# STRUCTURE AND FUNCTION OF MALE GENITALIA IN LIBELLULA, LADONA AND PLATHEMIS (ANISOPTERA: LIBELLULIDAE)

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Male genitalia of 25 spp. are studied using scanning electron microscopy, and the structure of the fourth penile segment is described. Remarkable diversity exists among spp., particularly in the size and shape of the lateral lobes, and the morphology of the cornua. There are also differences in the number of cornua among the taxa surveyed. The surface of the lobes of many spp. is covered with spines which anchor the penis during copulation, and may trap and remove sperm when the penis is collapsed and withdrawn following copulation. Spp. are categorized according to the morphology of the penis and inferred patterns of sperm removal. Type 1 taxa possess relatively large, broad, flat lateral lobes, and lack cornua, or possess cornua that are greatly reduced in size. These spp. are believed to displace sperm in the bursa copulatrix before depositing their own sperm, thereby gaining positional priority during oviposition. Type 2 spp. possess elongated lateral lobes and/or cornua. These taxa are believed to engage in a mixed strategy of sperm displacement and sperm removal. Optimization of these characters on a phylogeny of the 3 genera indicates that the Type 1 sperm displacement strategy is ancestral, and that the Type 2 strategy was subsequently derived within the majority of the Libellula s.s. taxa.

# INTRODUCTION

Among sexually reproducing organisms, there exists remarkable diversity of intromittent structures, even among closely related taxa (EBERHARD, 1985). The lock and key hypothesis, which holds that male and female genitalia evolve to ensure a mechanical fit reducing the risk of hybridization, has long been used to

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explain genitalic diversity (e.g. PAULSON, 1974). However, EBERHARD (1985) argues that there is little evidence for lock and key reproductive isolation, and instead suggests that male genitalia are under intense sexual selection by females. The cryptic female choice hypothesis maintains that females may exercise copulatory and post-copulatory mate choice decisions based on the morphology of the male genitalia. This hypothesis is intriguing, and there have been a few recent attempts to test its validity (ARNQVIST, 1997; TADLER, 1999). Another alternative is that genitalia may be evolving in response to sperm competition to manipulate or remove sperm from previous copulations more effectively.

Odonate genitalia are thought to have evolved rapidly and divergently in response to intense sperm competition. The structure and function of odonate genitalia and intromittent organs have been widely studied in males (PFAU, 1971; RESTIFO, 1972; MILLER, 1984, 1991, 1995; SIVA-JOTHY, 1984; WAAGE, 1979, 1984, 1986a) and females (MILLER, 1984, 1988; SIVA-JOTHY, 1987). The male penis in the Libellulidae is a four-segmented structure with the fourth distal segment of the penis often bearing hooks, bristles, spines and inflatable structures (MILLER, 1982). The female genital tract consists of a large sac-like bursa copulatrix and paired spermathecae (MILLER, 1982). The bursa is ventrally connected to the vagina and oviducts by a valved opening. WAAGE (1979) first suggested that male genitalia had two distinct functions in odonates; sperm transfer, and removal or displacement of stored sperm from previous copulations. Research on a number of odonate taxa has subsequently confirmed that male genitalia are the principal organs of sperm competition, and that sperm precedence is greatest for males that are last to mate with females prior to oviposition (FINCKE, 1984; McVEY & SMITTLE, 1984; WOLF et al., 1989; MICHIELS, 1992; HADRYS et al., 1993).

The dragonfly family Libellulidae has been the subject of several studies of male genitalia. KENNEDY (1922a, 1922b) surveyed the morphology of male genitalia in the genus *Libellula*, and inferred relationships based on similarities in morphology. This genus was examined again by RESTIFO (1972), who described the genitalia in greater detail. MILLER (1991) examined the genitalia and reproductive behavior of several species of libellulid dragonflies, and categorized them according to copulatory activity, genital morphology and the suggested mechanisms used in sperm competition. In another study, MILLER (1995) found that all eleven species of dragonflies studied either removed, displaced or diluted sperm from rival males, but that the intromittent structures used to accomplish this varied between species and genera.

The objectives of this study are (1) to examine and describe the functional structures of the male genitalia in the genera *Libellula, Ladona* and *Plathemis* using scanning electron microscopy (SEM), (2) to categorize the major types of genitalia and to discuss their potential functional significance, and (3) to examine patterns of sperm displacement inferred from genitalic morphology in a phylogenetic framework. This study is intended to supplement the previous works of KENNEDY

Species	Apical	lobe	Lateral lobes			Medial process					
	\$1	2	3	4	5	6	7	8	9	10	11
P. longipennis	x		x						x		2
E. simplicicollis		x	х							x	2
O. ferruginea		x	х							x	2
L. auripennis		x		х					x		1
L. axilena		x		x					x		1
L. comanche	х			x					х		1
L. composita		x		x				х			1
L. croceipennis		x				х				x	2
L. cyanea	x			x					x		1
Ladona deplanata		x	x				x				0
Ladona depressa	x		х				x				0
Ladona exusta		x	x				x				0
L. flavida		x			x				x		1
L. forensis		x				x		x			1
Ladona fulva		x	x				x				0
L. herculea		x				x				x	2
L. incesta		x		х						x	1
L. jesseana		x		x					x		1
Ladona julia		x	x				x				0
L. luctuosa	x				X ·				x		1
Plathemis lydia		x	х				x				0
L. needhami	x			x					x		1
L. nodisticta	x					x		x			1
L. pulchella		x				x		x			1
L. quadrimaculata	x					x		x			3
L. saturata	х					x				x	3
L. semifasciata		x				x		x			2
Plathemis subornata		x	x				x				0
L. vibrans	x			x					x		1

 Table I

 Summary of the genital structures on the fourth segment of the male penis in some libellulid species

<sup>6</sup> Descriptions of the lobes: (1) large and spinose; - (2) small, reduced or absent; - (3) large, round and flat; - (4) long and blade-like; - (5) long and spoon-like; - (6) reduced, rod-like; - (7) cornua absent; - (8) short and stout; - (9) long and straight; - (10) long and curved; - (11) number of cornua.

(1922a, 1922b), RESTIFO (1972), MILLER (1991) and MAY (1992). Specifically, this study provides a comprehensive survey of the morphology of male genitalia in three groups of closely related dragonflies. Scanning electron microscopy is used to provide greater resolution and higher magnification images than most previous studies. Finally, this study is the first to examine the evolution of sperm displacement strategies within a phylogenetic framework.

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# METHODS AND MATERIAL

Penes were dissected from ethanol or dried preserved specimens of male libellulid dragonflies. Nineteen species from the genus *Libellula s.s.* were examined together with members of the allied genera *Ladona* (incl. *fulva* and *depressa*; ARTISS et al., 2000) and *Plathemis*. Although the taxonomic status of these genera is the subject of some controversy, they shall be referred to by separate generic rank in this study. Three other libellulids were also examined; *Pachydiplax longipennis, Orthemis ferruginea,* and *Erythemis simplicicollis.* Data from these specimens were used to describe components of the fourth segment (Tab. I). However, in some cases data were



Fig. 1. Scanning electron micrographs of partially inflated penes of libellulid dragonflies: (A) Libellula croceipennis; - (B) L. herculea; - (C) L. saturata; - (D) L. forensis; - (E) L. pulchella; - (F) L. composita. - [Abbreviations: A, apical lobe; - C, cornua; - H, hood; - LL, lateral lobe; - M, medial lobe; - MP, medial process; - P, posterior lobe]

obtained from previous studies (KENNEDY, 1922a, 1922b; RESTIFO, 1972; WAAGE, 1984; MAY, 1992; MILLER, 1991).

During copulation, the fourth distal segment of the penis is swung out ca 180° ventrally, and genitalic lobes on this segment are exposed and inflated. To simulate genitalic inflation, penes were manipulated with dissecting pins to expose the ventral surface of the fourth distal segment. In some cases, penes were soaked in double distilled H<sub>2</sub>O and glycerol to facilitate manipulations. Specimens were air dried, or dried in hexamethyldisilizane (HMDS), mounted on a stub with carbon adhesive, sputter coated with gold palladium in a Polaron E5100 sputter coater for 1 minute, and examined in a Zeiss DSM982 field emission scanning electron microscope. All images were saved to disk as TIFF images, and printed on an Epson inkjet printer (600 dpi).

To examine the of evolution of patterns of sperm displacement, a phylogeny of the groups of interest is needed. A phylogeny of the odonate genera *Libellula, Ladona,* and *Plathemis* based on DNA sequence data from the mitochondrial genes, cytochrome oxidase I and 16S rRNA was generated (ARTISS, 1999; ARTISS et al., 2000). This phylogeny is largely consistent with the phylogeny of these groups produced by KAMBHAMPATI & CHARLTON (1999), but the former phylogeny includes more taxa. A maximum likelihood analysis of the combined data set using the general time reversible substitution model with rate heterogeneity accommodated using invariant sites plus gamma-distributed rates (GTR + I + G) produced a single tree (log likelihood of -7198.05338). This tree was used in subsequent analyses. For specific details on analyses used in phylogenetic reconstruction, see ARTISS et al. (2000).

To examine patterns of evolution in sperm manipulation strategies, species were categorized according to their morphology, and inferred patterns of sperm displacement (see SIVA-JOTHY, 1984). Inferred patterns were optimized on the phylogenetic tree using MacClade (MADDISON & MADDISON, 1992). Changes in patterns were unordered (changes from one state to another are equally probable, and equally weighted; viz. Fitch parsimony, FITCH, 1971; HARTIGAN, 1973).

# RESULTS

General descriptions of the genitalia of *Libellula* can be found in KENNEDY (1922a, 1922b) and RESTIFO (1972). Only the main features and structures not obvious from previous studies will be presented here. Descriptions will be limited to lobes on the fourth segment as they are interspecifically variable, and have formed the basis of previous taxonomic studies (KENNEDY, 1922a, 1922b; RESTIFO, 1972). General features of the male genitalia are seen in Figures 1-5.

The fourth segment is comprised of a sclerotized hood and a distal component consisting of several inflatable lobes. In some species, the hood is comparatively small (*L. semifasciata, saturata*), while in others it is prominent, and possesses numerous short spines (*L. luctuosa, incesta*). The distal component of the penis comprises an apical lobe found on the ventral surface, a medial process lying dorsal to the apical lobe, and paired lateral lobes. Each component of the fourth segment is described in greater detail below (see Tab. I). Descriptions of presumed functions of lobes of inflated penes follow MILLER (1991).

The apical lobe is located on the ventral surface of the fourth segment. It often possesses numerous spines (Fig. 5A). When inflated, the spines are erected at right angles or proximally. In most species, this lobe is small (*L. pulchella*, *P. lydia*); however in others it is large, as in *L. quadrimaculata*, where it is the largest of the

lobes (Fig. 4F).

The lateral lobes are well sclerotized, and during inflation they generally swing outwards and rotate ventrally. The lateral lobes are highly variable among the three genera. In some species they are greatly reduced (*L. quadrimaculata*, *croceipennis*). In others, the lateral lobes are large, oval, concave disks (*Plathemis* spp.; Figs 4A, B). In contrast, the lateral lobes of some species are quite long, and the tips are strongly curved resembling cornua (*L. composita*, *cyanea*) or flared and curved resembling scoops (*L. luctuosa*). The lateral lobes of several species possess a narrow groove running medially along the length of the interior edge (*L. forensis*; Fig. 5B).



Fig. 2. Scanning electron micrographs of partially inflated penes of libellulid dragonflies: (A) Libellula axilena; - (B) L. auripennis; - (C) L. needhami; - (D) L. luctuosa; - (E) L. incesta; - (F) L. vibrans. - [Abbreviations as in Fig. 1]

The medial process bears several structures that pivot dorsally with inflation. Most conspicuously, the medial process bears the inner (or posterior) lobes, and one or more cornua. In some libellulid genera, the medial process may possess flagella (MILLER, 1991), but flagella were absent in all species in this study. The surface of the medial process may be covered with numerous short spines (*L. cyanea*; Fig. 5D).



Fig. 3. Scanning electron micrographs of partially inflated penes of libellulid dragonflies: (A) Libellula jesseana; - (B) L. comanche; - (C) L. cyanea; - (D) L. flavida; - (E) Orthemis ferruginea; - (F) Erythemis simplicicallis. - [Abbreviations as in Fig. 1]

The cornua are typically long, curved, rod-like structures, although in some species they are greatly reduced (*L. quadrimaculata*). The cornua are generally smooth, slender extensions of the distal portion of the medial process, although in some cases, the cornua may possess short, proximally oriented or interlocking spines (*L. herculea, O. ferruginea*; Fig. 5E). The number of cornua varies between species. In some species, the cornua are absent (*Plathemis* spp., *Ladona* spp.; Figs. 4A-E), while others possess up to three cornua (*L. saturata*). The majority of species possess a single, relatively thick cornu which may be slightly forked (*L. auripennis*) or cupped (*L. vibrans*; Fig. 5F) at the tip. *Libellula croceipennis* and *L. herculea* possess short spines at the base of the cornua (Figs 1A, B).

In the majority of species examined, the posterior or inner lobes were not visible as they were obscured by the lateral lobes or medial process. The posterior lobes are typically highly inflatable, and are visible once the penis is inflated. In the few cases where the lobes were easily visible, they often bristled with a number stout spines on their ventral surface (*L. auripennis*, Fig. 5D).

# DISCUSSION

## FUNCTIONAL SIGNIFICANCE

The size, shape and features of the lobes on the fourth segment of the penis are highly variable among the species studied. The rapid and divergent evolution of genitalia in other odonate groups has been attributed to sperm competition (WAAGE, 1984; MILLER, 1991, 1995) and I am assuming this to explain the diversification of genitalic forms among species in the current study. However, EBERHARD's (1985) female choice hypothesis may also explain the diversity of genitalia among odonates (see SIVA-JOTHY & HADRYS, 1998). He maintains that male intromittent organs are the target of female copulatory and post-copulatory mate choice, and that female sexual selection has produced the variety of genitalia seen in many organisms. This hypothesis is unlikely to explain genitalic variation among taxa in this study as the structures considered herein are believed to function as sperm removing mechanisms (reviewed in CORBET, 1999). Moreover, there are several features that females may use as the basis of mate choice that would preempt post-copulatory mate choice such as territories and differences among males in body size and color (WOLF & WALTZ, 1988; MOORE, 1990; reviewed in CORBET, 1999).

Functional explanations of parts of the fourth segment of the male penis are offered below.

APICAL LOBE. – The apical lobe is large and blunt in the majority of species studied. Upon inflation, the apical lobe swings ventrally, helping to position the penis dorsally towards the bursa communis (MILLER, 1991). The surface of the apical lobe is generally covered with spines which presumably help anchor the

penis during copulation (Fig. 5A). MILLER (1991) suggests that these spines may also remove rival sperm displaced into the vagina.

LATERAL LOBE. - At rest, the lateral lobes are positioned on either side of the medial process (MILLER, 1991). Upon inflation, these lobes enter the genital canal forcing the ventral valve on the bursa communis open. When the penis is



Fig. 4. Scanning electron micrographs of partially inflated penes of libellulid dragonflies: (A) *Plathemis lydia*; - (B) *P. subornata*; - (C) *Ladona julia*; - (D) *L. depressa*; - (E) *L. fulva*; - (F) *Libellula quadrimaculata*. - [Abbreviations as in Fig. 1]

deflated and removed, the inner surfaces of the lateral lobes may remove sperm (WAAGE, 1986b; MILLER, 1991). In several of the species studied, the lateral lobes were reduced, and their function in protecting the penis or in assisting with sperm removal may be limited (e.g. *L. quadrimaculata*). However, the majority of the species studied had elongated lateral lobes. Given the absence or reduction in size of the cornua in many taxa, it is likely that the elongated lateral lobes have evolved to facilitate sperm removal. Moreover, the lateral lobes of some species are enlarged at the proximal end (e.g. *L. luctuosa*, Fig. 2D), possess a medial groove that may serve as a canal along which pre-existing sperm may travel (e.g. *L. forensis*; Fig. 5B), and/or have pitted surfaces (e.g. *L. composita*, Fig. 5C). All of these features would facilitate sperm removal if they accessed the bursa copulatrix or spermatheca.

MEDIAL PROCESS. – The medial process is the portion of the penis that has the most direct access to the bursa copulatrix and spermatheca making it the likely agent of sperm removal. Unfortunately, the ventral surface of the medial process is not easily visible unless inflation is achieved rather than simulated, so descriptions of this structure are generally lacking in this study. However, in some cases where the surface was visible penes in which inflation was simulated, the ventral surface contained numerous short spines (e.g. *L. auripennis*, Figs 2B, 5D). In addition to anchoring the penis during copulation, these spines may displace sperm from the bursa into the spermatheca, and catch and remove rival sperm when the penis is withdrawn (WAAGE, 1986b; MILLER, 1991; CORBET, 1999).

POSTERIOR OR INNER LOBES. – The structure of the inner lobes is similarly difficult to observe without genitalic inflation. However, in species where they were visible, they often appeared to be long, robust structures, bearing coarse spines on their ventral surface. These lobes may reposition sperm by moving it distally away from the region of fertilization, and the spines may trap and remove sperm upon withdrawal (SIVA-JOTHY, 1984; MILLER, 1991).

CORNUA. – Cornua are generally long, smooth, slender structures. In many odonates, flagella are used to remove sperm from the spermatheca (MILLER, 1982, 1990, 1991, 1995; SIVA-JOTHY, 1984). However, none of the species in this study possessed flagella, and it is likely that the cornua perform this function. The gross morphology of cornua was similar in many species. However some species possessed numerous spines (Fig. 5E), or had a tip that formed a fork or a cup (Fig. 5F). All of these structures could facilitate sperm removal. MILLER (1991) suggested that the stiff, curved cornua may also anchor the penis within the female genital tract during copulation.

# PATTERNS OF SPERM REMOVAL

Anisopteran penes have been categorized according to their morphology, and inferred patterns of sperm displacement (SIVA-JOTHY, 1984). Two basic sperm

displacing strategies exist based on the morphology of the genitalia; penes that are used to reposition sperm in the female bursa and spermatheca (cf. Type 1, SIVA-JOTHY, 1984), and those that possess apparatus for physically removing sperm (cf. Type 3, SIVA-JOTHY, 1984). A third category comprises species that are capable, to some extent, of both displacing and removing sperm (cf. Type 2, SIVA-JOTHY, 1984). Based on these descriptions, all of the species I studied could be classified as sperm displacers (Type 1), or capable of sperm removal and



Fig. 5. Scanning electron micrographs of parts of partially inflated penes of libellulid dragonflies: (A) apical lobe of *Libellula comanche* showing spines on surface; – (B) tip of lateral lobes of *L forensis* showing short spines and medial groove; – (C) lateral lobes of *L composita* showing curved tips and pitted surface; – (D) posterior lobe of *L auripennis* showing bristly dorsal surface. Spiny surface of medial process can be seen below posterior lobe; – (E) cornua of *L herculea* showing short spines; – (F) tip of cornua of *L vibrans*; note cup-shaped indentation on proximal end.

displacement (Type 2). Conclusions relating to functional morphology of sperm displacement are limited by the inability to observe fully inflated penes, and the lack of detailed information on the morphology of the female genitalia. In describing the mechanisms of sperm competition, SIVA-JOTHY (1984) described the ability to displace sperm in relation to the number of inflatable lobes and the degree to which they were inflated. Corresponding female genital morphology is also germane. For example, females of species in which males remove sperm typically possess long, paired spermatheca which are penetrated by flagella and/or cornua (SIVA-JOTHY, 1984; MILLER, 1991). Therefore, the results of this study are tentative, and require further examination of inflated penes and female genital morphology.

TYPE 1. – Species from the genera *Ladona* and *Plathemis* possess apical, medial processes and lateral lobes, and the dorso-medial lobes are likely capable of large changes in volume during inflation. Upon inflation, the lobes may enter the bursa, repositioning rival sperm before new sperm is added. This strategy would only be effective if sperm precedence worked on a "last in, first out" basis, and there is evidence to suggest that sperm precedence is given to males that are the last to mate with females prior to oviposition (McVEY & SMITTLE, 1984; HADRYS et al., 1993). Males in this category lack long modified lateral lobes and cornua capable of entering the bursa or spermatheca, and appear incapable of any direct physical sperm removal



Fig. 6. Phylogenetic distribution of character changes for sperm manipulation strategy on a hypothesis of phylogeny for *Libellula, Ladona* and *Plathemis*. Unshaded lines = lineages of sperm displacers (Type 1), shaded lines = lineages of sperm displacers and removers (Type 2). The phylogeny is based on the most likely tree derived from molecular cytochrome oxidase I and 16S rRNA data (length = 1193, C.I. = 0.392, and R.I. = 0.560; ARTISS et al., 2000).

apart from incidental removal of sperm displaced into the vagina.

Of the remaining species, several lack elongated lateral lobes, and although they possess cornua, these structures are typically short and stout and are likely incapable of direct sperm removal. Specifically, *L. quadrimaculata, semifasciata, forensis, nodisticta* and *pulchella* do not possess structures capable of physical sperm removal, and they are characterized as Type 1 sperm displacers.

TYPE 2. – In addition to the lobes possessed by Type 1 males, Type 2 males are distinguished by the presence of one or more elongated cornua and lateral lobes. Given the length of the lateral lobes and cornua, it is likely that these structures are capable of accessing the bursa, and possibly the spermatheca, and the spines on the cornua of some species likely facilitate sperm removal. Moreover, the apical lobe, medial process and inner lobes are often spinose. In addition to anchoring the penis during copulation, these spines are believed to trap and remove rival sperm when the penis deflates (SIVA-JOTHY, 1984; MILLER, 1991). Therefore, males in this category probably engage in a mixed strategy of sperm displacement and sperm removal.

Recent phylogenetic work indicates that the genera *Ladona* and *Plathemis* are sister groups to *Libellula* s.s. (KAMBHAMPATI & CHARLTON, 1999; ARTISS et al., 2000). Since these genera are sperm displacers, the evolution of the Type 2 strategy appears to be derived within *Libellula* s.s.. Optimization of these characters on a phylogeny derived from independent molecular data indicates that the Type 2 strategy is the derived condition (Fig. 6). Species that possess structures apparently modified for both sperm removal and displacement include the majority of *Libellula* s.s., while basal taxa (*L. quadrimaculata* and *semifasciata*) and the closely related genera *Ladona* and *Plathemis* appear to be limited to sperm displacement (Type 1). Moreover, the presence of short, stout cornua in *L. quadrimaculata* and *semifasciata* may indicate the transition from sperm displacement to sperm removal. An apparent reversion to the ancestral Type 1 strategy occurs in the clade comprised of *L. forensis, nodisticta* and *pulchella* (Fig. 6). These species have large medial processes, but possess reduced lateral lobes and cornua needed for effective sperm removal.

Species in this study have been generally categorized into those that displace sperm, and species that engage in a mixed strategy. Extensive variation does, however, exist between species within strategies, and individual lobes have been differentially modified. This suggests functional convergence, and would indicate that the genitalia may be inappropriate structures on which to base taxonomic relationships (MILLER, 1991). Future studies on these groups should be further directed at evaluating the evolution of the genitalia in a phylogenetic framework.

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