the CA on longevity costs as a function of sexual interactions.

4.1. CA and survival of virgins

Consistent with earlier results (Hodkova, 2008), ablating the CA remarkably prolonged life span of virgin females of *P. apterus* (Fig. 1A). In addition we showed that a less extensive, but significant increase of life span was produced by ablating the CA in males (Fig. 1B). A higher survival cost of the CA in females relative to males might be related to the CA volume, which is nearly two times larger in females than in males (Hodkova and Socha, 2006; Socha and Hodkova, 2006).

Although it is clear that JH plays a role in the control of longevity, the way it does so remains unknown. Metabolic rate and egg production (both stimulated by JH in females) seem not to be important in determining longevity, because oxygen consumption is similar in CA-ablated and ovary-ablated females of *P. apterus* (Slama, 1964a), while ovary-ablation has no effect on life span in either intact or allatectomized females (Hodkova, 2008). Furthermore, allatectomy has no effect on oxygen consumption in males of *P. apterus* (Slama, 1964b).

A tissue that may potentially link CA-signalling and the physiological processes related to aging is the fat body. In females of many insects, including *P. apterus*, JH promotes vitellogenin synthesis in the fat body (Socha et al., 1991; Wyatt, 1997; Soller et al., 1999). Interestingly, antiDAF-16 of *C. elegans* (the homolog of dFOXO) represses vitellogenin mRNA, and the knockout of vitellogenin genes prolongs life span (Murphy et al., 2003). However, the absence of vitellogenins cannot explain life span extension by the CA ablation in males of *P. apterus*, because they do not produce vitellogenins (Socha et al., 1991). Life span of *D. melanogaster* is extended by transgenic over-expression of the insulin signalling responsive transcription factor encoded by *dfoxo* in the fat body (Ginnakou et al., 2004; Hwangbo et al., 2004). Defining interactions between JH and FOXO will be the subject for the next phase of our study of the endocrine regulation of aging in males and females of *P. apterus*.

4.2. CA and survival of females exposed to males

As expected, continuous exposure to males significantly reduced the life span of control females. Relative benefit of virginity on life span was similar in isolated virgins vs. females kept in isolated pairs (44.7%, Fig. 1A) and in females kept in homosexual vs. heterosexual groups (44.3%, Fig. 5A), although the absolute longevities were different (females originated from different laboratory colonies). An unexpected finding was that the reduction of life span (50% survival) caused by sexual interactions was more extensive in allatectomized than in control females (Fig. 2). A possibility that the presence of males stimulates the production of JH in intact females cannot be excluded. However, the benefit of allatectomy on life span was higher in virgin than in non-virgin females (Fig. 2), indicating that the negative impact of the CA on female survival was rather enhanced by virginity. Interestingly, mating as well as *D. melanogaster* sex peptide inhibit JH biosynthesis in the Mediterranean fruit fly (*Ceratitis capitata*) CA

in vitro (Moshitzky et al., 2003). These results contrast with the stimulatory effect of *D. melanogaster* sex peptide on the CA of *D. melanogaster* and a moth *Helicoverpa armigera*, where JH production by CA in vitro increases after mating in these and other species (Moshitzky et al., 1996; Shu et al., 1998; Fan et al., 1999; Holbrook et al., 2000). In any case, our results provide direct evidence that enhanced activity of the CA, including JH production, is not the likely mechanism by which sexual interactions shorten the life span in females of *P. apterus*. On the contrary, the cost of sexual interactions (the difference between survivals of virgins and non-virgins) was enhanced by the absence of the CA (Fig. 2).

The present findings imply that downstream consequences of JH effects, such as the synthesis of vitellogenins by fat body or eggs production are not causally related to the sex-dependent decrease of female life span, because these activities are absent in allatectomized females (Socha et al., 1991). This conclusion is also supported by observations that the rate of egg production is similar in mated and virgin females and the lifetime production of eggs was even higher in the latter (Table 2). Consequently, the difference in longevity between non-virgin and virgin females must be due to the costly effects of sexual interactions as such.

A cost of mating that is independent of the rate of egg production was also identified in other insect species. For example mated females have a shortened life span even if the cost of egg production is excluded in *D. melanogaster* (Fowler and Partridge, 1989; Chapman et al., 1995), *C. capitata* (Chapman et al., 1998) or the seed beetle *Callosobruchus chinensis* (Yanagi and Myiatake, 2003). In these species, manipulations of egg production by genetic interventions, X-radiation or the availability of oviposition sites revealed a separate cost of egg production, in addition to the cost of mating (Chapman et al., 1998; Sgro and Partridge, 1999; Yanagi and Myiatake, 2003). In *P. apterus*, an additional (mating-independent) cost of egg production seems unlikely, because ablating the ovary does not prolong female life span (Hodkova, 2008), but additional costs of vitellogenin synthesis and/or other reproduction related processes have not been excluded.

In *D. melanogaster*, the negative effect of mating on female longevity is caused by peptides made in male accessory glands (Acps) (Chapman et al., 1995, Wigby and Chapman, 2005). The accessory glands of allatectomized males of *P. apterus* are involuted and contain about four times lower amount of proteins than the accessory glands of control males (Socha et al., 2004). Consequently, it was likely that females exposed to allatectomized males received lower amounts of Acps, because the allatectomy of males did not enhance their mating activity (Table 1, Fig. 4). Therefore, we expected a strong negative effect of the male CA on female life span. In contrast to our expectation, females exposed to allatectomized males lived only slightly longer than females exposed to control males (Fig. 3A). These results suggest that the negative effect of males on female life span is principally independent of the male CA, leaving thus the effect of Acps on female survival open to question. Either the cost of mating depends on factors other than Acps or a low amount of Acps produced in the absence of CA is sufficient to reduce female life span. Several possible causes of a negative effect of mating on female life span have recently been discussed (De Loof, 2011). Alternatively, sexual interactions without mating may be responsible for the survival cost. Further studies should unravel the relative importance of mating and mating-independent effects of males on female survival.

Another question for future research concerns the role of Acps for reproductive success. Females do not benefit from the male CA through increased egg production (Table 2). In contrast, a critical role for JH in the regulation of male reproduction, especially through the expression of Acps, was suggested in *Tenebrio castaneum* (Parthasarathy et al., 2009).

4.3. CA and survival of males exposed to females

Continuous exposure to females significantly reduced the median life span of control males (Figs. 1B and 5B), and this effect was independent of the male's own CA (endogenous JH) (Fig. 2). Given that the growth of accessory gland size is impaired in allatectomized males (Socha et al., 2004), our results indicate that the production of accessory fluid is not important in determining longevity of mated males of *P. apterus*. In contrast, the extra production of accessory gland products were thought to be responsible for much of the cost of reduced longevity associated with high mating rates in males of the stalk-eyed flies *Cyrtodiopsis dalmani* (Pomiankowski et al., 2005).

The most striking effect in our study was that males continuously exposed to allatectomized females had significantly longer life span than males exposed to control females (Fig. 3B), although numbers of copulation bouts were similar and the copulation duration was even longer in the former (Table 1). Hence, it is not the mating itself that mediates the negative effect of the female CA on male longevity. On the other hand, the shorter genital associations (lower female receptivity) caused by the female CA allow males to spend longer time courting and that may be ultimately responsible for the cost of male survival associated with the female CA. Notably, the main cost of sexual interactions arises from courtship e.g. in males of *D. melanogaster* (Cordts and Partridge, 1996) or *Ceratitis capitata* (Papadopoulos et al., 2010).

An alternative or additional mechanism responsible for the effect of the female CA on life span of males might involve pheromones. In *C. elegans*, sugar-like chemicals (acarosides) at very low concentrations act as a sex pheromone that attract males to hermaphrodites and the same group of compounds at higher concentrations trigger young worms to enter the long-lived dauer stage (Srinivastan et al., 2008). The dauer pheromone may also increase the life span of adult worms (Kawano et al., 2005). Interestingly, exogenously applied JH analogue, fenoxycarb, suppressed sex-pheromone production and receptivity in adult females of a moth *H. armigera* (Rafaeli and Bober, 2005), but allatectomy prevented pheromone production and calling, e.g. in a moth *Pseudaletia unipuncta* (Cusson and McNeil, 1989) or a cockroach *Supella longipalpa* (Smith and Schal, 1990). Although the regulation of pheromone production by JH appears to be widespread among insects, a potential role for pheromones in connecting sexual activity with longevity has not yet been examined. In any case, the effect of a female reproductive hormone on the male life span observed in our study is presently unique and desires further attention.

Acknowledgement

The study was supported by the grant Agency of the Czech Republic (Grant P502/10/1612).

References

Arnqvist, G., Nilson, T., 2000. The evolution of polyandry: multiple mating and female fitness in insects. Animal Behaviour 60, 145–164.

Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F., Partridge, L., 1995. Cost of mating in *Drosophila melanogaster* is mediated by male accessory gland products. Nature 373, 241–244.

Chapman, T., Myiatake, T., Smith, H.K., Partridge, L., 1998. Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. Proceedings of the Royal Society of London B 265, 1879–1894.

Chen, P.S., 1984. The functional morphology and biochemistry of insect male accessory glands and their secretions. Annual Review of Entomology 29, 233–255.

Cordts, R., Partridge, L., 1996. Courtship reduces longevity of male *Drosophila melanogaster*. Animal Behaviour 52, 269–278.

Cusson, M., McNeil, J., 1989. Involvement of juvenile hormone in the regulation of pheromone release activities in a moth. Science 243, 210–212.

De Loof, A., 2011. Longevity and aging in insects: is reproduction costly; cheap; beneficial or irrelevant? A critical evaluation of the "trade-off" concept. Journal of Insect Physiology 57, 1–11.

Fan, Y.L., Rafaeli, A., Gileadi, C., Kubli, E., Applebaum, S.W., 1999. Drosophila melanogester sex peptide stimulates juvenile hormone synthesis and depresses sex pheromone production in *Helicoverpa armigera*. Journal of Insect Physiology 45, 123–127.

Fowler, K., Partridge, L., 1989. A cost of mating in female fruitflies. Nature 338, 760–761.

Gillot, C., 2003. Male accessory gland secretions: modulators of female reproductive physiology and behavior. Annual Review of Entomology 48, 163–184.

Ginnakou, M.E., Goss, M., Jünger, M.A., Hafen, E., Leevers, S.J., Partridge, L., 2004. Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. Science 305, 361.

Herman, W.S., Tatar, M., 2001. Juvenile hormone regulation of longevity in the migratory monarch butterfly. Proceedings of the Royal Society of London B 268, 2509–2514.

Hodkova, M., 2008. Tissue signaling pathways in the regulation of life-span and reproduction in females of the linden bug, *Pyrrhocoris apterus*. Journal of Insect Physiology 54, 508–517.

Hodkova, M., Socha, R., 2006. Endocrine regulation of the reproductive arrest in the long-winged females of a flightless bug, *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae). European Journal of Entomology 103, 523–529.

Holbrook, G.L., Bachmann, J.A., Schal, C., 2000. Effects of ovariectomy and mating on the activity of the corpora allata in adult female *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Physiological Entomology 25, 27–34.

Hwangbo, D.S., Gershman, B., Tu, M.-P., Palmer, M., Tatar, M., 2004. Drosophila dFOXO controls lifespan and regulates insulin signaling in brain and fat body. Nature 429, 562–566.

Kawano, T., Kataoka, N., Abe, S., Ohtani, M., Honda, Y., Honda, S., Kimura, Y., 2005. Lifespan extending activity of substances secreted by the nematode *Caenorhabditis elegans* that include the dauer-inducing pheromone. Bioscience, Biotechnology, Biochemistry 69, 2479–2481.

Kubli, E., 2003. Sex-peptides: seminal peptides of the *Drosophila* male. Cellular and Molecular Life Sciences 60, 1689–1704.

Moshitzky, P., Fleischmann, I., Chaimov, N., Saudan, P., Klauser, S., Kubli, E., Applebaum, S.W., 1996. Sex-peptide activates juvenile hormone biosynthesis in the *Drosophila melanogaster* corpus allatum. Archives of Insect Biochemistry and Physiology 32, 363–374.

Moshitzky, P., Gilbert, L.I., Applebaum, S.W., 2003. Biosynthetic maturation of the corpus allatum of the female adult medfly, *Ceratitis capitata*, and its putative control. Journal of Insect Physiology 49, 603–609.

Murphy, C.T, McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Kenyon, C., 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature 424, 277–284.

Papadopoulos, N.T., Liedo, P., Müller, H.-G., Wang, J.-L., Molleman, F., Carey, J.R., 2010. Cost of reproduction in male medflies: the primacy of sexual courting in extreme longevity reduction. Journal of Insect Physiology 56, 283–287.

Parthasarathy, R., Tan, A., Sun, Z., Chen, Z., Rankin, M., Palli, S.R., 2009. Juvenile hormone regulation of male accessory gland activity in the red flour beetle, *Tribolium castaneum*. Mechanisms of Development 126, 563–579.

Pener, M.P., 1972. The corpus allatum in adult acridids: the inter-relation of its functions and possible correlations with the life cycle. In: Hemming, C.F., Taylor, T.H.C. (Eds.), Proceedings of the International Study Conference on the Current and Future Problems of Acridology. Centre for Overseas Pest Research, London, pp. 135–147.

Pomiankowski, A., Denniff, M., Fowler, K., Chapman, T., 2005. The costs and benefits of high early mating rates in male stalk-eyed flies, *Cyrtodiopsis dalmanni*. Journal of Insect Physiology 51, 1165–1171.

Rafaeli, A., Bober, R., 2005. The effect of the juvenile hormone analog, fenoxycarb on the PBAN-receptor and pheromone production in adults of the moth Helicoverpa armigera: an "aging" hormone in adult females? Journal of Insect Physiology 51, 401–410.

Ramaswamy, S.B., Shu, S., Mbata, G.M., Rachinsky, A., Park, Y.I., Crigler, L., Donald, S., Srinivasan, A., 2000. Role of juvenile hormone-esterase in mating-stimulated egg development in the moth *Heliothis virescens*. Insect Biochemistry and Molecular Biology 30, 785–791.

Rolff, J., Siva-Jothy, M.T., 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. Proceedings of the National Academy of Sciences of the United States of America 99, 1999–9916.

Scott, M.P., Panaitof, S.C., 2004. Social stimuli affect juvenile hormone during breeding in biparental burying beetles (Silphidae: *Nicrophorus*). Hormones and Behavior 45, 159–167.

Sgro, C.M., Partridge, L., 1999. A delayed wave of death from reproduction in *Drosophila*. Science 286, 2521–2524.

Shu, S., Park, Y.I., Ramaswamy, S.B., Srinivasan, A., 1998. Temporal profiles of juvenile hormone titers and egg production in virgin and mated females of *Heliothis virescens* (Noctuidae). Journal of Insect Physiology 44, 1111–1117.

Slama, K., 1964a. Hormonal control of respiratory metabolism during growth, reproduction, and diapause in female adults of *Pyrrhocoris apterus* L. (Hemiptera). Journal of Insect Physiology 10, 283–303.

Slama, K., 1964b. Hormonal control of respiratory metabolism during growth, reproduction, and diapause in male adults of *Pyrrhocoris apterus* L. (Hemiptera). Biological Bulletin 127, 499–510.

Smith, A.F., Schal, C., 1990. Corpus allatum control of sex pheromone production and calling in the female brown-banded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). Journal of Insect Physiology 36, 251–257.

Socha, R., Hodkova, M., 2006. Corpus allatum volume-dependent differences in accessory gland maturation in long- and short-winged males of *Pyrrhocoris apterus* (Hemiptera: Pyrrhocoridae). European Journal of Entomology 103, 27–32.

Socha, R., Sula, J., Kodrik, D., Gelbic, I., 1991. Hormonal control of vitellogenin synthesis in *Pyrrhocoris apterus* (L.) (Heteroptera). Journal of Insect Physiology 37, 805–816.

Socha, R., Sula, J., Kodrik, D., 2004. Wing morph-related differences in developmental pattern of accessory gland proteins in adult males of *Pyrrhocoris apterus* (L.) and their endocrine control. Journal of Insect Physiology 50, 893–901.

Soller, M., Bownes, M., Kubli, E., 1999. Control of oocyte maturation in sexually mature *Drosophila* females. Developmental Biology 208, 337–351.

Sréng, L., Léoncini, I., Clément, J.L., 1999. Regulation of sex pheromone production in the male *Nauphoeta cinerea* cockroach: role of brain extracts, corpora allata (CA), and juvenile hormone (JH). Archives of Insect Biochemistry and Physiology 40, 165–172.

Srinivastan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H.T., Teal, P.E.A., Malik, R.U., Edison, A.S., Sternberg, P.W., Schroeder, F.C., 2008. A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. Nature 454, 1115–1118.

Teal, P.E.A., Gomez-Simuta, Y., Proveaux, A.T., 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Carribean fruit flies. Proceedings of the National Academy of Sciences of the United States of America 97, 3708–3712.

Trabalon, M., Campan, M., Porcheron, P., Clement, J.-P., Baehr, J.-C., Moriniere, M., Joulle, C., 1990. Relationships among hormonal changes, cuticular hydrocarbons, and attractiveness during the first gonadotropic cycle of the female *Calliphora vomitoria* (Diptera). General and Comparative Endocrinology 80, 216–222.

Trumbo, S.T., Robinson, G.E., 2004. Nutrition, hormones and life history in burying beetles. Journal of Insect Physiology 50, 383–391.

Wigby, S, Chapman, T., 2005. Sex peptide causes mating costs in female *Drosophila melanogaster*. Current Biology 15, 316–321.

Wilson, T.G., DeMoor, S., Lei, J., 2003. Juvenile hormone involvement in *Drosophila melanogaster* male reproduction as suggested by the Methoprene-tolerant^{27} mutant phenotype. Insect Biochemistry and Molecular Biology 33, 1167–1175.

Wyatt, G.R., 1997. Juvenile hormone in insect reproduction – a paradox? European Journal of Entomology 94, 323–333.

Yanagi, S., Myiatake, T., 2003. Costs of mating and egg production in female *Callosobruchus chinensis*. Journal of Insect Physiology 49, 823–827.

Yamamoto, K., Chadarevian, A., Pellegrini, M., 1988. Juvenile hormone action mediated in male accessory glands of *Drosophila* by calcium and kinase C. Science 239, 916–919.

Zdarek, J., 1966. Hormonal control of mating behaviour in *Pyrrhocoris apterus* L. In: Proceedings of the International Symposium in Insect Endocrines, Brno, pp. 51–61.

Zdarek, J., 1968. Le comportement d'accouplement a la fin de la diapause imaginale et son controle hormonal dans le cas de la punaise *Pyrrhocoris apterus* L. (Pyrrhocoridae, Heteroptera). Annales d'Endocrinologie, Paris 29, 703–707.

Zdarek, J., 1970. Mating behaviour in the bug, *Pyrrhocoris apterus* L. (Heteroptera): ontogeny and its environmental control. Behaviour 37, 253–268.