15

Selective Forces Propelling Genitalic Evolution in Odonata

ADOLFO CORDERO-RIVERA AND ALEX CÓRDOBA-AGUILAR

INTRODUCTION

Odonates are considered to be among the oldest insects (Silsby 2001), with fossil odonatoid insects known from the upper Carboniferous (about 300 million years ago). Although they have changed very little in morphology since the Jurassic (65 million years), their behavior is by no means simple, showing pre-copulatory courtship, intense male–male contests, post-copulatory associations between males and females, and other elaborate behaviors (figure 15.1). Odonates are the only insect group whose males do not have the penis directly connected to the testis, but use a seminal vesicle (figure 15.2) for temporary sperm storage. This fact explains the need for intra-male sperm transfer (figure 15.1c), before each mating, when the male translocates his sperm from the testis, opening at the end of the abdomen, to the seminal vesicle, situated under the second and third abdominal segments (figure 15.2). In some species, males perform elaborate precopulatory courtship, slowly flying around the female, and simultaneously exposing wing and body coloration (figure 15.1a,b), for instance in Calopterygidae (Heymer 1973). In other cases, males simply capture mature females with their anal appendages forming the precopulatory tandem, and then perform an "invitation" to copulate (Robertson and Tennessen 1984), by elevating the abdomen and vibrating their wings. Only if the female touches the male's secondary genitalia, does the male proceed to sperm translocation and copulation.

When a mating couple finishes copulation, the male may or may not guard the female during oviposition (either in tandem or by remaining close to her). Reproductive behavior in odonates is "classical" in the sense that males compete for females, who are the limiting resource for reproduction, and sexual selection is intense, especially on males (Banks & Thompson 1985;Conrad and Pritchard 1992; Córdoba-Aguilar 2002b; Fincke 1986; Fincke and Hadrys 2001).

Odonates are popular for research perhaps because their reproductive behavior is a typical text-book example for postcopulatory sexual selection: males use their penis for a dual function, removal of rivals' sperm during the first part of copulation, and transfer of their own sperm, during the final part (Córdoba-Aguilar et al. 2003b). The description of this fact by Waage (1979) opened a new era in sexual selection studies, clearly under the influence of the *seminal* work on sperm competition by Parker (1970). Removing or repositioning rivals' sperm is obviously advantageous for males, and there is no doubt that selective forces (sperm competition, see below) have contributed to the evolution of sperm removal behavior, not only in odonates but other insects as well (Haubruge et al. 1999; Kamimura 2000; Ono et al. 1989;Yokoi 1990; reviewed by Simmons 2001). However, research on genitalic evolution and sexual biology

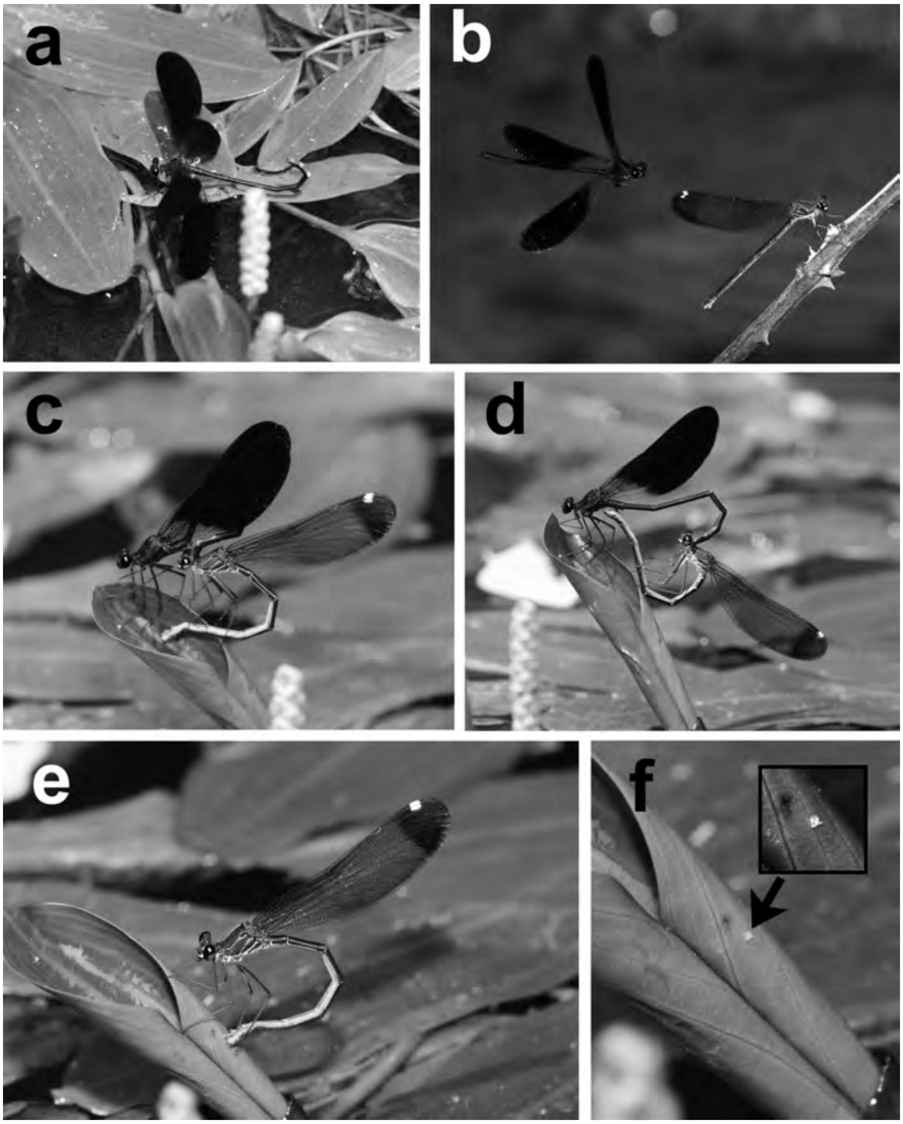

FIGURE 15.1 Reproductive behavior of a typical territorial odonate (*Calopteryx haemorrhoidalis*, Calopterygidae). Males perform precopulatory courtship, sometimes landing over water or appropriate oviposition substrates (a), and slowly flying around the female (b). If the female remains motionless, the male is able to grasp her, and perform the intra-male sperm translocation (c), transferring the sperm from the testes, whose opening is at the end of the abdomen, to the secondary genitalia, situated under the segments 2 and 3. Copulation follows (d) and is usually divided into a first stage, where rivals' sperm removal takes place, and a stage II, when insemination occurs. At the end of copulation the male flies directly to the territory, and the female sometimes remains in a "postcopulatory rest" (e). In some cases females release a drop of sperm (f) after mating. Photos by A. Cordero Rivera.

in general (see for example explanations for male postcopulatory behavior by Waage 1984) has been biased or shaped by sperm competition theories, and sexual selection in general (Fincke et al. 1997), and tests for other selective forces have been rare. For example, what about female interests during sperm competition in odonates? Fincke (1997) provides a comprehensive review of the potential for female choice and benefits to females of mating multiply, and offers alternative (or complementary) explanations for mating patterns in odonates. If the last male to copulate with a female always removes the sperm, females would certainly lose when remating with a low quality male after having mated with a good male. Therefore we should expect females to retain control over fertilization, and exercise cryptic female choice (Eberhard 1996). The fact that female odonates mate multiply (e.g., Córdoba-Aguilar et al. 2003a) is the key for the existence of a conflict of interests between the sexes (Arnqvist and Rowe 2005).

This chapter reviews the available evidence for sexual selection forces shaping genitalic evolution. We also review briefly other hypotheses that have not been tested in odonates and that are not related to sexual selection. These are the lock and key (Shapiro & Porter 1989) and pleiotropy hypotheses (Mayr 1963). Odonate genitalia have been mainly studied from the point of view of sexual selection, as secondary sexual characters, and little has been done from the standpoint of natural selection, which is bizarre given that genitalia are traditionally considered primary sexual characters. This is probably due to the existence of secondary genitalia in this order, which is a unique character of odonates.

GENITALIC MORPHOLOGY AND FUNCTION

The copulatory apparatus of male odonates is not homologous with any organ in the Animal Kingdom (Tillyard 1917). It is developed from the second sternite, and consists of a penis, a seminal vesicle (*vesica spermalis*), and a series of accessory structures (hamuli anteriores, hamuli posteriores) that protect the penis, and presumably help to achieve genital connection during copulation (figure 15.2). Little is known about the evolution of these structures, but they seem to be already present in the Mesozoic fossil *Tarsophlebia eximia*, and can be observed in some Protozygoptera (Fleck et al. 2004). In the odonate literature, these structures are known as the "secondary genitalia", to distinguish them from the "primary genitalia" found at abdominal segment 9 (in males, reduced to two scales closing the genital pore). This distinction is not related with the primary and secondary sexual characters which are the focus of this book. For further morphological details, the reader can consult Tillyard's (1917) monograph, which remains a rich source of information for the anatomy of this order, and the detailed functional morphology work of Pfau (1971, 1991, 2005). Fleck et al. (2004) discuss the possible origin of the secondary genital apparatus in the Odonata, and suggest that the stem species of Odonata did not remove rival's sperm, but rather transferred a spermatophore to the female. The oldest dragonflies known from the fossil record (Odonata-like insects from the Upper Carboniferous) seem to have had a paired penis with a pair of lateral parameres and a pair of segmented, leaf-like gonopods at the end of the abdomen, and therefore were unlikely to form a copulatory wheel like modern odonates (Bechly et al. 2001). This makes it possible for several interpretations of the origin of the secondary copulatory apparatus of modern odonates to be constructed (Bechly et al. 2001). Unfortunately, the fossil record cannot say much about precopulatory behavior.

Three different structures act as intromittent organ (hereafter penis) in the Odonata. Given their use for sperm removal, the most parsimonious explanation implies that this specialized behavior has evolved three times independently in modern odonates (Bechly et al. 2001). In damselflies (Zygoptera), the intromittent organ is the *ligula* or aedeagus (figure 15.2). It is a chitinized arc, with an inflatable membrane, and variable morphology at the distal part (spoon-like, a variable number of stout appendages, "horns", flagella, and so on). It has no direct connection with the *vesica spermalis*. The sperm is conducted in a furrow of the *ligula* during insemination (figure 15.2) (Pfau 1991). In contrast with the penis of the Anisoptera, which is supplied with internal muscles and tracheae, the penes of the Zygoptera seem not to have such structures (Tillyard 1917), but in both suborders some nerves are present inside the penis (Uhía & Cordero Rivera 2005).

In dragonflies (Anisoptera), the distal part of the *vesica spermalis* acts as a penis (figure 15.2), and

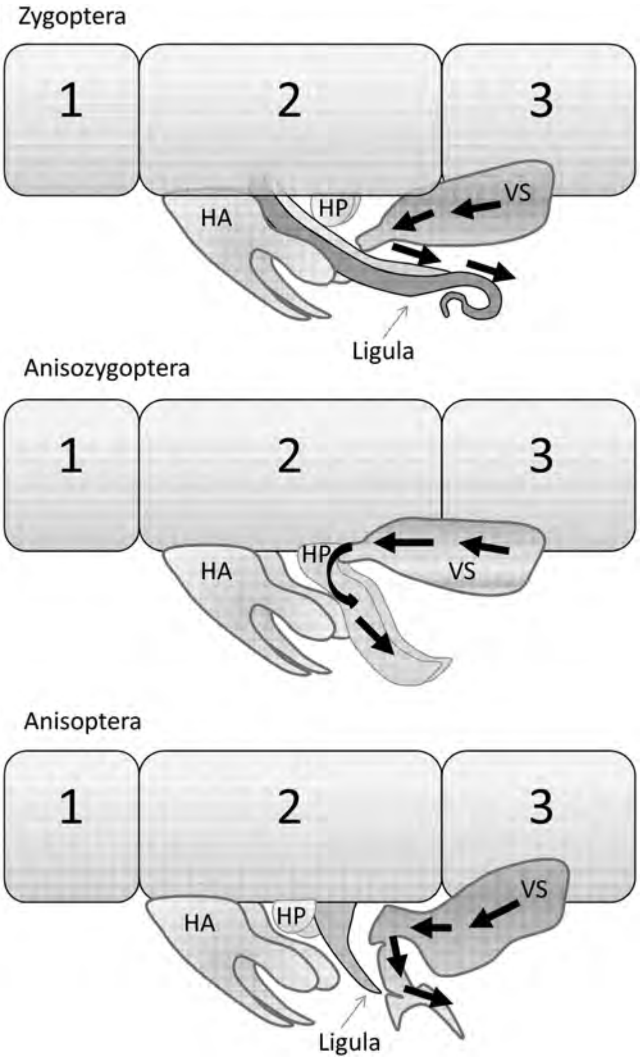

FIGURE 15.2 External genitalic structures of male odonates, in schematic representation. Note that the intromittent organ (the functional penis) is a different structure in each taxon. Numbers indicate abdominal segments, and the arrows show the way of sperm during insemination. HA: *hamuli anteriores*, HP: *hamuli posteriores*, VS: *vesica spermalis*.

has therefore been modified into three parts, a basal joint, which is strongly chitinized, a second element, usually curved, and with the orifice at the end, and a third element, very variable in form, and sometimes with flagella (figure 15.3c, e) or short lateral flaps, only visible when the penis is erected. The penis projects forwards from the seminal vesicle, situated ventrally on the third abdominal segment (figure 15.2), to which it is directly connected. The sperm, which in dragonflies is usually transferred in groups or "spermatodems" (see below), is temporally stored in the vesicle, and transferred to the female at the end of copulation.

In the Anisozygoptera (previously considered a separate suborder but now included among the Anisoptera; Bybee et al. 2008), a group which only has two extant species, the penis consists of the paired *hamuli posteriores* (figure 15.3), which are pressed against each other and form a tube of two halves (Pfau 1991).

During copulation, nerve cells in the penis are likely used to detect the presence of sperm inside the female, probably by means of chemical sensilla (Andrés & Cordero Rivera 2000; Uhía & Cordero Rivera 2005). The first part of copulation (named "stage I") is characterized by rhythmic movements of the penis, which remove sperm from the bursa and spermatheca (figure 15.1d) (Miller & Miller 1981). This stage takes up most of the copulation time in odonates (Córdoba-Aguilar et al. 2003b; Córdoba-Aguilar and Cordero Rivera 2008). As we discuss below, this phase could serve not only to remove sperm, but might also be prolonged due to copulatory courtship. A few minutes (or seconds) before the end of copulation, males change their behavior, flex their abdomen, and inseminate. This is stage II, which, in some species, slowly progresses into a motionless phase known as stage III, when insemination ends (Miller & Miller 1981). In some species, females show a "postcopulatory rest", occasionally associated with sperm ejection (figure 15.1e, f). The copulatory process has been studied in detail in a number of Zygoptera (Córdoba-Aguilar 2003a; Miller 1987a, b). Figure 15.1 shows these phases in a model species, *Calopteryx haemorrhoidalis*.

Male genitalic diversity and function in the Anisoptera have been recently reviewed in detail (Pfau 2005). No such study is available for the Zygoptera (but see Pfau 1971). Some genitalic structures, like the hamuli (figure 15.2), are considered to function in guidance of the ovipositor (Tillyard 1917), and as such, might be examples of naturally selected traits. Here we focus on structures likely to have arisen as sexually selected traits, because a comprehensive analysis of genitalic diversity of the order is premature.

Anisoptera (Dragonflies)

Pfau (2005) has shown that the distal part of the penis in Anisoptera functions like a pressure pump or a two-way tap, that allows males to wash out rivals' sperm and simultaneously inject their own sperm into the female tract. This organ is inflated during copulation, and therefore its three-dimensional configuration is not easily deduced from dried specimens. The diversity of this structure does not seem related to the taxonomic position of the species (Miller 1991). For instance, *Gomphus pulchellus* (figure 15.3a, b) has a penis with two distal tubes (perhaps a two-way tap), but *Onychogomphus uncatus*, from the same family (Gomphidae), has a complex three-dimensional penis, with no clear tubes (figure 15.3c, d). Many Libellulidae have a well-developed distal segment of the penis, with inflatable parts and a variable number of flagella (Garrison et al. 2006; Siva-Jothy 1984). Some species have one distal tube, which is accompanied by a long thin flagellum, presumably used to remove sperm from the spermatheca (Miller 1991); *Oxygastra curtisii* (Corduliidae) is a typical example (figure 15.3e, f), but this is also found in other cordulids like *Macromia splendens* (Córdoba-Aguilar & Cordero Rivera 2008).Two flagella are found in the penis of some of the Libellulidae (Córdoba-Aguilar et al. 2003b), Austropetaliidae, Aeshnidae, Gomphidae and Corduliidae (Garrison et al. 2006).

Female anisopterans have a large bursa copulatrix and from none to two spermathecae, variable in size and shape, but with uniformity in histology, musculature and sensory structures, at least in the Libellulidae (Siva-Jothy 1987). There are even examples of species without a bursa copulatrix (Miller 1991) which is very interesting as this structure seems less derived than the spermathecae (the bursa is more widespread across species, and the spermatheca usually emerges from the bursa). In *Anax*, the cuticular intima of the spermathecae is thin and lightly corrugated while that of the bursa copulatrix is thick and heavily folded (Andrew and Tembhare 1997). Many representatives of this suborder ejaculate sperm in groups ("spermatodesms"), particularly those larger-bodied taxa that utilize non-defendable resources (Siva-Jothy 1997), and these spermatodesms are dissociated inside the bursa copulatrix, probably under the action of compounds produced by the female. If that is the case, female control over this process is highly likely. The variability of genitalic structures in female anisopterans is far from being well studied, and the possible functions of accessory glands (Andrew and Tembhare 1997) need to be established. Siva-Jothy (1997) discusses several hypotheses as to why some species of Anisoptera use spermatodesms, including their possible function as a nutritional gift to the female and their evolution as sexually-selected traits in the context of postcopulatory sexual selection.

Zygoptera (Damselflies)

The intromittent organ in the Zygoptera, the *ligula* (figure 15.2 and 15.4), has arisen from a sternal

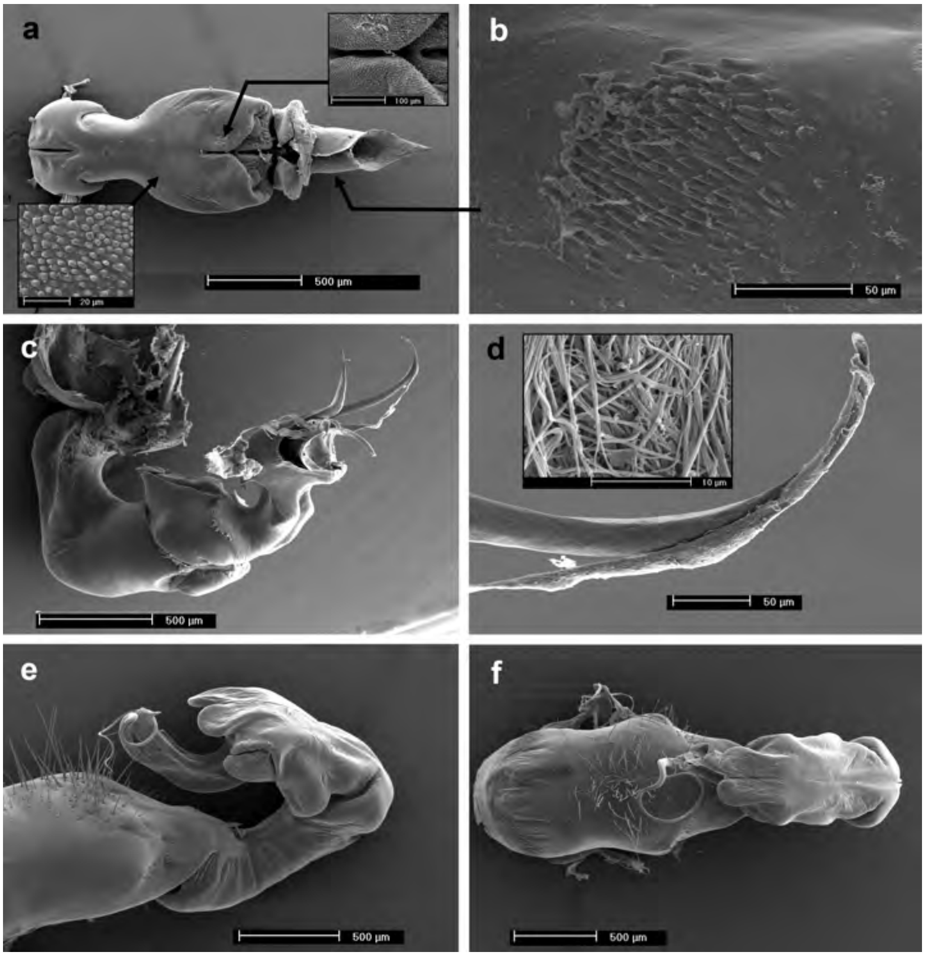

FIGURE 15.3 Typical examples of male genitalia in Anisoptera. (a) Ventral view of penis in *Gomphus pulchellus* (Gomphidae), with details of the microstructure. In this species, the head of the penis ends in two tubes, only one visible in this picture. (b) A field of short spines found in the sides of the penis in *G. pulchellus*. The arrow indicates the approximate position. (c) Lateral view of the penis in *Onychogomphus uncatus* (Gomphidae), showing details of the sperm (d) trapped in one of the horns. (e) Lateral and (f) ventral view of the genitalia in male *Oxygastra curtisii* (Corduliidae), an example of genitalia with just one flagelum.

abdominal appendix (Pfau 1971), and is used to transfer sperm to the female, and to remove sperm stored from previous matings (Waage 1979). In general, zygopterans have a long *ligula* whose head is a mobile element, like a hinge, allowing a flexible joint between both parts. The lateral distal parts of the *ligula*, before the junction of the head, are usually covered with microspines (figure 15.4b, d, f), which presumably help in removing sperm from the bursa copulatrix. Some species lack spines on the penis head, but have, nevertheless, an extensive covering of micro-spines on the *ligula*. One example is the Cuban endemic *Protoneura capillaris* (A. Cordero, personal observation). In *Coenagrion scitulum* this morphology is associated with limited sperm removal ability (Cordero et al. 1995).

A recent paper has experimentally demonstrated that, in two species of Calopterygidae, the head of the *ligula* is used to remove sperm from the bursa copulatrix, and the lateral processes are used to remove sperm from the spermatheca (Tsuchiya & Hayashi 2008). Surgically removing the lateral processes of the *ligula* produced a reduction of movements during copulation in *Calopteryx cornelia*, and no sperm removal from the spermatheca, while in *Mnais pruinosa*, a species whose males are unable to physically remove sperm from the spermatheca, cutting these processes had no effect on copulation or sperm removal from the bursa (Tsuchiya and Hayashi 2008). This is the best direct evidence we have for the function of male genitalia as a device to remove sperm in an odonate.

The head of the *ligula* can be classified into four main types (Waage 1986) (pending a description of genitalia of some tropical families). Kennedy (1920) also describes four groups of damselflies, based on

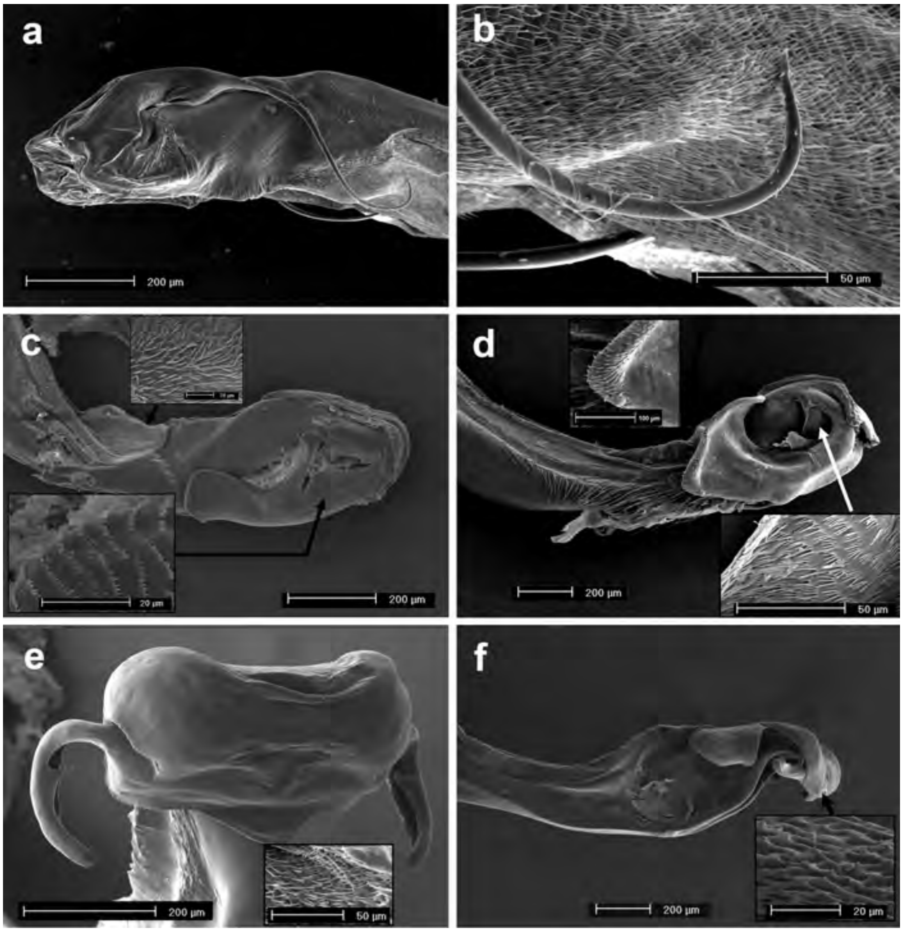

FIGURE 15.4 Typical examples of male genitalia in Zygoptera. (a) Lateral view of the penis in *Coenagrion mercuriale* (Coenagrionidae), showing two long thin flagella. The tip ends in a hook (b) presumably used to trap and remove sperm. (c) Lateral view of the penis in *Telebasis dominicana* (Coenagrionidae), a typical example of "spoon-shaped" aedeagus, with back-orientated microspination. (d) The aedeagus of *Hetaerina vulnerata* (Calopterygidae), representative of morphologies with short prolongations, also covered by fine spination. (e) The penis head of *Chalcolestes viridis* (Lestidae), with no flagella, but with a complex tridimensional structure. The insert shows a zone with some structures that seem chemical sensilla, and could be used to detect the presence of sperm inside the female. (f) Detail of the penis head of *Platycnemis pennipes* (Platycnemididae), a species with two short flagella, with a detail of the microspination found at the basis of the aedeagus.

the morphology of the ligula, and illustrates many representatives of each group, although his grouping is not exactly the same as the proposed here. Many of the Coenagrionidae (e.g., *Ischnura elegans, I. graellsii, I. ramburii, I. hastata, Coenagrion mercuriale*, personal observation; *Argia translata*, Von Ellenrieder & Lozano 2008), have two long thin flagella (figure 15.4a, b), corresponding with the presence of a long thin spermathecal duct. This morphology is also found in representatives of other families (e.g., Platycnemididae; Gassmann 2005, Protoneuridae; Pessacq 2008). In *Ischnura graellsii* and *I. senegalensis* there is experimental evidence for males being able to introduce these flagella into the spermatheca, and removing sperm in this way (Cordero & Miller 1992; Sawada 1995), but in *Ischnura elegans* males are apparently unable to introduce their flagella into the spermatheca (Miller 1987b). There is also at least one species which has only one long thin flagellum (*Podopteryx selysi*; see figures 105 and 106 in Kennedy 1920). A second group of species has a wide head, with a well developed flexible joint, and back-oriented spines (figure 15.4c). This morphology is common in the Coenagrionidae, like *Ceriagrion tenellum* (Andrés & Cordero Rivera 2000), *Enallagma cyathigerum, Telebasis dominicana* (figure 15.4), and species of the genera *Acanthagrion, Aceratobasis, Argentagrion, Cyanallagma, Enallagma, Homeoura, Hylaeonympha, Oxyagrion, Phoenicagrion, Schistolobos* and *Telagrion* (Von Ellenrieder 2008; Von Ellenrieder & Garrison 2008a,c; Von Ellenrieder & Lozano 2008), but is also found in the protoneurid *Epipleoneura venezuelensis* (Pessacq 2008), and some Southeast Asian Platycnemididae (Gassmann 2005; Gassmann & Hämäläinen 2002). These species probably remove sperm by using the head of the *ligula* like a spoon. The third morphology shows a wide head, which ends into a variable number (2 or 4) of short appendages, finely covered with spines. This is typical of the Calopterygidae (Adams & Herman 1991; Cordero Rivera et al. 2004; Garrison 2006; Orr & Hämäläinen 2007; Waage 1984) (figure15.4d) and Platycnemididae (Dijkstra et al. 2007; Gassmann 1999, 2000, 2005; Gassmann and Hämäläinen 2002; Uhía and Cordero Rivera 2005) (figure 15.4e). Some of the Protoneuridae also show this morphology, in agreement with their phylogenetic affinity with the Platycnemididae (Pessacq 2008; Von Ellenrieder & Garrison 2008b). Finally, some species have no flexible joint, and a penis head with a variable number of lobules, and very little spination, like the Lestidae (Uhía & Cordero Rivera 2005; Waage 1982) (figure 15.4f).

The genitalia of female zygopterans consist of a weakly chitinized vagina, that has two chitinized plates with embedded sensilla, where the oviducts

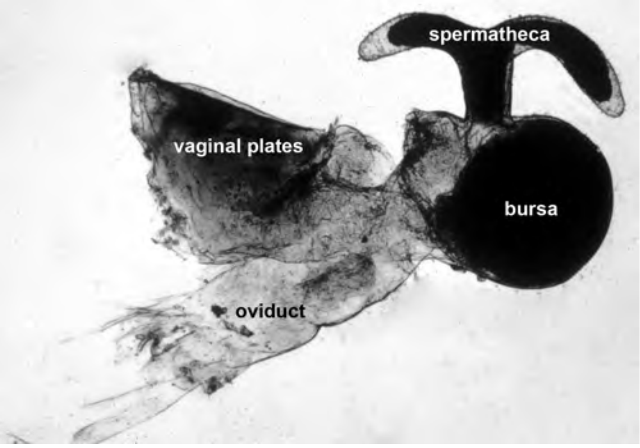

FIGURE 15.5 Female genitalia in the zygopteran *Calopteryx haemorrhoidalis*, showing the typical configuration for odonates. Variations on this pattern include the presence of one spheroid spermatheca, its complete absence, and the presence of accessory glands, whose function is poorly known.

open, together with the openings of the bursa copulatrix and spermatheca (figure 15.5). Histological evidence has shown that sperm maintenance is the primary function for these organs (e.g., Córdoba-Aguilar 2003a). During the fertilization of eggs, the vaginal plates are deformed by the egg that is about to be laid, and this elicits the release of sperm (Miller 1987a). This fact allows males to exploit this sensory channel, by stimulating the sensilla, and thereby eliciting the ejection of sperm during copulation, even if no eggs are laid at that moment (Córdoba-Aguilar 2002a). As with Anisoptera, there is substantial interspecific variation in the size and number of spermathecae (Córdoba-Aguilar et al. 2003b), and part of this variation might be due to an arms race between sexes to control the fertilization process (see below).

HYPOTHESES OF GENITALIC EVOLUTION

Sperm Competition: Tests, Predictions and Results

Sperm competition may occur when two or more males mate with a female during a single reproductive event. This competition is particularly important in insects because females store sperm in special organs, and fertilize eggs only at the moment of oviposition (Parker 1970). Sexual selection theory predicts that males should either (1) reduce the likelihood of their sperm competing with rivals' sperm, by minimizing female mating rate, or (2) maximize their probability of fertilization by removing, displacing, or incapacitating rivals' sperm, when sperm competition is unavoidable.

The first hypothesis predicts the evolution of claspers or other structures to maintain a secure hold of the female, as in water striders (Arnqvist and Rowe 2002) or aquatic beetles (Bergsten et al. 2001). Odonates have two anal appendages, which are used to grasp the prothorax (Zygoptera) or head (Anisoptera) of females. These structures are species specific, and are therefore good taxonomic characters. In some Zygoptera, male anal appendages stimulate particular areas of the prothorax of the female, and may contribute to species recognition (Robertson & Paterson 1982). As far as we know, odonate genitalia do not have internal claspers used to secure females during copulation, but the *hamuli* (figure 15.2) could be externally used for this. Some stout spines found at the basis of the *ligula*, like in *Ischnura* (see figure 3 in Córdoba-Aguilar & Cordero Rivera 2008), might be an example of such structures.

The second hypothesis has stimulated a fruitful field of research in odonates. The pioneering work of Jonathan Waage and Peter Miller (among others) in the 1980s and 1990s, showed that the penis is covered by spines, oriented backwards, which trap sperm stored in the female genitalia (figures 15.3 and 15.4) that can therefore be removed during copulation (Miller 1987b, 1991, 1995; Miller & Miller 1981; Waage 1979, 1984, 1986). In some cases, the penis lack spines, but is used to reposition rivals' sperm far from the fertilization sites (Siva-Jothy 1988). In other cases, spines are present but the penis cannot physically remove sperm from the spermatheca (Cordero et al. 1995).

Predictions derived from sperm competition theory have been very successful at explaining odonate genital diversity. In general, there is a good concordance between male genitalic structure and female sperm storage organs (Waage 1984). Two possibilities have been recognized. First, in many species, notably in the Calopterygidae and Coenagrionidae, the penis has a form and a size that enables males to situate the *ligula* well inside the females' bursa copulatrix, and sometimes, spermatheca. In these cases, sperm competition theory predicts the evolution of spines and other structures that trap sperm and eject it to the outside during genitalic movements of stage I (figure 15.3c, d and 15.4c). Second, in some cases males have genitalia that cannot be inserted inside the spermatheca, thereby making the presence of spines useless for sperm removal. Nevertheless, if male and female genitalia are in an evolutionary arms race, the evolution of sperm storage organs in females that cannot be accessed by males, may explain cases of "useless" spines in males, as primitive characters that have not been lost possibly because selection against them is weak.

Cryptic Female Choice: Tests, Predictions and Results

Females can exert postcopulatory choice only if males are unable to remove sperm from the bursa and spermatheca completely. The fact that many studies on odonate reproductive biology have reported that P_2 values, the proportion of sperm fertilized by the second of two males mated to the same female, is nearly 100% (for a review see Córdoba-Aguilar et al. 2003b) is strong evidence in favor of male control of fertilization, that is sperm competition. Nevertheless, the last male advantage is only clear when eggs are laid shortly after mating. The pattern is less clear for eggs fertilized some days after copulation (Cordero & Miller 1992; McVey & Smittle 1984; Sawada 1998; Siva-Jothy & Tsubaki 1989). Behavioral studies have shown that females sometimes lay eggs without re-mating, even in the presence of territorial males (Siva-Jothy & Hooper 1996), and this is certainly common in species that lay eggs unguarded, like many *Ischnura* (Cordero 1994) or *Calopteryx* (Cordero Rivera & Andrés 2002; Hooper & Siva-Jothy 1997; Waage 1987). Therefore, the potential for cryptic female choice (CFC) in odonates is high. Cryptic female choice mechanisms are possible when some of the sperm stored inside the genitalic trait of females remain unreachable for males. The evolution of two sperm storage organs, the bursa and the spermatheca, is consistent with predictions of CFC theories (but certainly not the only possibility for females retaining some control of sperm stores).

Variation in copulation duration (figure 15.6) has been used to infer mechanisms of genital evolution (Córdoba-Aguilar & Cordero Rivera 2008). Cordero (1990) experimentally showed that copulation duration in some damselflies varies with female mating history. Apparently males control copulation duration (Miller 1987a), and perform short copulations with virgin females and long copulations with previously mated females (Andrés & Cordero Rivera 2000; Cordero 1990). Males can physically remove sperm from both the bursa and the spermatheca(e), in only a fraction of odonate species. In these cases, females seem to have lost their control over fertilization, and male and female genitalia diversity is well explained by sperm competition predictions.

In many cases, nevertheless, males have a spoon-shaped *ligula* (figure 15.4c), as in the Lestidae (Uhía & Cordero Rivera 2005; Waage 1982), and some of the Coenagrionidae (Andrés & Cordero Rivera 2000; Uhía & Cordero Rivera 2005), or short genitalic processes, that clearly cannot remove sperm from the spermatheca, as in the Platycnemididae (Uhía & Cordero Rivera 2005). Some species have no spermatheca (Uhía & Cordero Rivera 2005). This diversity is well suited for controlled experiments to test predictions of CFC and sperm competition hypotheses. It is easy to see that if females do not have spermatheca, males can remove sperm from the bursa, and if sperm removal is fast, then males should not prolong copulation with mated females compared to virgins (figure 15.6; case of *Lestes barbarus* and *L. virens*). This assumes that sperm removal from the bursa is fast. This seems reasonable, because removing sperm from the bursa needs only 5 minutes in *Ceriagrion tenellum* (Andrés & Cordero Rivera 2000), and *Calopteryx* males are able to completely empty the bursa in less than two minutes (Cordero Rivera et al. 2004). The behavior of species without a spermatheca and with a bursa accessible to male genitalia could be explained by sperm competition alone, which suggests that females have little control over the process. If sperm removal were slow, then males should mate for longer periods with mated females, to maximize the amount of sperm removed, irrespective of the presence/absence of spermathecae. We are unaware of any example of this in Odonata. On the contrary, if males cannot remove sperm from the spermatheca, but they remove it from the bursa, then sperm competition predicts the same copulation duration with virgin and mated females, but cryptic female choice predicts longer matings with mated females, because males should perform "copulatory courtship" (Eberhard 1994) to increase their paternity success, that is, mated females can cryptically choose, but virgins do not (although if the virgin is going to store sperm before egg-laying she may choose later). In this case, the sperm stored in the spermatheca, which is inaccessible to male genitalia, is the key that allows females to exercise cryptic choice. There is experimental evidence in Coenagrionidae, Lestidae, and Platycnemididae in agreement with CFC predictions (figure 15.6; Uhía & Cordero Rivera 2005).

Even after copulation females might exert cryptic choice, for instance by selectively ejecting sperm from a particular male (figure 15.1f). The ejection of sperm after copulation has been overlooked in studies of odonate behavior, until Eberhard (1996) highlighted observations on *Paraphlebia quinta* females, that were seen to expel a drop of sperm after copulation (González Soriano & Córdoba-Aguilar 2003). This led to further experimental and observational work that suggested this is a case of cryptic female choice of sperm (Córdoba-Aguilar 2006). Even females of *Ischnura graellsii* have been observed to expel sperm after their first mating, before oviposition, in a laboratory environment

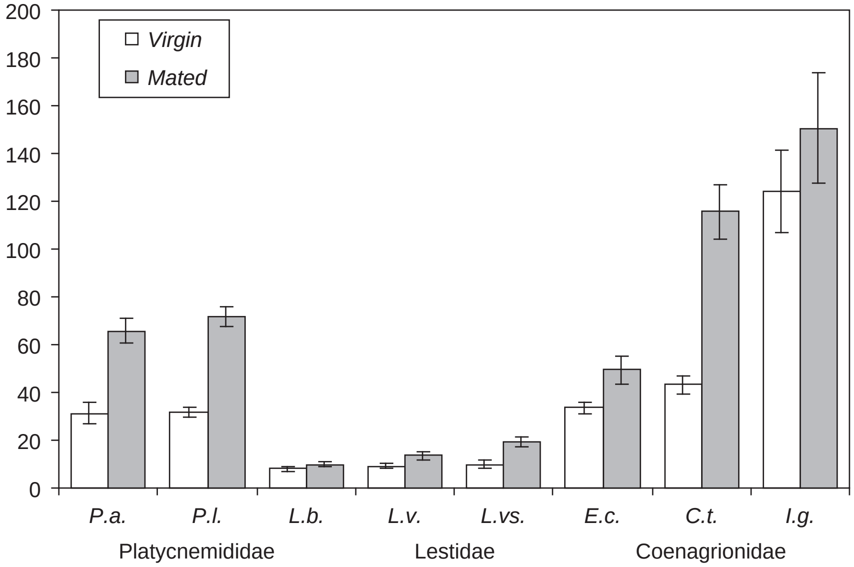

FIGURE 15.6 Copulation duration (mean±SE) in laboratory matings of virgin and mated females of three families of Zygoptera: *Platycnemis acutipennis (P.a.), P. latipes (P.l.)* (Platycnemididae); *Lestes barbarus (L.b.), L. virens (L.v.), Lestes viridis (L.vs.)* (Lestidae), and *Enallagma cyathigerum (E.c.), Ceriagrion tenellum (C.t.), Ischnura graellsii (I.g.)* (low density) (Coenagrionidae). Note that copulation lasts longer when females have stored sperm, but this effect is not significant for species without a spermatheca (*L.b.* and *L.v.*). data from Andrés & Cordero Rivera 2000; Cordero 1990; Uhía & Cordero Rivera 2005).

(A. Cordero, personal observation). The control of sperm release from the spermatheca is so good that females have apparently evolved a mechanism by which they can control from which spermathecal duct they can eject more sperm, in *C. haemorrhoidalis*, a species whose females have two spermathecal ducts (figure 15.5) (Córdoba-Aguilar & Siva-Jothy 2004). Nevertheless, this sperm ejection behavior could be a by-product of sperm competition, if females were simply ejecting the sperm removed by their mate during copulation (Lindeboom 1998). Future studies should use molecular markers to determine the identity of the sperm, by comparing DNA fingerprints of the last male and the ejected sperm. If a CFC mechanism is at work, then in many cases the sperm ejected should belong to the copulating male.

Sexual Conflict: Tests, Predictions and Results

The interpretation of sexual selection as a conflict of interests is not an alternative to the sperm competition and CFC. Rather, conflict will be ubiquitous given that males and females have different interests. In some cases, males seem to be ahead in the interaction, and a sperm competition approach is then the best to explain and predict patterns (for instance in *Ischnura graellsii;* Cordero & Miller 1992). In other cases, although sperm competition, in the form of sperm removal, occurs, CFC mechanisms prevail (a good example is *Ceriagrion tenellum;* Andrés & Cordero Rivera 2000). Nevertheless, there are some novel predictions about genitalic evolution that cannot be derived from the male or female standpoint alone. From the previous discussion, the sperm stores in the spermatheca seem to be the focus of sexual conflict. Long term maintenance of sperm in this organ allows females to lay fertile eggs over their whole life, even after just one mating (Cordero 1990; Fincke 1987;Grieve 1937). Females can control which organ releases sperm during fertilization, and it has been shown that they use both organs in different contexts (Nakahara & Tsubaki 2007, Siva-Jothy & Hooper 1996). An elegant and recent paper on *Ischnura senegalensis* has revealed that the spermatheca is a safer place (in terms of mortality) for sperm than the bursa, perhaps because sperm in the bursa are more likely to be removed (Nakahara & Tsubaki 2007). This may explain patterns of sperm use in this species in which females use bursal sperm immediately after copulation while spermathecal sperm are used over the long-term (Nakahara & Tsubaki 2007). Furthermore, patterns of sperm survival are associated with whether sperm are removed or not. In *Mnais pruinosa*, where spermathecal sperm is not removed, sperm survival in the spermatheca is reduced as compared to *Calopteryx cornelia* where spermathecal sperm are removed (Hayashi & Tsuchiya 2005). If males are able to reach the spermatheca to remove sperm, one possible evolutionary response by females is a reduction of the size of this organ, as it is no longer useful for sperm storage. This is what Hayashi and Tsuchiya (2005) found for *Mnais pruinosa*, where the spermatheca is almost vestigial. Nevertheless, other alternatives do exist that allow females to regain control: longer spermathecal ducts, which impede males from reaching the tip of the spermatheca (a likely case is *Calopteryx splendens*; A. Cordero, personal observations), the evolution of mechanical barriers in the ducts, larger spermathecae internally convoluted, and so on. In any case, such an interspecific difference in the function of sperm storage organs suggests antagonistic coevolution between the sexes (Holland & Rice 1998) as females may derive benefits from storing sperm and such benefits are lost once males are able to have access to the spermatheca. In fact, a reduction in female mating rate has been associated with increased male ability to displace sperm (Córdoba-Aguilar 2009). This means that when females accrue no more benefits of storing sperm, there is no need to mate multiply.

In relation to genitalic evolution, a straightforward prediction is that male traits that manipulate females in ways that reduce the female's direct fitness (Eberhard 2006), like reducing re-mating frequency, or increasing egg-laying even if this has survival costs for females, are to be expected. Examples are hooks and other structures that damage the internal female genital tract (Crudgington & Siva-Jothy 2000), or seminal products that are toxic to females (Rice 1996). Nevertheless, damaging females would be a bizarre male adaptation. Morrow et al. (2003) experimentally showed in two beetles and *Drosophila melanogaster* that females do not delay re-mating or increase their reproductive rate after being harmed, but re-mate sooner and lay fewer eggs in some cases. This suggests that male harmful "adaptations" in the context of sexual conflict are more parsimoniously explained as byproducts or pleiotropic side effects of other male adaptations.

Nothing is known about the composition of odonate ejaculates, but hooks and spines are common in the penis (figure 15.3 and 15.4). Nevertheless, these structures seem better explained as sperm removal devices rather than "sexual weapons" in odonates. There is little evidence for negative effects on females of multiple mating, except in some polymorphic *Ischnura* (Cordero et al. 1998;Gosden & Svensson 2007; Sirot & Brockmann 2001), and no evidence for genital damage during mating in odonates. Dunkle (1991) found dragonfly females whose heads were damaged, presumably by the male abdominal appendages during mating attempts prior to secure tandem formation, but this would be a "weaponry" case for a non-intromittent genitalic trait. This topic merits further study.

Other Hypotheses: Lock and Key and Pleiotropy

Rooted in evolution textbooks and traditional evolutionary thinking, is the idea that animals may continuously face the risk of mating with members of a different species. This should promote the evolution of physiological, morphological and behavioral traits aimed to reduce such risk and complex genitalia may be a such a set of traits (Dufour 1844 in Mayr 1963). Complex genitalia, being species-specific, prevent males from mating with females of different species. This lock and key hypothesis "purports to explain species-specific genitalic morphology in terms of mechanical reproductive isolation" (Shapiro & Porter 1989), and although defended by a few people (e.g., Nagata et al. 2007; Takami et al. 2007), has not been supported either by recent tests (Arnqvist et al. 1997; Arnqvist 1998; Arnqvist & Thornhill 1998), or by comparative evidence (Eberhard 1985). The lock and key hypothesis, however, has not been tested in odonates. Watson (1966) in a study of the size of secondary genitalia in five species of *Tramea* (Libellulidae), concluded that there is a clear correlation between the size of male hamuli and female vulvar scales, and suggested that this is an example of a lock and key mechanism in the Odonata. This example is suggestive but manipulative experiments are needed for a formal test of the hypothesis.

On the other hand, Paulson (1974) performed experiments with five species of male Coenagrionidae and ten species of females, and only in one of the possible combinations was the male able to achieve the tandem position easily, suggesting that the primary genitalia in this family may act as a mechanical barrier to interspecific matings.

In fact, there is evidence for a match between male anal appendages and female mesostigmal plates in the Zygoptera, acting as a mechanical barrier to interspecific tandems (Robertson & Paterson 1982), although it never prevents all interspecific tandems (Corbet 1999, reviewed in Utzeri & Belfiore 1990). The fact that interspecific matings and hybrids (Leong & Hafernik 1992; Monetti et al. 2002; Tynkkynen et al. 2008) may be more common than usually thought, supports the assumption that there is a risk of heterospecific mating.

Genitalia may have a role in avoiding heterospecific matings, at least in those species that do not show pre-copulatory courtship. Whether the lock and key hypothesis can be the prime explanation for genitalic evolution in these animals is still open to discussion. It may also be that a lock and key process is incidentally reinforced by a sexual selection mechanism. It may actually be that the two mechanisms are not mutually exclusive. Take the case of two sister, sometimes sympatric, species for which interspecific matings and hybrids have been documented: *Ischnura graellsii* and *I. elegans*. It has been shown that these species produce hybrids and are undergoing an incipient isolation (Monetti et al. 2002). Thus, one would expect that there must be selection to avoid mating mistakes. There is evidence which suggests that secondary male genitalia in both species are under strong sexual selection (Cordero & Miller 1992; Miller 1987a, b). But also, a lock and key mechanism may apply for the primary genitalia of these species: the prothoracic tubercle of female *I. elegans* impedes males of *I. graellsii* from achieving a firm grasp for a precopulatory tandem. As a consequence, these hybrid matings are almost never observed (Monetti et al. 2002). The opposite is nevertheless not true: male *I. elegans* have no physical impediments in grasping female *I. graellsii*, and matings between the two species are frequent in the laboratory and the field, resulting in viable hybrids (R.A. Guillén & A. Cordero, unpublished).

The pleiotropy hypothesis supposes a nonfunctional basis for genitalic traits. It implies that the same genes that control other adaptive traits, incidentally control genitalic morphology. Thus, genitalic evolution is driven by the evolution of other adaptive traits (Arnold 1973; Mayr 1963). Although this hypothesis is tremendously difficult to test, the few tests performed with insects have not rejected it (Arnqvist et al. 1997; Arnqvist & Thornhill 1998). In fact, in one group of Jamaican millipedes, this hypothesis seems to match the gradual evolution of male genitalia (Bond et al. 2003). Whether the pleiotropy hypothesis operates on odonate genitalic traits also needs to be checked although we cannot foresee an easy experimental test using these animals. (Evidence that specific genital traits are strongly correlated with fitness would argue against a pleiotropy hypothesis).

SEASONAL EFFECTS: AN OVERLOOKED SOURCE OF GENITAL VARIATION?

Body size in insects is affected by seasonality: adults that emerge early in the reproductive season are larger than those emerging late in the season (Roff 1980, 1986). This pattern is mainly due to the time, accrued food, and developmental strategies, that larvae use depending on when they were laid as eggs (for a review of this in odonates, see Stoks et al. 2008). In those temperate places in which animals have a restricted season (e.g., a few months), this effect is particularly strong as early emerging individuals have spent nearly a year as larvae. This has given them more time to acquire more food, unlike late emerging individuals which may have started and completed their development in the same season that they emerge. Little is known about the effect of this change on genital size and, if this is the case, how genital functions are affected. Some evidence in a dung fly indicates that the seasonal effect may have an evolutionary impact on sperm competition: late emerging individuals have smaller testes which produce less sperm (Ward & Simmons 1991). These late emerging males are less successful as in this species the more successful males are those that transfer relatively more sperm (Simmons & Parker 1992). A recent study in two calopterygids, *Calopteryx haemorrhoidalis asturica* and *Hetaerina americana*, has uncovered more details (Córdoba-Aguilar 2009). These species vary considerably in the extent of their reproductive seasons: from three to four months for *C. h. asturica* and nearly the

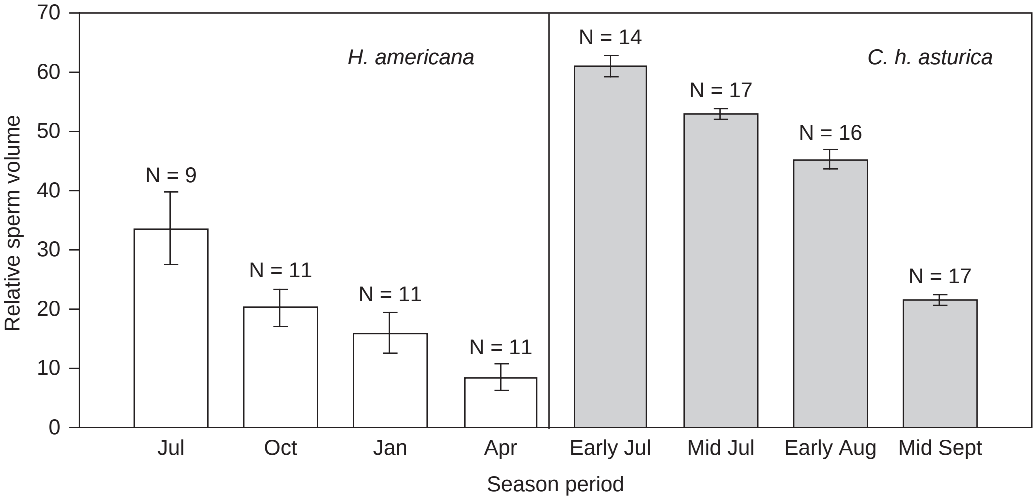

FIGURE 15.7 Variation (mean ± SE) in relative sperm remaining in the spermatheca (the sperm that males were unable to displace after aedeagal stimulation within the female vagina) according to representative months during the reproductive season in *H. americana* and *C. h. asturica*.

whole year for *H. americana*. Since the width of the aedeagus is important in displacing sperm in both species (the wider the aedeagus, the more intense the stimulation, which induces females to eject previously stored sperm) and this trait correlates with body size, seasonality has extensive effects. Interestingly, larger males tend to obtain the territories in which females arrive to mate (for *C. h. asturica* see Córdoba-Aguilar 2009; for *H. americana* see Serrano-Meneses et al. 2007) so that, in general, large males are more successful. However, early emerging males that gain a territory tend to mate with females that match their size, which is a situation different to late emerging males which mate with females that vary considerably in size (as different cohorts overlap) but in general are relatively smaller. In fact, if one measures the sexual size dimorphism of mating couples over the season, skew toward larger males becomes more pronounced at the end of the season. In terms of copulation, this means that late in the season males with larger aedeagi with respect to the female zone that the aedeagus stimulates, become more successful in eliciting female sperm ejection than males early in the season (see figure 15.7). Thus, late in the season females are less able to keep away the sperm they stored. The fact that this pattern is consistent in two species that differ in the extent of their reproductive season, implies that this phenomenon may apply to other species including non-odonate species. In terms of sperm competition and/or sexual conflict, then there will be varying regimes of selection intensity along the season and the female benefits of storing sperm and keeping it unreachable during male displacement will vary depending on when females emerge. In fact, late emerging females tend to mate less frequently and male harassment increases both as possible consequences of the reduction in mating frequency (Córdoba-Aguilar 2009).

EVOLUTION OF GENITALIA AS AN ENGINE FOR SPECIES DIVERGENCE?

Genitalic diversification has long ago been proposed as an important engine for species divergence via sexual selection (Eberhard 1985). In many animal groups there is a general pattern for male genitalia to be more diverse than female genitalia, and this has been interpreted in terms of cryptic female choice (Eberhard 1996). In odonates, there is evidence for allopatric divergence of male and female genitalia in the Calopterygidae (Cordero Rivera et al. 2004). The genus *Calopteryx* has been enlightening in this respect. Research in different species has shown that males have evolved different genitalic morphologies aimed to displace spermathecal sperm (but never seem able to remove all) and that, due to sexual co-evolution, there may be representatives of different "situations". For example, in *C. maculata*, the penis is narrow enough to penetrate the spermatheca and remove the sperm located in this site (Córdoba-Aguilar 2003b; Waage 1979). This does not apply to *C. splendens xanthostoma* where the *ligula* is larger than the spermatheca (Córdoba-Aguilar 2003b) which explains why males cannot remove the sperm present in this organ (Siva-Jothy and Hooper 1996). These differences are not only inter- but also intraspecific. In *C. haemorrhoidalis*, there is variation in sperm displacement mechanisms with males of some populations being able to displace spermathecal sperm while in other populations, males are unable to do so (Cordero Rivera et al. 2004). These interpopulational differences are only present in genitalia and not in other traits which suggests that sexual selection, at the copulatory level, has been key in species divergence. It may be that post-copulatory sexual selection may be stronger than pre-copulatory sexual selection and this is why the evolution of genitalic traits has been the engine of species divergence. This idea can be tested now with the genus *Calopteryx* where information is available as to characters being selected during pre- (i.e., pigmentation; Córdoba-Aguilar & Cordero Rivera 2005) and post-copulatory events.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Although it seems that sexual selection, particularly sperm competition, is an important force shaping genital morphology and function, other selective forces cannot be disregarded. Other sexual selection forces are cryptic female choice and sexual conflict. A similar argument can be made for natural selection hypotheses, especially the lock and key hypothesis. Further investigations should test hypotheses from both sexual and natural selection.

Our knowledge of genital functional morphology is still rather poor for many families of Anisoptera (but see Pfau 2005; for a comprehensive work see Siva-Jothy 1997), and this is especially true for females. Another research priority is tropical families, and also species-poor and primitive taxa, like the Hemiphlebiidae or Petaluridae. Furthermore, study of the genital morphology of highly diverse and localized taxa, like *Megalagrion* in Hawaii (Polhemus and Asquith 1996) or *Nesobasis* in Fiji (Donnelly 1990), both with more than 20 species, would be appropriate tests of hypotheses of genital evolution and speciation on islands.

As we have mentioned above, there is limited evidence for mating frequency having negative effects on females, and we lack direct evidence for genital damage, two predictions derived from sexual conflict hypotheses, and therefore open to future studies. Finally, the lock-and-key and pleiotropy hypotheses are still not formally tested with odonates, a group that offers high rewards for future studies of genital diversity.

Acknowledgments This work was directly related to part of the goals of the research grants CGL2005-00122 and CGL2008-02799 (Dirección General de Proyectos de Investigación, Ministerio de Ciencia e Innovación de España including FEDER funding). We are very grateful to the reviewers for their clever suggestions to improve this manuscript.

REFERENCES

Adams JA, Herman TH (1991). A comparison of the male genitalia of three *Calopteryx* species (Odonata: Calopterygidae). Canadian Journal of Zoology, 69, 1164–1170.

Andrés JA, Cordero Rivera A (2000). Copulation duration and fertilization success in a damselfly: An example of cryptic female choice? Animal Behaviour, 59, 695–703.

Andrew RJ, Tembhare DB (1997). The post-ovarian genital complex in *Anax guttatus* (Burmeister) (Anisoptera: Aeshnidae). Odonatologica, 26, 385–394.

Arnold EN (1973). Relationships of the palaeartic lizards assigned to the genera *Lacerta, Algyroids* and *Psammordromus* (Reptilia: Lacertidae). Bulletin of the British Museum of Natural History (Zoology), 25, 291–366.

Arnqvist G (1998). Comparative evidence for the evolution of genitalia by sexual selection. Nature, 393, 784–785.

Arnqvist G, Rowe L (2002). Correlated evolution of male and female morphologles in water striders. Evolution, 56, 936–947.

Arnqvist G, Rowe L (2005). Sexual Conflict. Princeton University Press, Princeton, NJ.

Arnqvist G, Thornhill R (1998). Evolution of animal genitalia: Patterns of phenotypic and genotypic variation and condition dependence of genital and non-genital morphology in water strider (Heteroptera: Gerridae: Insecta). Genetical Research, 71, 193–212.

Arnqvist G, Thornhill R, Rowe L (1997). Evolution of animal genitalia: Morphological correlates of fitness components in a water strider. Journal of Evolutionary Biology, 10, 613–640.

Banks MJ, Thompson DJ (1985). Lifetime mating success in the damselfly *Coenagrion puella*. Animal Behaviour, 33, 1175–1183.

Bechly G, Brauckmann C, Zessin W, Groning E (2001). New results concerning the morphology of the most ancient dragonflies (Insecta: Odonatoptera) from the Namurian of Hagen-Vorhalle (Germany). Journal of Zoological Systematics and Evolutionary Research, 39, 209–226.

Bergsten J, Toyra A, Nilsson AN (2001). Intraspecific variation and intersexual correlation in secondary sexual characters of three diving beetles (Coleoptera: Dytiscidae). Biological Journal of the Linnean Society, 73, 221–232.

Bond JE, Beamer DA, Hedin MC, Sierwald P (2003). Gradual evolution of male genitalia in a sibling species complex of millipedes (Diplopoda: Spirobolida: Rhinocricidae: *Anadenobolus*). Invertebrate Systematics, 17, 711–717.

Bybee SM, Ogden TH, Branham MA, Whiting MF (2008). Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. Cladistics, 24, 477–514.

Conrad KF, Pritchard G (1992). An ecological classification of odonate mating systems: the relative influence of natural, inter- and intra-sexual selection on males. Biological Journal of the Linnean Society, 45, 255–269.

Corbet PS (1999). Dragonflies. Behaviour and Ecology of Odonata. Harley Books, Essex, UK.

Cordero A (1990). The adaptive significance of the prolonged copulations of the damselfly, *Ischnura graellsii* (Odonata: Coenagrionidae). Animal Behaviour, 40, 43–48.

Cordero A (1994). Inter-clutch interval and number of ovipositions in females of the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). Etología, 4, 103–106.

Cordero A, Miller PL (1992). Sperm transfer, displacement and precedence in *Ischnura graellsii* (Odonata: Coenagrionidae). Behavioral Ecology and Sociobiology, 30, 261–267.

Cordero A, Santolamazza Carbone S, Utzeri C (1995). Male disturbance, repeated insemination and sperm competition in the damselfly *Coenagrion scitulum* (Zygoptera: Coenagrionidae). Animal Behaviour, 49, 437–449.

Cordero A, Santolamazza Carbone S, Utzeri C (1998). Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata). Animal Behaviour, 55, 185–197.

Cordero Rivera A, Andrés JA (2002). Male coercion and convenience polyandry in a Calopterygid damselfly (Odonata). Journal of Insect Science, 2, 14—Available online: insectscience.org/2.14.

Cordero Rivera A, Andrés JA, Córdoba-Aguilar A, Utzeri C (2004). Postmating sexual selection: allopatric evolution of sperm competition mechanisms and genital morphology in calopterygid damselflies (Insecta: Odonata). Evolution, 58, 349–359.

Córdoba-Aguilar A (2002a). Sensory trap as the mechanism of sexual selection in a damselfly genitalic trait (Insecta: Calopterygidae). American Naturalist, 160, 594–601.

Córdoba-Aguilar A (2002b). Wing pigmentation in territorial male damselflies, *Calopteryx haemorrhoidalis*: a possible relation to sexual selection. Animal Behaviour, 63, 759–766.

Córdoba-Aguilar A (2003a). A description of male and female genitalia and a reconstruction of copulatory and fertilisation events in *Calopteryx haemorrhoidalis* (Vander Linden) (Zygoptera : Calopterygidae). Odonatologica, 32, 205–214.

Córdoba-Aguilar A (2003b). Predicting mechanisms of sperm displacement based on genitalic morphometrics in the Calopterygidae (Odonata). Journal of Insect Behavior, 16, 153–167.

Córdoba-Aguilar A (2006). Sperm ejection as a possible cryptic female choice mechanism in

Odonata (Insecta). Physiological Entomology, 31, 146–153.

Córdoba-Aguilar A (2010). Seasonal variation in genital and body size, sperm displacement ability, female mating rate and male harassment in two calopterygid damselflies (Odonata: Calopterygidae). Biological Journal of the Linnean Society, 96, 815–829.

Córdoba-Aguilar A, Cordero Rivera A (2005). Evolution and ecology of Calopterygidae (Zygoptera: Odonata): Status of knowledge and future research perspectives. Neotropical Entomology, 34, 861–879.

Córdoba-Aguilar A, Cordero Rivera A (2008). Cryptic female choice and sexual conflict. In A Córdoba-Aguilar, ed. Dragonflies. Model Organisms for Ecological and Evolutionary Research, pp 189–202. Oxford University Press, Oxford.

Córdoba-Aguilar A, Siva-Jothy MT (2004). Sperm displacement ability in *Calopteryx haemorrhoidalis* (Vander Linden): Male and female roles, male limits in performance, and female neural control (Zygoptera: Calopterygidae). Odonatologica, 33, 245–252.

Córdoba-Aguilar A, Salamanca-Ocaña JC, Lopezaraiza M (2003a). Female reproductive decisions and parasite burden in a calopterygid damselfly (Insecta: Odonata). Animal Behaviour, 66, 81–87.

Córdoba-Aguilar A, Uhía E, Cordero Rivera A (2003b). Sperm competition in Odonata (Insecta): the evolution of female sperm storage and rivals' sperm displacement. Journal of Zoology, 261, 381–98.

Crudgington HS, Siva-Jothy MT (2000). Genital damage, kicking and early death. Nature, 407, 855–856.

Dijkstra KDB, Clausnitzer V, Martens A (2007). Tropical African *Platycnemis* damselflies (Odonata: Platycnemididae) and the biogeographical significance of a new species from Pemba Island, Tanzania. Systematics and Biodiversity, 5, 187–198.

Donnelly TW (1990). The Fijian genus *Nesobasis* Part 1: Species of Viti Levu, Ovalau, and Kadavu (Odonata: Coenagrionidae). New Zealand Journal of Zoology, 17, 87–117.

Dunkle SW (1991). Head damage from mating attempts in dragonflies (Odonata: Anisoptera). Entomological News, 102, 37–41.

Eberhard W (2006). Sexually antagonistic coevolution in insects is associated with only limited morphological diversity. Journal of Evolutionary Biology, 19, 657–681.

Eberhard WG (1985). Sexual Selection and Animal Genitalia. Harvard University Press, Cambridge, Massachusetts.

Eberhard WG (1994). Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. Evolution, 48, 711–733.

Eberhard WG (1996). Female Control: Sexual Selection by Cryptic Female Choice. Princeton University Press, Princeton.

Fincke OM (1986). Lifetime reproductive success and the opportunity for selection in a nonterritorial damselfly (Odonata: Coenagrionidae). Evolution, 40, 791–803.

Fincke OM (1987). Female monogamy in the damselfly *Ischnura verticalis* Say (Zygoptera: Coenagrionidae). Odonatologica, 16, 129–143.

Fincke OM (1997). Conflict resolution in the Odonata: implications for understanding female mating patterns and female choice. Biological Journal of the Linnean Society, 60, 201–220.

Fincke OM, Hadrys H (2001). Unpredictable offspring survivorship in the damselfly, *Megaloprepus coerulatus*, shapes parental behavior, constrains sexual selection, and challenges traditional fitness estimates. Evolution, 55, 762–772.

Fincke OM, Waage JK and Koenig WD (1997). Natural and sexual selection components of odonate mating patterns. In JC Choe, BJ Crespi, eds. The Evolution of Mating Systems in Insects and Arachnids, pp 58–74. Cambridge University Press, Cambridge.

Fleck G, Bechly G, Martínez-Delclòs X, Jarzembowski EA, Nel A (2004). A revision of the Upper Jurassic–Lower Cretaceous dragonfly family Tarsophlebiidae, with a discussion on the phylogenetic positions of the Tarsophlebiidae and Sieblosiidae (Insecta, Odonatoptera, Panodonata). Geodiversitas, 26, 33–60.

Garrison RW (2006). A synopsis of the genera *Mnesarete* Cowley, *Bryoplathanon* gen. nov., and *Ormenophlebia* gen. nov. (Odonata: Calopterygidae). Contributions in Science, 506, 1–84.

Garrison RW, Von Ellenrieder N and Louton J (2006). Dragonfly Genera of the New World.

The Johns Hopkins University Press, Baltimore.

Gassmann D (1999). Taxonomy and distribution of the inornata species-group of the Papuan genus *Idiocnemis* Selys (Odonata: Zygoptera: Platycnemididae). Invertebrate Taxonomy, 13, 977–1005.

Gassmann D (2000). Revision of the Papuan *Idiocnemis bidentata*-group (Odonata: Platycnemididae). Zoologische Mededelingen, 74, 375–402.

Gassmann D (2005). The phylogeny of Southeast Asian and Indo-Pacific Calicnemiinae (Odonata, Platycnemididae). Bonner zoologische Beiträge, 53, 37–80.

Gassmann D, Hämäläinen M (2002). A revision of the Philippine subgenus *Risiocnemis* (*Igneocnemis*) Hämäläinen (Odonata: Platycnemididae). Tijdschrift voor Entomologie, 145, 213–266.

González Soriano E, Córdoba-Aguilar A (2003). Sexual behaviour in *Paraphlebia quinta* calvert: Male diamorphism and a possible example of female control (Zygoptera: Megapodagrionidae). Odonatologica, 32, 345–353.

Gosden TP, Svensson EI (2007). Female sexual polymorphism and fecundity consequences of male mating harassment in the wild. PLoS ONE, 2, e580.

Grieve EG (1937). Studies on the biology of the damselfly *Ischnura verticalis* Say, with notes on certain parasites. Entomologica Americana, 17, 121–153.

Haubruge E, Arnaud L, Mignon J, Gage MJG (1999). Fertilization by proxy: Rival sperm removal and translocation in a beetle. Proceedings of the Royal Society of London Series B Biological Sciences, 266, 1183–1187.

Hayashi F, Tsuchiya K (2005). Functional association between female sperm storage organs and male sperm removal organs in calopterygid damselflies. Entomological Science, 8, 245–252.

Heymer A (1973). Étude du comportement reproducteur et analyse des méchanismes déclencheurs innés (MDI) optiques chez les Calopterygidae (Odon. Zygoptera). Annales de la Societé entomologique de France (Nouvelle Série), 9, 219–255.

Holland B, Rice WR (1998). Perspective: Chase-away sexual selection: Antagonistic seduction versus resistance. Evolution, 52, 1–7.

Hooper RE, Siva-Jothy MT (1997). “Flybys”: A prereproductive remote assessment behavior of female *Calopteryx splendens xanthostoma* (Odonata: Calopterygidae). Journal of Insect Behavior, 10, 165–175.

Kamimura Y (2000). Possible removal of rival sperm by the elongated genitalia of the earwig, *Euborellia plebeja*. Zoological Science, 17, 667–672.

Kennedy CH (1920). The phylogeny of the zygopterous dragonflies as based on the evidence of the penes. Ohio Journal of Science, 21, 19–32.

Leong JM, Hafernik JEJ (1992). Hybridization between two damselfly species (Odonata: Coenagrionidae): Morphometric and genitalic differentiation. Annals of the Entomological Society of America, 85, 662–670.

Lindeboom M (1998). Post-copulatory behaviour in *Calopteryx* females (Insecta, Odonata, Calopterygidae). International Journal of Odonatology, 1, 175–184.

Mayr E (1963). Animal Species and Evolution. Harvard University Press, Harvard.

McVey ME, Smittle BJ (1984). Sperm precedence in the dragonfly *Erythemis simplicicollis*. Journal of Insect Physiology, 30, 619–628.

Miller PL (1987a). An examination of the prolonged copulations of Ischnura elegans (Vander Linden) (Zygoptera: Coenagrionidae). Odonatologica, 16, 37–56.

Miller PL (1987b). Sperm competition in *Ischnura elegans* (Vander Linden) (Zygoptera: Coenagrionidae). Odonatologica, 16, 201–207.

Miller PL (1991). The structure and function of the genitalia in the Libellulidae (Odonata). Zoological Journal of the Linnean Society, 102, 43–73.

Miller PL (1995). Sperm competition and penis structure in some libellulid dragonflies (Anisoptera). Odonatologica, 24, 63–72.

Miller PL, Miller CA (1981). Field observations on copulatory behaviour in Zygoptera, with an examination of the structure and activity of male genitalia. Odonatologica, 10, 201–218.

Monetti L, Sánchez-Guillén RA, Cordero Rivera A (2002). Hybridization between *Ischnura graellsii* (Vander Linder) and *I. elegans* (Rambur) (Odonata: Coenagrionidae): are they different species? Biological Journal of the Linnean Society, 76, 225–235.

Morrow EH, Arnqvist G, Pitnick S (2003). Adaptation versus pleiotropy: why do males harm their mates? Behavioral Ecology, 14, 802–806.

Nagata N, Kubota K, Yahiro K, Sota T (2007). Mechanical barriers to introgressive hybridization revealed by mitochondrial introgressive patterns in *Ohomopterus* ground beetles assemblages. Molecular Ecology, 16, 4822–4836.

Nakahara M, Tsubaki Y (2007). Function of multiple sperm-storage organs in female damselflies (*Ischnura senegalensis*): Difference in amount of ejaculate stored, sperm loss, and priority in fertilization. Journal of Insect Physiology, 53, 1046–1054.

Ono T, Siva-Jothy MT, Kato A (1989). Removal and subsequent ingestion of rivals' semen during copulation in a tree cricket. Physiological Entomology, 14, 195–202.

Orr AG, Hämäläinen M (2007). *The Metalwing Demoiselles* (*Neurobasis and Matronoides*) *of the Eastern Tropics*. Natural History Publications, Borneo.

Parker GA (1970). Sperm competition and its evolutionary consequences in the insects. Biological Reviews, 45, 525–567.

Paulson DR (1974). Reproductive isolation in damselfies. Systematic Zoology, 23, 40–49.

Pessacq P (2008). Phylogeny of Neotropical Protoneuridae (Odonata: Zygoptera) and a preliminary study of their relationship with related families. Systematic Entomology, 33, 511–528.

Pfau HK (1971). Struktur und Funktion des sekundären Kopulationsapparates der Odonaten (Insecta, Palaeoptera), ihre Wandlung in der Stammesgeschichte und Bedeutung für die adaptive Entfaltung. Zeitschrift fuer Morphologie und Oekologie der Tiere, 70, 281–371.

Pfau HK (1991). Contributions of functional morphology to the phylogenetic systematics of the Odonata. Advances in Odonatology, 5, 109–141.

Pfau HK (2005). Structure, function and evolution of the 'glans' of the anisopteran vesica spermalis (Odonata). International Journal of Odonatology, 8, 259–310.

Polhemus D, Asquith A (1996). Hawaiian Damselflies. A Field Identification Guide. Bishop Museum Press, Hawaii.

Rice WR (1996). Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature, 381, 232–234.

Robertson HM, Paterson HEH (1982). Mate recognition and mechanical isolation in *Enallagma* damselflies (Odonata: Coenagrionidae). Evolution, 36, 243–250.

Robertson HM, Tennessen KJ (1984). Precopulatory genital contact in some zygoptera. Odonatologica, 13, 591–595.

Roff DA (1980). Optimizing development time in a seasonal environment: The 'ups and downs' of clinal variation. Oecologia, 45, 202–208.

Roff DA (1986). Predicting body size with life history models. Bioscience, 36, 316–323.

Sawada K (1995). Male's ability of sperm displacement during prolonged copulations in *Ischnura senegalensis* (Rambur) (Zygoptera: Coenagrionidae). Odonatologica, 24, 237–244.

Sawada K (1998). Sperm precedence in the damselfly *Ischnura senegalensis* (Rambur): Is prolonged copulation advantageous to sperm precedence? (Zygoptera: Coenagrionidae). Odonatologica, 27, 425–431.

Serrano-Meneses MA, Córdoba-Aguilar A, Méndez V, Layen SJ, Székely T (2007). Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. Animal Behaviour, 73, 987–997.

Shapiro A, Porter A (1989). The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. Annual Review of Entomology, 34, 231–245.

Silsby J (2001). Dragonflies of the Wold.The Natural History Museum, London.

Simmons LW (2001). Sperm Competition and Its Evolutionary Consequences in the Insects. Princeton University Press, Princeton.

Simmons LW, Parker GA (1992). Individual variation in sperm competition success of yellow dung flies, *Scatophaga stercoraria*. Evolution, 46, 366–375.

Sirot LK, Brockmann HJ (2001). Costs of sexual interactions to females in Rambur's forktail damselfly, *Ischnura ramburi* (Zygoptera: Coenagrionidae). Animal Behaviour, 61, 415–424.

Siva-Jothy MT (1984). Sperm competition in the family Libellulidae (Anisoptera) with special reference to *Crocothemis erythraea* (Brullé) and *Orthetrum cancellatum* (L.). Advances in Odonatology, 2, 195–207.

Siva-Jothy MT (1987). The structure and function of the female sperm-storage organs in libellulid dragonflies. Journal of Insect Physiology, 33, 559–567.

Siva-Jothy MT (1988). Sperm “repositioning” in *Crocothemis erythraea*, a libellulid with a brief copulation. Journal of Insect Behavior, 1, 235–245.

Siva-Jothy MT (1997). Odonate ejaculate structure and mating systems. Odonatologica, 26, 415–437.

Siva-Jothy MT, Hooper RE (1996). Differential use of stored sperm during oviposition in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). Behavioral Ecology and Sociobiology, 39, 389–393.

Siva-Jothy MT, Tsubaki Y (1989). Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata: Calopterygidae) 2. Causal factors. Behavioral Ecology and Sociobiology, 25, 261–267.

Stoks R, Johansson F and De Block M. (2008). Life-history plasticity under time stress in damselfly larvae. In A Córdoba-Aguilar, ed. Dragonflies and Damselflies: Model Organisms for Ecological and Evolutionary Research, pp 39–50.Oxford University Press, Oxford.

Takami M, Nagata N, Sasabe M, Sota T (2007). Asymmetric in reproductive isolation and its effects on directional mitochondrial introgression in the parapatric ground beetles *Carabus yamato* and *C. albrechti*. Population Ecology, 49, 337–346.

Tillyard RJ (1917). The Biology of Dragonflies. Cambridge University Press, Cambridge.

Tsuchiya K, Hayashi F (2008). Surgical examination of male genital function of calopterygid damselflies (Odonata). Behavioral Ecology and Sociobiology, 62, 1417–1425.

Tynkkynen K, Grapputo A, Kotiaho JS, Rantala MJ, Vaananen S, Suhonen J (2008). Hybridization in *Calopteryx* damselflies: the role of males. Animal Behaviour, 75, 1431–1439.

Uhía E, Cordero Rivera A (2005). Male damselflies detect female mating status: importance for postcopulatory sexual selection. Animal Behaviour, 69, 797–804.

Utzeri C, Belfiore C (1990). Tandem anomali fra Odonati. Fragmenta Entomologica, 22, 271–287.

Von Ellenrieder N (2008). *Phoenicagrion* gen. nov. for *Leptagrion flammeum*, with description of a new species, *P. paulsoni*, from Peru (Odonata: Coenagrionidae). International Journal of Odonatology, 11, 81–93.

Von Ellenrieder N, Garrison RW (2008a). A redefinition of *Telagrion* Selys and *Aceratobasis* Kennedy stat. rev. and the description of *Schistolobos* gen. nov. for *Telagrion boliviense* Daigle (Odonata: Coenagrionidae). Transactions of the American Entomological Society, 134, 1–22.

Von Ellenrieder N, Garrison RW (2008b). *Drepanoneura* gen. nov. for *Epipleoneura letitia* and *Protoneura peruviensis*, with descriptions of eight new Protoneuridae from South America (Odonata: Protoneuridae). Zootaxa, 1842, 1–34.

Von Ellenrieder N, Garrison RW (2008c). Oreiallagma gen. nov. with a redefinition of *Cyanallagma* Kennedy 1920 and *Mesamphiagrion* Kennedy 1920, and the description of *M. dunklei* sp. nov. and *M. ecuatoriale* sp. nov. from Ecuador (Odonata: Coenagrionidae). Zootaxa, 1805, 1–51.

Von Ellenrieder N, Lozano F (2008). Blues for the red *Oxyagrion*: a redefinition of the genera *Acanthagrion* and *Oxyagrion* (Odonata: Coenagrionidae). International Journal of Odonatology, 11, 95–113.

Waage JK (1979). Dual function of the damselfly penis: sperm removal and transfer. Science, 203, 916–918.

Waage JK (1982). Sperm displacement by male *Lestes vigilax* Hagen (Zygoptera: Lestidae). Odonatologica, 11, 201–209.

Waage JK (1984). Sperm competition and the evolution of odonate mating systems. In RL Smith, ed. Sperm Competition and the Evolution of Animal Mating Systems, pp 251–209. Ademic Press, Orlando.

Waage JK (1986). Evidence for widespread sperm displacement ability among Zygoptera (Odonata) and the means for predicting its presence. Biological Journal of the Linnean Society, 28, 285–300.

Waage JK (1987). Choice and utilization of oviposition sites by female *Calopteryx maculata* (Odonata: Calopterygidae). I. Influence of site size and the presence of other females. Behavioral Ecology and Sociobiology, 20, 439–446.

Ward PI, Simmons LW (1991). Copula duration and testes size in the yellow dung fly, *Scathophaga stercoraria* (L)—The effects of diet, body size, and mating history. Behavioral Ecology and Sociobiology, 29, 77–85.

Watson JAL (1966). Genital structure as an isolating mechanism in Odonata. Proceedings of the Royal Entomological Society of London Series A—General Entomology, 41, 171–174.

Yokoi N (1990). The sperm removal behavior of the Yellow Spotted Longicorn Beetle *Psacothea hilaris* (Coleoptera: Cerambycidae). Applied Entomology and Zoology, 25, 383–388.