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Trauma, disease and collateral damage: conflict in cimicids

M. T. Siva-Jothy*

Department of Animal & Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

The bed bugs and bat bugs (Hemiptera: Cimicidae) are unusual in being a gonochorist (separate male and female genders) taxon with obligate traumatic insemination. Males of all the species in this family have a lanceolate paramere (intromittent organ) which they use to pierce the female's body wall and inseminate directly into her haemocoel, despite the presence of a functional female genital tract. Mating is tightly linked to the feeding cycle in *Cimex lectularius*, the common bed bug. In this paper, I examine key aspects of the reproductive anatomy and behaviour of *C. lectularius* that underpin the nature of the conflict over mating rate in this species. I then examine the consequences of traumatic insemination for female fitness and examine potential mechanisms that might underpin those costs. Finally, the collateral consequences of the male reproductive tactic on other males of *C. lectularius* and the African bat bug, *Afrocimex constrictus* are examined.

Keywords: bed bug; traumatic insemination; spermalege; *Cimex*; *Afrocimex*; sexual conflict

1. INTRODUCTION

The evolutionary interests of each gender typically differ (Parker 1979) and the resultant conflict results in the evolution and subsequent coevolution of conflict traits such as seminal fluid proteins that alter female physiology (e.g. Rice 1996; Chapman 2001), and/or anatomical and behavioural traits associated with mate-securing and mating (e.g. Arnqvist & Rowe 2002a,b). However, despite the likelihood that sexual conflict is a general phenomenon affecting many taxa, it and its effects are notoriously difficult to observe. There are probably two main reasons for this difficulty. First, although the selection pressures may be strong and the resultant coevolution rapid (e.g. Holland & Rice 1999) at any one point in time there may be very little observable consequences on the genders (since the counter response will reduce the advantage of the selecting conflict trait). Second, it is likely that many conflict traits are physiological since these offer the opportunity for relatively rapid evolutionary change (compared to anatomical or behavioural traits).

As a result of these and other factors, sexual conflict has relatively few empirically amenable model systems with which to study the predictions from the relative wealth of sophisticated theory.

My aim in this paper is to outline the biology and consequences of traumatic extragenital insemination in the cimicids (Hemiptera: Insecta) in order to examine the suitability of this understudied taxon as a model for studies of sexual conflict.

*m.siva-jothy@sheffield.ac.uk

2. REPRODUCTIVE BIOLOGY

(a) *Anatomy*

The two aspects of reproductive biology of most interest in this family are the anatomical adaptations to traumatic insemination and the dynamics of mate encounter and unison. An understanding of both of these aspects of cimicid biology is important before considering the nature of the conflict between the genders over mating frequency.

The male intromittent organ of *Cimex lectularius* consists of a strongly sclerotized paramere (Davis 1956; Usinger 1966) and is relatively featureless (figure 1): its striking needle-like structure reflects its specialized function of traumatic extragenital insemination. Copulation consists of the male piercing the female's abdominal wall and transferring sperm into her haemocoel (despite the presence of a functional female genital tract; Carayon 1966). Recent work has revealed that the paramere has chemosensory abilities, which the male uses to detect his mate's current mating status: the information is used to adjust ejaculate size (see Siva-Jothy & Stutt (2003) for detailed discussion).

The function of the female's 'normal' reproductive tract is confined to post-insemination events where, among other things, it functions as an egg conduit during oviposition. In the vast majority of cimicids the internal genitalia are supplemented by the mesodermally derived 'paragenitalia'. This unique anatomical feature shows a spectrum of complexity across the family, with one species (*Primicimex cavernis*) showing very simple, almost non-existent, paragenitalia, while others (e.g. *Crassicimex sexualis*) have paragenitalia as if not more complex than their internal genitalia (figure 2). In *C. lectularius* the most prominent compartment of the female paragenitalia is the spermalege (or 'organ of Berlese', Carayon 1966). This is manifest externally as a dorso-ventral groove in the fifth abdominal sternite (figure 3) termed the

Figure 1. Scanning electron micrograph (courtesy of Andrew Syred, MicroscoPix) of the male intromittent organ (paramere) of *Cimex lectularius*.

'ectospermalege': it represents the site at which the male inseminates the female. With the exception of *P. cavernis* all members of the taxon possess a well defined ectospermalege through which the male inserts his intromittent organ (Carayon 1966). Exactly why males 'comply' with this aspect of female control over the site of mating is unclear, especially as male *P. cavernis* appear to be able to penetrate the abdomen at a number of points independent of the presence of an ectospermalege. One possibility is that mating outside the ectospermalege reduces female fecundity to such an extent that the mating male's paternity is significantly reduced (see 'palliative adaptations' in Lessells (2006)). The ectospermalege appears to act as a mating guide, directing the male's copulatory interest, and therefore damage, to a restricted area of the female's abdomen. Underlying the ectospermalege is the mesospermalege, a mesodermal bag filled with haemocytes (blood cells associated with immune function and wound healing). Semen is injected into this structure during mating, from where it migrates into the haemolymph, swims to and penetrates the base of the oviducts and eventually makes its way up the oviducts to fertilize eggs in the ovaries (Usinger 1966). The haemocytes in the mesospermalege undergo rapid morphological change soon after insemination: they show a large increase in cell volume and, in their post-mating state, have distinct morphological forms in electron micrographs (see Klein & Kallenborn 2000; Steigner 2001). There are several reports of sperm phagocytosis in these cells (e.g. Usinger 1966), which have lead to the proposal that these cells may be an agent of cryptic female choice (Eberhard 1985, 1996). However, electron microscope examination of *ca* 600 sections taken from *ca* 125 specimens prepared under controlled mating circumstances revealed only one unequivocal, and two equivocal, examples of a single haemocyte containing internalized sperm (M. T. Siva-Jothy 2005, unpublished work). If sperm phagocytosis was an agent of female choice we would predict its frequency to be high soon after remating. However, sperm have a finite lifespan and it is likely that haemocytes may also internalize sperm as part of a 'housekeeping' function. This latter, more parsimonious explanation better fits our observations (since relatively little internalization occurs immediately after insemination when dead sperm are expected to be at a minimum).

In conclusion, the key aspects of cimicid reproductive anatomy are that males have an intromittent organ that is sclerotized and drawn to a point, that females guide this piercing organ to a particular site on their abdomen, and that underneath this site is a tissue containing haemocytes that undergo rapid morphological change
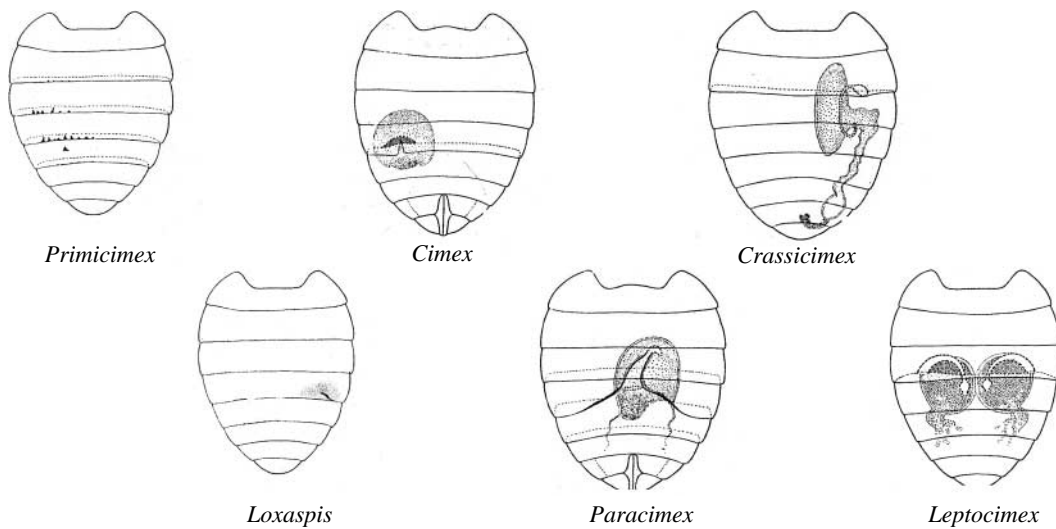
Figure 2. A schematic illustrating the range of spermalege morphologies in cimicid females. The figure shows a stylized abdomen from each of the six represented genera. In *Primicimex* there appears to be no distinct ectospermalege—the black mating scars occur over much of the surface of the left-hand side of the female's abdomen. Female *Loxaspis* have a slightly concave ectospermalege on the right-hand side of the abdomen underneath which lies a relatively poorly developed mesospermalege (represented by shading). *Cimex* females have a well developed mesospermalege underneath a well defined notch-like ectospermalege (see also figure 3). *Paracimex* females have a very prominent ectospermalege and a mesospermalege, which is beginning to extend towards the common oviduct. *Crassicimex* and *Leprocimex* have no visible ectospermalege, but have instead developed well defined invaginated conduits that males inseminate into, before sperm migrate along the ducts and into the mesospermalege.
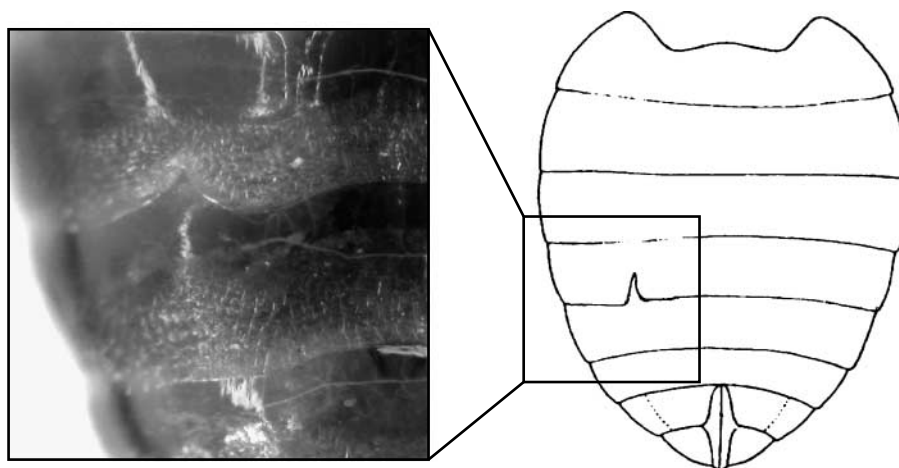

Figure 3. A schematic of the ventral surface of the abdomen of an adult female *C. lectularius* with an enlarged photograph of the ectospermalege.

soon after insemination. The female 'paragenitalia' (mainly the spermalege) are unique among the insects and are a strong candidate for a female response to a male sexually agonistic trait.

(b) *Behaviour*

Video taping of bed bugs (*C. lectularius*) under dim red light allowed the quantification of several aspects of reproductive behaviour (Stutt & Siva-Jothy 2001). Mellanby (1939) showed that starved bed bugs do not mate: Stutt & Siva-Jothy's (2001) observations showed that copulation always followed the female's ingestion of a blood meal after which females mated 5.0 ± 3.16 (mean $\pm$ s.d.; $n = 20$) times with a median remating interval of 17 min (Stutt & Siva-Jothy 2001; the mating male does not guard his mate and usually leaves immediately after finishing; remating therefore, rarely occurs with the same male). Fed females were,

therefore, subjected to repeated matings in the short space of time following mating, when they were still engorged with blood. By isolating *ad libitum* fed singly mated females, Stutt & Siva-Jothy (2001) were able to show that these females laid fertilized eggs for four weeks after a single mating. Under natural feeding rates and mating rates a female would take four blood meals and, therefore, receive 20 matings during this period: the optimum female remating rate that maintains natural levels of fertility was, therefore, 20 times lower than the mating rate imposed by males under natural conditions. Under *ad libitum* feeding conditions (once a week in *C. lectularius*; Mellanby 1939) females produce eggs at a relatively constant rate (relationship between fecundity and longevity $r^2 = 0.93$, $t = 38$, $p < 0.001$; Reinhardt *et al*. 2003) regardless of whether they received the male imposed mating frequency, or the minimum that maintains fertility (Stutt & Siva-Jothy
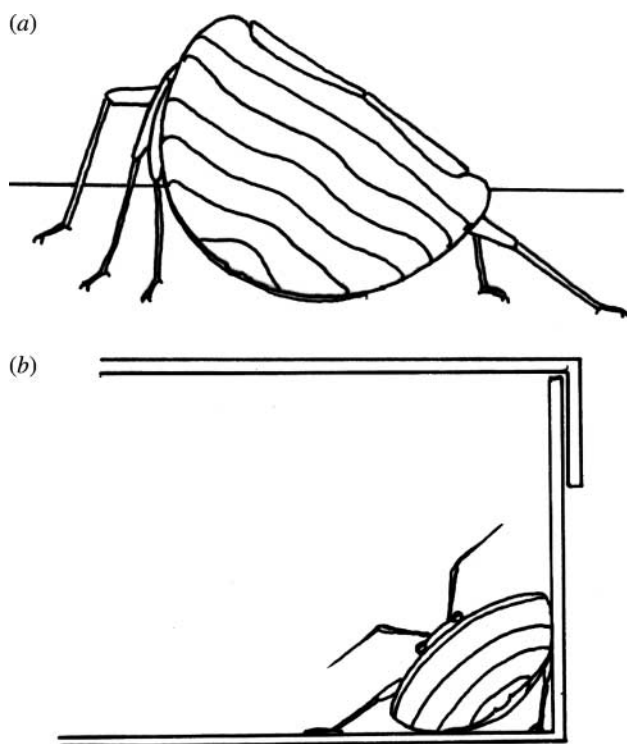
Figure 4. Two posterior views of the 'refusal' posture in unfed adult female *C. lectularius*. (*a*) The female pressing the side of her abdomen that bears the ectospermalege towards the substrate thereby making it inaccessible to males. (*b*) The same behaviour in the context of the female's refugia, where she can effectively prevent any access to the ectospermalege in her unfed state.

2001). The reason recently fed females are the subject of male mating interests is probably because once they are engorged they are relatively slow moving and unable to perform the 'refusal' posture, which unfed females use to conceal the ectospermalege (figure 4).

3. THE COST OF CONFLICT OVER REMATING FREQUENCY

Given that female *C. lectularius* receive 20 times more traumatic inseminations than are required to maintain fertility, and that the insemination requires the physical wounding of the female, it is reasonable to assume that a conflict over remating rate exists in this insect. To quantify this cost, Stutt & Siva-Jothy (2001) compared survival rates and fecundity in females subjected to 'natural' mating rates (i.e. 5 times per blood meal) and females subjected to the minimum rate that maintained fertility (i.e. once per four blood meals). They found that females in the natural remating regime died at a significantly higher rate (log rank test: $\chi^2 = 20.48$, $p < 0.0001$) and produced significantly ($t_{88} = 3.69$, $p < 0.001$) fewer eggs (224.7 ± 16 eggs; mean $\pm$ s.e.) than females in the minimum remating rate group (294.3 ± 9 eggs; mean $\pm$ s.e.). The difference in lifetime fecundity between these treatments represents a 24% reduction in female fitness as a consequence of the male imposed mating rate. It is important to bear in mind that this substantial cost is only evident under ideal feeding conditions and when lifetime fecundity is compared with the female's optimum remating frequency.

Figure 5. (*a*) Adult female *C. lecularius* immediately after feeding to satiation. (*b*) Adult male *C. lecularius* immediately after feeding to satiation.

4. THE OPPORTUNITY FOR INFECTION

With the exception of internal parasites, most animals live in septic environments. Cimicids are obligate haematophages, that engorge during a single feeding visit after which they return to their refugia. The blood meal is largely processed in the refugia which, when established, is full of other bugs, exuvia, and the excreta from digested blood meals (Mellanby 1939, see fig. 5(2) in Usinger 1966). Examination of laboratory refugia and the body surface of *C. lectularius* has revealed the presence of a range of fungi and bacteria in the bug's habitat as well as on the male's intromittent organ (Reinhardt *et al.* 2005). Because of their presence on the male's intromittent organ these microbes can potentially enter the female's haemocoel during traumatic insemination and stimulate the female's immune system. Immune system activation in the absence of pathogens has been shown to be costly in a number of studies (e.g. Armitage *et al.* 2003 and references therein): it is likely to be even costlier if the microbes introduced during mating stimulate higher levels of immune system activation. Consequently, wound healing as well as the immunological response(s) to microbes introduced during traumatic insemination, are candidate mechanisms contributing to the mating cost documented by Stutt & Siva-Jothy (2001). It is also possible that components of this cost also arise from other routes. The most likely is the effect of seminal fluid proteins that alter female physiology and behaviour, as documented in *Drosophila melanogaster* (e.g. Chapman 2001). However, if such proteins are transferred in *C. lectularius*, they do not result in elevated oviposition rates (see fig. 2 in Stutt & Siva-Jothy 2001) or enhanced female refractoriness (since females cannot/do not avoid remating).

5. THE ROLE OF THE SPERMALEGE

Since the spermalege is intimately associated with traumatic insemination and contains haemocytes (immune and repair cells), which show rapid morphological change upon wounding it is unsurprising that biologists have sought to examine its function in reducing the costs of mating in an immunological
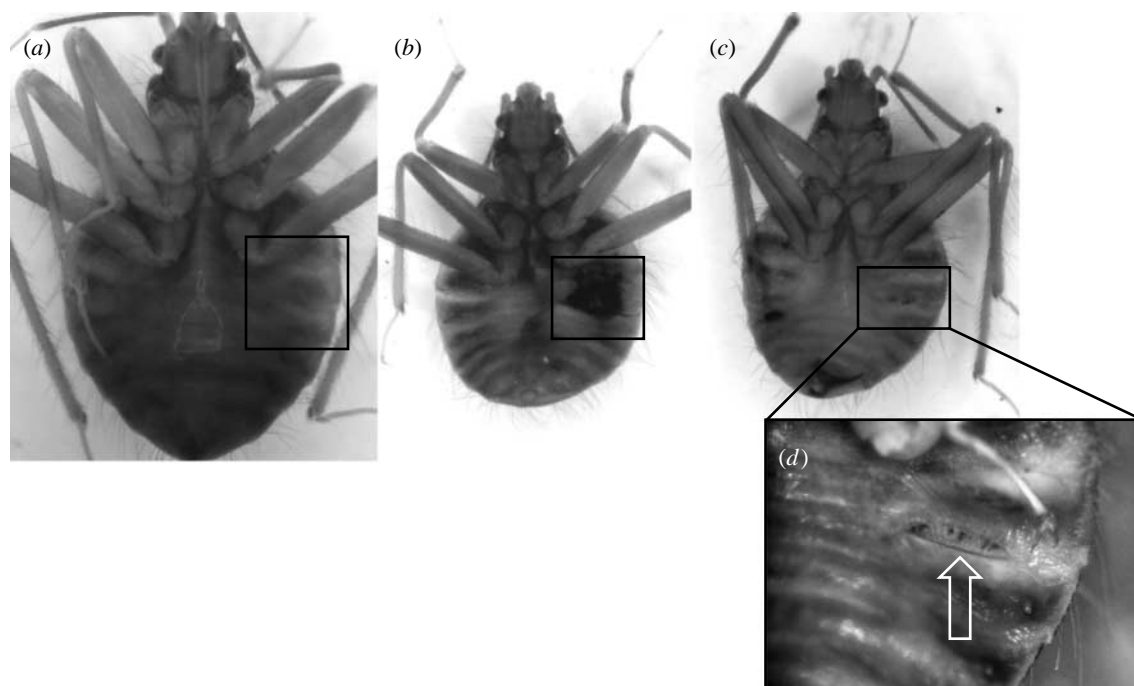
Figure 6. (*a*) Virgin adult female *Afrocimex constrictus*. The box encloses the region of the paired ectospermalege, which bears no mating scars in this unmated individual. (*b*) An adult female *A. constrictus* collected from the field. The box encloses the ectospermalege, which shows considerable melanization resulting from conflated wounding scars. The scar tissue in this insect had formed a hardened sphere, which could not be dissected open. (*c*) An adult male *A. constrictus* collected from the field showing wounding scars in the ectospermalege. (*d*) An enlarged view of the same area of another male: the white arrow indicates the lower ectospermalege with clearly visible melanized mating scars.

context. Two studies (Morrow & Arnqvist 2003; Reinhardt *et al.* 2003) have examined the role of the spermalege as a female counter-adaptation to traumatic insemination. Both studies used needles to inflict copulation-like trauma on females, and both examined the consequence of aseptic and septic trauma into the spermalege as well as avoiding the spermalege. Both studies concluded that the spermalege functioned to ameliorate the costs of mating associated with the introduction of microbes during traumatic insemination. However, the subtle differences in design between the studies allowed Morrow & Arnqvist (2003) to additionally conclude that repeated treatment (needle-piercing of the spermalege) did not affect fecundity over the time scale of their experiments. Reinhardt *et al.* (2003) concluded additionally that the mesosperma-lege did not provide any elevated wound-healing function since females in their aseptic treatments did not differ in survivorship or fecundity whether they were stabbed in the spermalege or alongside it. These independent studies support the notion that the spermalege evolved as a female counter-response to traumatic insemination and functions to ameliorate the immune costs associated with it. The variation in complexity in the spermalege throughout the family, therefore, represents a unique opportunity to examine the trajectories of female counteradaptation to male conflict traits. Unfortunately, no complete molecular phylogeny of this taxon currently exists.

6. COLLATERAL CONSEQUENCES OF TRAUMATIC INSEMINATION

An urban myth among zoologists states that male bed bugs traumatically inseminate other males and gain

a fitness benefit from doing so. The benefit arises, so the story goes, because the 'donor's' sperm migrate to the recipient's testes and subsequently form part of the recipient's ejaculate.

Observation of recently fed adult males and females shows they are very similar in size (M. T. Siva-Jothy 2005, unpublished data) and appearance (figure 5). When reproductively active males are given access to recently fed individuals of both genders they show no tendency to mount one gender more than the other ($\chi^2 = 3.3$; $p > 0.05$). The observation that fed males are rapidly and frequently mounted by other males may well have been the origin of the 'ejaculate parasitism' story. However, despite the apparent inability of males to distinguish fed males from fed females prior to mounting, it is clear that they do not treat the genders similarly once mounted. Mounting males remain on virgin females for longer (93 ± 6 s, $n = 50$; mean$\pm$s.d.) than they do on fed virgin males (4.4 ± 0.6 s, $n = 20$; mean$\pm$s.d.; $U = 1020$, $p < 0.0001$). Moreover, examination of the mounted individuals after this process revealed all the mounted females ($n = 50$) contained sperm in the mesospermalege, while none of the mounted males ($n = 20$) showed mating scars or any sign of sperm in the haemocoel. It, therefore, appears that male *C. lectularius* assess the gender of potential mates after they have mounted, but do not impose collateral costs other than sequestering relatively short periods of time on the fed males they mount.

In contrast, males of the cimicid *Afrocimex constrictus* have a clearly defined ectospermalege (figure 6*c*), the external manifestation of the female's paragenitalia. In this species selection appears to have been sufficiently strong on males to select for the development of

functional external female paragenitalia. Comparisons of mated and virgin adults collected from the wild show that females bear prominent melanized mating scars (figure 6). Dissection of the female in figure 6b revealed the scar tissue in the posterior ectospermalege (this species has two ectospermaleges) to be a very hard plug of melanized tissue that would have been difficult to penetrate. Randomly captured adult males also showed 6.3 ± 2.9 (mean $\pm$ s.d.; $n = 31$) visually prominent 'mating' scars in their ectospermalege (figure 6d). Although males show fewer scars than females (female scars were so numerous that, it was impossible to distinguish individual scars and so score the damage accurately) there is clear evidence that males are wounded. Exactly why males insert their paramere into other males is unclear at present, but field observations suggest pre-mating behaviour is similar to *C. lectularius*, and fed males are very similar in appearance to fed females. Whatever the causal basis of 'intrasexual traumatic intromission', it is clear that male *A. constrictus* are at least paying wound healing costs and are likely to be paying immune system activation costs as well. This species represents the first example of a male conflict trait that causes collateral damage in a gonochorist species.

7. SUMMARY

Traumatic extra genital insemination is largely confined to the cimicids within gonochorist taxa. Males use their sclerotized lanceolate intromittent organ to pierce the female's abdomen soon after she has fed, and adult females are subjected to multiple traumatic inseminations after each feeding bout. There appears to be little or no courtship and females appear to have little or no control over mating frequency in the period immediately following a blood meal. Experimental evidence suggests that the natural remating rates are 20 times greater than the remating rate that maintains maximum female fecundity. There therefore appears to be strong conflict over remating frequencies, especially because females pay a high fecundity cost because of the traumatic insemination rates they are subjected to.

It appears that at least part of this cost is paid because the female must respond immunologically to the opportunistic microbes that are introduced into her haemocoel during traumatic insemination and, as a response to this selection pressure, female cimicids have evolved a unique organ, the spermalege. The spermalege, therefore, probably represents one of the most tangible female evolutionary responses to conflict over remating rates and provides an ideal opportunity to examine how females ameliorate the physiological costs males impose on them (despite the fact that the amelioration is incomplete in *C. lectularius*). By confining wounding to a restricted part of their abdomen females may select for an increased efficiency in the systemic immune system at this site—a proposal in keeping with the presence, and rapid activation, of the haemocytes in the mesospermalege. Their mating system and haematophagous lifestyle appear to combine and result in frequent intrasexual mountings in *C. lectularius*: these were never observed to result in intromission. However, in *A. constrictus* adult males bear multiple mating scars in their distinct

spermalege. The possession of female paragenitalia by males in a species, where males are also subjected to traumatic insemination clearly requires more detailed scrutiny. However, if the observations of intrasexual behaviour in *C. lectularius* hold for *A. constrictus* the most parsimonious explanation is that males are responding to selection from traumatic insemination (rather than encouraging it).

The cimicids provide a unique opportunity to examine a conflict over the rates and consequences of traumatic insemination in males and females. While it is not as amenable for experimental evolution studies as *D. melanogaster* (e.g. Holland & Rice 1999) the nature of the conflict and the conflict traits in cimicids means it is relatively easy to observe, measure and manipulate the basis of the conflict in this understudied taxon.

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