

## Slow-motion analysis of female refusal behaviour in dragonflies

Georg Rüppell<sup>a\*</sup> and Dagmar Hilfert-Rüppell<sup>b</sup>

<sup>a</sup>An der Wasserfurche 32, D-38162 Cremlingen, Germany; <sup>b</sup>Institut für Fachdidaktik der Naturwissenschaften, Technische Universität, Bienroder Weg 82, D-38106 Braunschweig, Germany

(Received 22 March 2014; accepted 24 September 2014)

By means of slow-motion film analysis we found new female refusal behaviour patterns against male harassment in a variety of Odonata species. Often, females could escape simply by flying faster than males. Due to the morphological preconditions, there were differences in the two suborders. In Anisoptera, several behavioural specialities were analysed: (a) females of *Aeshna cyanea*, which oviposit solitarily and endophytically, clung to the substrate with great force when being pulled away by attacking males. (b) *Anax imperator* females showed a very fast, characteristic bending of the abdomen causing sudden U-turns for escape. (c) Solitary *Libellula quadrimaculata* females flew loops to escape pursuing males or to shake them off. They either used the impact of the crashing male for the turning moment or they generated it themselves by an abrupt change of the wing beat direction. In Zygoptera we investigated different *Calopteryx* species, which all oviposit alone. Fleeing was most common but wing clapping, not cooperating to build a tandem, tandem separation, fast diving for submerged oviposition and threatening and attacking the male were also documented. Fast water current prevented submerged oviposition by *Calopteryx xanthostoma* and increased refusal behaviour by females.

**Keywords:** Odonata; sexual conflict; coercion; communication; male harassment; dragonfly flight; flight loops; flight turns; submerged oviposition; wing clapping

### Introduction

Sexual conflict is a common consequence of sexual reproduction (Arnqvist & Rowe, 2005; Clutton-Brock & Parker, 1995) and is particularly distinct in Odonata (Fincke, 1997). For males of many species there can be intense competition for females, especially when densities of conspecific males are high at mating sites, and thus strategies are used to coerce females (e.g. Cordero, 1999). Coloured-winged species at normal densities solve this conflict mainly through a signalling system by which sexual selection can work to optimize reproduction. In coloured-winged species, displaying males are holding valuable territories for reproduction and are chosen by females, while in clear-winged species, males at the reproduction sites mostly fight at encounters and try to seize females (Hilfert-Rüppell & Rüppell, 2013; Waage, 1984a). Females, in contrast to males, are choosy and try to restrict fertilization of their eggs to 'good fathers' (Alcock, 1993; Andersson, 1994; Koenig, 1991; Waage, 1984a). Consequently, there is a large gender inequality in the optimal mating frequency of the sexes. As the operational sex ratio at the reproduction site is mostly male-biased, mated females may try to avoid other males.

This seems to be useful for females because it reduces the additional costs of extra pairing, such as energy expenditure, the risk of being caught by predators, being drowned, or of receiving

\*Corresponding author. Email: [rueppell-film@t-online.de](mailto:rueppell-film@t-online.de)

lower-quality sperm (Arnqvist & Row, 2005; Fincke, 1997). Odonata females avoid harassing males by using different behavioural adaptations:

- (1) *Resistance behaviour* such as wing spreading, abdomen-raising or abdomen-curling downwards. Wing clapping by females can signal unreadiness to mate to approaching males (Bick & Bick, 1978; Waage, 1984b) or it can be used to reject a male physically (Günther, Hilfert-Rüppell, & Rüppell, 2014). *Leucorrhinia rubicunda* females use their upwardly stretched forelegs to repel attacking males (Rüppell, 1989b). Females of some species have been seen struggling or pushing when a male tried to seize them (see Corbet, 2004, p. 471). Furthermore, females may not cooperate in forming the copulation wheel by keeping their abdomen stiff (Fincke, 1997) or may separate from the tandem position (Xu & Fincke 2011). In extreme cases the female may kill the attacking male; this was reported in *Ischnura elegans* (Cooper, Holland, & Miller, 1996) and *Libellula quadrimaculata* (Rüppell & Hilfert-Rüppell, 2013).
- (2) *Fleeing*. Many females are able to fly faster and have been seen to flee and to hide, remaining perched and motionless (Kaiser, 1974, 1985; Miller, 1991; Rehfeldt, 1989, 1991; Rowe, 1988; Rüppell & Hilfert-Rüppell, 2009; Ubukata, 1973). *Anax imperator* females in flight curve their abdomen downward (Moore, 1960), thus decreasing the area of the abdomen available for attaching or landing of the male, and also facilitating some flight manoeuvres, as described below.
- (3) *Spatial avoidance*. The most common female adaptation to avoid unwanted male attacks is to visit the water only when they have a batch of eggs to lay. Females of some Zygoptera submerge for oviposition (Fincke, 1986; Waage, 1984b), and some Anisoptera oviposit in dense bank vegetation (Ubukata, 1984; Wildermuth, 2008).
- (4) *Temporal avoidance*. Many females oviposit when males are absent, in low temperatures (Hilfert, 1997) or when males are occupied by intrasexual contests (Corbet, 2004, p. 470).

The active role that females play in mating decisions has, after Fincke (1997), often been ignored, perhaps because males are easier to observe given that they mostly appear in great numbers at water bodies for reproduction (Debano, 1996; Conrad & Hermann, 1990).

Our aim is to show, especially in Anisoptera, which are more difficult to observe visually than Zygoptera, that females employ a number of mechanisms to avoid unwanted matings. The aim of this descriptive slow-motion study is to document new behavioural patterns with which females avoid or repel approaching males. Filming when reproductive activity in different species was high, it was possible to analyse some very fast running behaviour. All females in the footage are those that were either at the water because they had eggs to lay, or were females that had already laid eggs and wanted to escape back to shore. By describing new patterns of refusal behaviour we provide more evidence for anti-harassment traits of odonate females. Our observations strengthen the argument that females may manage their reproduction behaviour to a considerable extent and are not passive objects of males' unselective pairing attempts (Fincke, 1997).

## Material and methods

All filming was done when intense dragonfly behaviour took place at a site with many reproductively active males and female, mostly in a reproductive context. The specific purpose was to find new behaviour that occurs over a relatively large area within a very short time frame. The large amount of footage was viewed and organized topically. All behaviour concerning female refusal was collected and analysed in as much detail as possible. Good filming opportunities depended on available research time, size of the site, the density of dragonflies and weather conditions. The results presented here are anecdotal.

Encounters between male and female odonates usually happen so fast that it is impossible to follow them with the human eye. In order to analyse behavioural details we used slow-motion filming. However, it is difficult to obtain records of the unpredictable and fast movements of flying dragonflies. This difficulty may be overcome by choosing a narrow site and by studying the actions of the insects intensively in advance. The slow-motion filming slowed the action by 12–24 times, revealing data in a temporal range similar to odonate optical perception (Stange, Chal, & Mizutani, 2001). Since the availability of digital cameras that can record slow-motion pictures for many minutes, there has been a realistic chance of obtaining rare behaviour in a resolution sufficiently detailed for analysis. For this, photographic cameras capable of slow-motion filming (Casio EX F1, Casio Computer, Tokyo, Japan) were used. The filming frequencies were 300 and 600 frames per second (fps). Figure 6 was taken by a Canon 650 D camera (Canon, Tokyo, Japan) and a Tamron 180 mm macro lens (Tamron, Hirosaki, Japan). From 2010 to 2013, adults of *Aeshna cyanea* and *Libellula quadrimaculata* were filmed at a garden pond (9 × 4 m), 15 km east of Braunschweig (52°26' N, 10°23' E), as well as at a forest pond (15 × 10 m), 10 km north-east of Braunschweig, where *Anax imperator* was also filmed. *Calopteryx splendens* was filmed at the river Oker in 2004–2013 near Braunschweig, while *C. xanthostoma* was investigated in south-west France at the river Célé (44°55' N, 1°76' E) in 2013. Additional observations on *C. haemorrhoidalis* were made at the Canal de Vergière near Arles, Southern France (43°40' N, 4°37' E). *Leucorrhinia rubicunda* was filmed at a bog 50 km north-west of Braunschweig in 1988.

The single frames of the slow-motion films were analysed by using the computer program Quick Time version 7.7.1 (Apple, Cupertino, CA, USA), which offers frame-by-frame viewing and counting (by clicking to the time marks at bottom left). To obtain the duration of behavioural sequences, the frames were counted from the first measure for an event or manoeuvre to the end of it. The number of frames was taken as the equivalent duration by dividing the number by the filming rate. When filmed at 300 fps, each frame was exposed for 1/300 s, so 10 frames lasted 1/30 s and 300 frames 1 s.

Individual flight velocities were measured by marking the shift of the individual's image on a movable foil placed on the screen of the computer (Asus Essentio CM 6730-DEMM 19 SILE, ASUSTeK Computer, Taipei, Taiwan). When the camera was moved, prominent structures of the background were drawn on the foil and were covered by these structures on the film when measuring the dragonfly's body displacement. These distances were some mm to 1 cm per time intervals of 0.03 s and were calculated to 1 s. All measured distances were corrected by a factor derived from comparisons of morphological lengths of bodies from the literature (Dijkstra & Lewington, 2006) with the lengths of bodies measured on the screen. Only those cases with the flight path perpendicular to the direction of filming were used for analysis.

Turn durations were compared by a *t*-test with PAST (Øyvind Hammer, Natural History Museum, University of Oslo). The numbers of individuals are indicated by *N*, whereas the numbers of wing beats by an individual are indicated by *n*.

All investigations were performed under sunny, calm weather conditions. To obtain data from undisturbed behaviour it was necessary to sit and wait for long periods. The camera was pre-focused to the expected flight range and moved parallel to the flying dragonflies, without looking for the dragonflies through the viewfinder and controlling only from moment to moment the orientation of the camera by the screen. The holding force of *Aeshna cyanea* females and the pulling force by flight of the males were measured using a spring balance (Kern, Balingen, Germany) calibrated in 0.1 g. Several measurements gave true results only by tilting the balance quickly, so that no friction could distort the measurement.

The drawings were made by copying single frames from the film and enlarging them. After drawing the shape and laying a transparency on the picture, details were included by moving

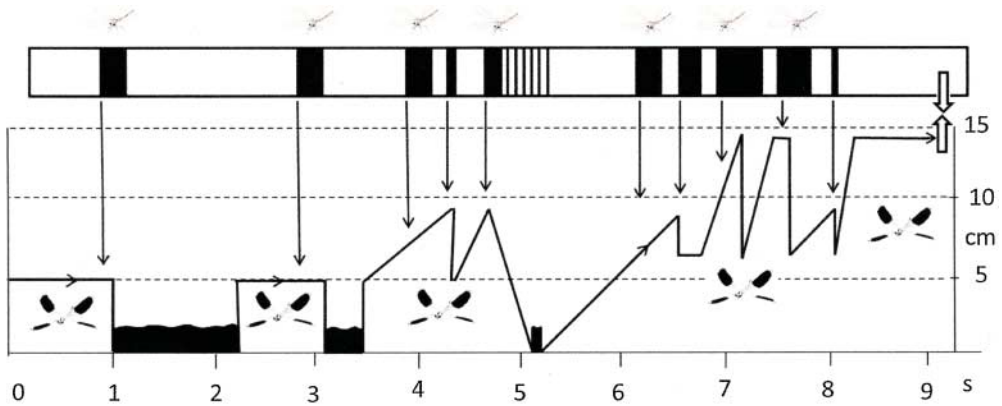


Figure 1. Temporary refusal behaviour by wing clapping of a female of *Calopteryx splendens* (figures and black bars at top, vertical lines in between = female flying, white parts of bar = female perching, landing afterwards on the same leaf to a courting male (figures below). The distances of the male from the water surface (black line with arrows in between) are depicted over 9 s (abscissa). The female was perching at about 15 cm on a leaf of arrowhead (*Sagittaria* sp.). Vertical downward arrows = beginning of wing clapping of the female. Three times the male settled on the water (black waves at bottom, at 1, 3 and 5 s) while the wings were held still. Vertical white arrows = pair formation. (Filmed at 300 fps.)

the film very slowly fore- and backwards. Unclear morphological details were finally added by studying photographs.

## Results

### 1. *Calopteryx* females rejected males by wing clapping

At low male densities with territorial behaviour, males of *C. splendens*, *C. haemorrhoidalis* and *C. xanthostoma* (Zygoptera) approaching in courting flight could be temporarily refused by wing clapping of solitary, ovipositing females .

In one case in *C. splendens* female wing clapping was exhibited at distances of 5–20 cm from the male (Figure 1). The male performed 328 wing beats at a mean frequency of 48.5 Hz before pair went into copula. When the female was near to him at the beginning and when he approached the end his beat frequency reached 60 Hz ( $n = 9$ ). Of the total recording time of 9 s the female showed wing clapping for 2.4 s (i.e. 27.2%). The female performed 10 episodes of wing-clapping (mean beat frequency: 18.9 Hz) with a total of 22 wing claps each lasting on average 0.2 s (with a maximum of 0.45 s) and one short flight lasting 0.46 s with nine wing beats. The male responded by eight retreating downwards flights, always courting. Only twice he did not respond and continued his approach. For the first retreats he flew down to the water where he floated, lasting the first time more than 1 s, the second time less than 0.5 s and the third time only about 0.1 s. His flight path, in spite of periodic retreating, showed a rising tendency (see supplemental material).

At densities higher than 4–6 males per 10 m bank stretch, females of *C. splendens* in northern Germany did not show wing clapping but fled immediately from males pursuing them.

### 2. *Anax imperator* female repelled male physically by wing clapping

An *Anax imperator* (Anisoptera) female ovipositing in a floating stem of reed was attacked by a *Brachytron pratense* male (Figure 2). He crashed into her spread wings and only 0.02 s later she began to clap her wings and shook him off.





Figure 2. Attack of a male *Brachytron pratense* on an ovipositing *Anax imperator*. The sequence starts at the top left and ends at bottom right. The female kicked him away by wing clapping and escaped (last picture shows her abdomen tip in air) while the *Brachytron* male falls on the water. The duration of the whole sequence was 0.8 s. (From 600 fps film.)

The female performed 15 wing beats lasting 0.4 s altogether. Wing clapping had a mean frequency of 43.4 Hz (maximum 50 Hz) and amplitudes of beating angles of 20–30°, finally increasing to 45°. The down- and upstrokes were nearly equally long (1.03:1,  $n = 15$ ). During beating the phase-relationship of the forewings and hind wings shifted. For the first four beats, the hind wings led the beating, the forewings following with a delay of 16–25% of the whole beating cycle; the next nine beats were done mostly in parallel with a slight advance of the hind wings of 8–10%, and the last two beats when the *Brachytron* male was shaken off, were done by nearly counterstroking beats.

The *Anax* female then took off, beating the first three strokes in parallel. The *Brachytron* male fell on his back on the water's surface, turned to face downwards and saved himself by swimming. During this, the forewings were moved through air and reached a frequency of 39 Hz, while at the same time the hind wings rowing in the water reached only 9 Hz. With each of nine rowing strokes the male covered about 3 cm (see supplemental material).

### 3. Anisoptera females held onto the substratum avoiding mating

In *Leucorrhinia rubicunda* in two recorded cases a single male could not detach the female from the water, although successful detachment was possible when two or three males pulled together (Table 1).

*Aeshna cyanea* males often spotted solitary ovipositing females and hovered near to them but only in two cases did they seize the female. In one of these, after having patrolled about 10 minutes at a distance of 20–30 cm from a female, a male *Aeshna* seized her and fastened his anal appendages to her head against her resistance by wing clapping. After several wing

Table 1. Cases of filmed refusal behaviour of females of Odonata species.

Female refusal behaviour	Species	Successful	Unsuccessful
Wing clapping	<i>Calopteryx</i> sp. <sup>a</sup> <i>Anax imperator</i>	Frequent <i>N</i> = 1 (against <i>Brachytron</i> <i>pratense</i> male)	Often (HD) <sup>b</sup>
Holding substrate	<i>Calopteryx</i> sp. <i>Aeshna cyanea</i> <i>Leucorrhinia rubicunda</i>	Sometimes (HD) <i>N</i> = 2 <i>N</i> = 3 (HD)	Often (HD) (+ males) <sup>d</sup> : <i>N</i> = 3 (HD)
Fleeing	<i>C. splendens</i> <i>C. xanthostoma</i>	Very frequent (HD) Frequent (HD)	<i>N</i> = 3 (HD)
Flying loops	<i>Orthetrum cancellatum</i> <i>Libellula quadrimaculata</i>	<i>N</i> = 2 <i>N</i> = 20 (HD)	<i>N</i> = 1 (HD)
Fast turns	<i>Anax imperator</i>	<i>N</i> = 12	
Tandem separations	<i>C. xanthostoma</i>	<i>N</i> = 2 (HD) (1 passive, 1 active) <sup>c</sup>	
Female attacking male	<i>C. xanthostoma</i>	<i>N</i> = 1 (HD)	
Female threatening male	<i>C. xanthostoma</i>	<i>N</i> = 1 (HD)	

<sup>a</sup>*Calopteryx* sp. means mainly *Calopteryx splendens* and *C. xanthostoma* and to a minor extent *C. virgo* and *C. haemorrhoidalis*.

<sup>b</sup>HD = high densities (several males simultaneously approached one female), all other cases normal densities.

<sup>c</sup>Passive tandem separation means not cooperating to form the tandem; active tandem separation means female was working to break the male's grasp.

<sup>d</sup>“+ males” means that other males seized the tandem and all pulled together.

beats – all four wings beating together in parallel – he paused while she continued egg-laying. After 10 minutes of pulling attempts he gave up and flew away. She continued egg-laying for another 20 minutes. He came back after 4–5 minutes and inspected her, but without any attempt to seize her again. Experiments on other individuals using a spring balance showed that the pulling force that males can produce by flying is 18–20 mN (*N* = 4) and the holding force of females is 200–300 mN (*N* = 8), depending on the substratum (Figure 3).

Another female which was chilled and wet, however, was seized and pulled off by a male and brought into pairing (“wheel”) position.

#### 4. Females of Zygoptera and Anisoptera fled to escape males

Females of *C. splendens* (Zygoptera), when attacked by males, usually fled successfully. In nearly all observed cases, females managed to separate from the male (*N* > 70). Only at very high male densities (> 10 males per 10 m bank stretch) females were finally seized (*N* = 3).

Flight velocity was very variable, ranging from 2 m s<sup>-1</sup> to a maximum of 4.65 m s<sup>-1</sup>. The beat frequency was lower in females (11.5 Hz, *N* = 19, *n* = 298, *p* < 0.01) than in males (18 Hz, *N* = 20, *n* = 439). In all observed cases the females accelerated with a burst of beats or changed their flight direction in the moment just before capture. In one case, a female, pursued by four males and having almost been caught, flew a U-turn in 1/10 s and, after another 1/10 s, she was already about 20 cm away from the males which only then were beginning their turn.

Females fled either inland or plunged at a high speed of about 2 m s<sup>-1</sup> onto the water to grasp floating leaves of water plants to submerge for oviposition (see supplemental material). Males pursuing the female inland decreased in number until only one or two were left. Often a remaining male pursued the female, changing his flight style with synchronous parallel beats of all four wings to the high-frequency courting flight, with counter-stroke beating of both wing pairs. Females that tried to submerge for oviposition were pursued by up to 10 males. In some



Figure 3. An ovipositing female of *Aeshna cyanea* clinging to the substratum by a force of 200–300 mN while a male is trying to pull her off by a force of 18–20 mN (see text). The forces are not the associated forces of the filmed event but those measured from single individuals (mN = milli Newton, measurements: male  $N = 4$ , female  $N = 8$ ). (Drawn from 300 fps film.)

cases ( $N = 6$ ) *C. splendens* and *C. xanthostoma* males nearly got the female but then seized other males nearby, while the female escaped (Figures 4, 10).

Fleeing by females was also observed and filmed in Anisoptera. In *Libellula quadrimaculata* females mostly fled when pursued by males at high densities (5–7 males at a pond of 30 m<sup>2</sup>). When females were flying forward, males could rarely seize them, because females usually accelerated when a male approached closely. When dipping onto the water for ovipositing, females could escape mostly by sudden turns and fast accelerations, but sometimes they were seized. Then pairing of the same couple could take place repeatedly.

##### 5. *Libellula quadrimaculata* females flew loops to escape males

At the same pond and density as described above, females of *L. quadrimaculata* (Anisoptera) escaped harassing males by flying loops. Fifteen out of 20 filmed loops qualified for analysis. Only in two cases the male copulated with the female, in all other cases the females escaped. In one case the female flew three loops, and in two cases two loops and in all other only one loop. Some loops were enhanced by the rotation moment of the impact of the crashing male.

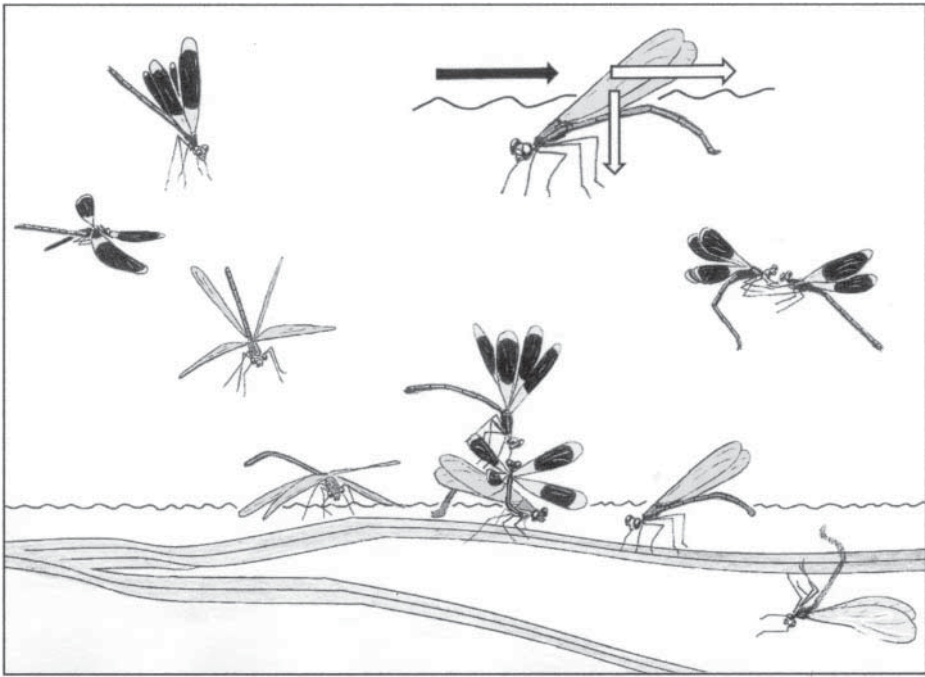


Figure 4. A female of *Calopteryx splendens* escapes two males by a power dive (beginning top left), then grasps a water plant, U-turns and lets the water current press her under water to oviposit. The diagram at the top right shows the same phase as below at the water with added arrows: black arrow = water current, white arrows = induced forces by the current at the inclined shape of thorax and wings. As the holding (legs) compensate the drag (horizontal white arrow), only the negative lift (vertical white arrow) is working and is pressing the damselfly under water. The two males grasped each other and fought. Parallel lines = water plant (floating leaves of reed sweetgrass *Glyceria maxima*). (Drawn from 300 fps film.)

When a male had already seized a female, the escape by flying the loop was facilitated by struggling with the legs, pushing with the abdomen and twisting the body (Figure 5; see supplemental material). Each loop was performed using six wing beats ( $N = 7$ ) at a mean beat frequency of  $44.4 \text{ s}^{-1}$  (females  $N = 5$ ) and  $54.1 \text{ s}^{-1}$  (males  $N = 5$ ). The duration of the 15 analysed loops was variable (mean  $150.7 \text{ ms}$ ,  $N = 15$ ), depending on whether the male had already grasped the female or not. If both rotated, the loop lasted longer ( $180 \text{ ms}$ ,  $N = 2$ ). When the female exhibited two loops one after the other, it did not last twice the time of one loop, but only  $190 \text{ ms}$  (for two loops,  $N = 1$ ). The rotation frequency was calculated to be 6.6 loops per second (mean from 15 loops).

The velocities after the loops were different in one flight: male ( $5.04 \text{ m s}^{-1}$ ), female ( $3.45 \text{ m s}^{-1}$ ), the latter having a head start of some decimetres. The wing beat frequencies in pursuing flights after the loops were also different: females:  $44.4 \text{ Hz}$ , males:  $54.1 \text{ Hz}$  ( $N = 5$ ).

### 6. *Anax imperator* female performed fast turns to escape males

*Anax imperator* males flew sharp turns with stretched abdomen (normal position) ( $N = 9$ ).

These conventional turns were done by banking and beating the right and left wings at different angles and amplitudes and led to turns of about  $90^\circ$  ( $N = 1$ ),  $120^\circ$  ( $N = 2$ ) and  $180^\circ$  ( $N = 6$ ). Extrapolating all turns to  $180^\circ$ , a mean duration of  $0.21 \text{ s}$  would be required to accomplish a full reversal. Females, when pursued by males, showed a special turning manoeuvre, which was observed several times and filmed six times. They curled their abdomen down and forward



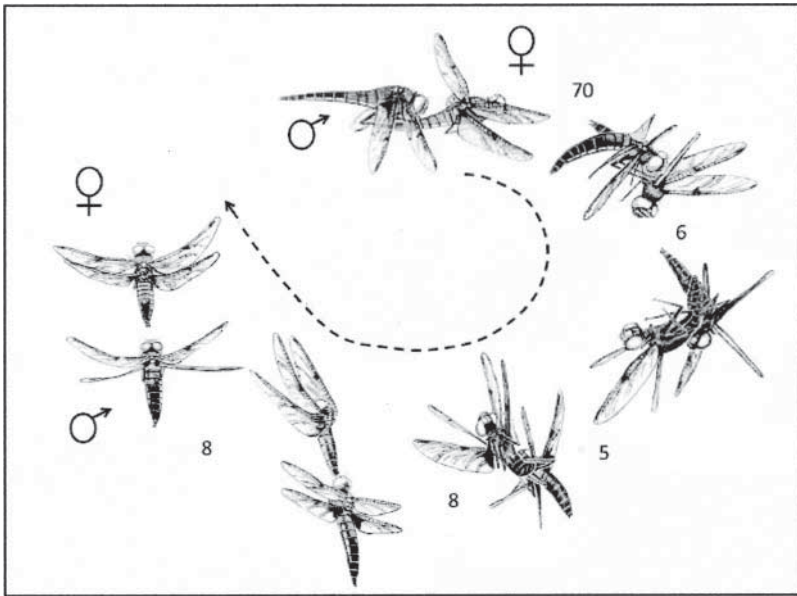


Figure 5. A seized female of *L. quadrimaculata* flew a loop and shook off the male, beginning at the top. The rotation (lasting 0.2 s) started by the female bending her thorax down and shifting the beating plane forward (1st to 2nd phase). Stretching of the bent abdomen (3rd to 4th phase) and twisting (black arrow, 5th phase), in addition to the centrifugal force of the loop delivered by the male, enabled her to flee (left). Duration from seizing to escaping = 0.74 s. Numbers between the phases are frame numbers between the phases; for example five frames = duration of 5/300 s. (Drawn from 300 fps film.)

very fast (duration from beginning of bending to maximal bend: 0.037 s, 0.047 s) and then flew one ( $N = 3$ ) or several turns (up to nine) continuously ( $N = 1$ ) or with short breaks in between ( $N = 2$ ) (Figures 6, 7, 8; see supplemental material). When breaks between the turns occurred the abdomen was bent before some turns once more. Conspecific males, and even a harassing male of *Libellula quadrimaculata*, could not follow these turns of the females and were left behind. The turns of the females with arched abdomen lasted only 0.079 s ( $N = 5$ ), with significant difference to the conventional turns of the males ( $p = 0.0004$ ,  $t$ -test). The turns of the females were narrower in each case than those of the males (Figure 8). In none of the filmed cases could the male seize the female. In two other observed cases he seized her, but she escaped after struggling on the water's surface.

### 7. Intense female refusal behaviour of *Calopteryx xanthostoma* under extreme ecological conditions

At a shallow site, the River Célé in July 2013 was fast-running ( $1\text{--}2\text{ m s}^{-1}$ ). The density of *C. xanthostoma* was high (estimated 50–60 individuals in an area of  $15 \times 10\text{ m}$ ). Although the normal threat display of males, with waving, pendulum and circling threatening flight or frontal threatening, could be seen, escalated fights (fast pursuing, speed up to  $5.4\text{ m s}^{-1}$ ) and physical fights were more frequent. Male competition was intense and could be seen in injuries of the male's wings. About 30% of males were injured ( $N = 28$ ). Females were not submerging for oviposition. Only one female did amidst a patch of common water crowfoot (*Ranunculus aquaticus*), generating low water speed.

Flying females were mostly pursued in fast flight. Ovipositing females were also seized by males in full flight. But sometimes males tried to approach a female slowly by courting flight.



Figure 6. A female (above) *Anax imperator* pursued by a conspecific male flew a U-turn in the moment just before being seized. She then pushed the male off with her middle and hind legs and escaped. After the next 0.2 s the distance of the female from the pursuing male was about 0.4 m. (Photo taken at 1/2500 s.)

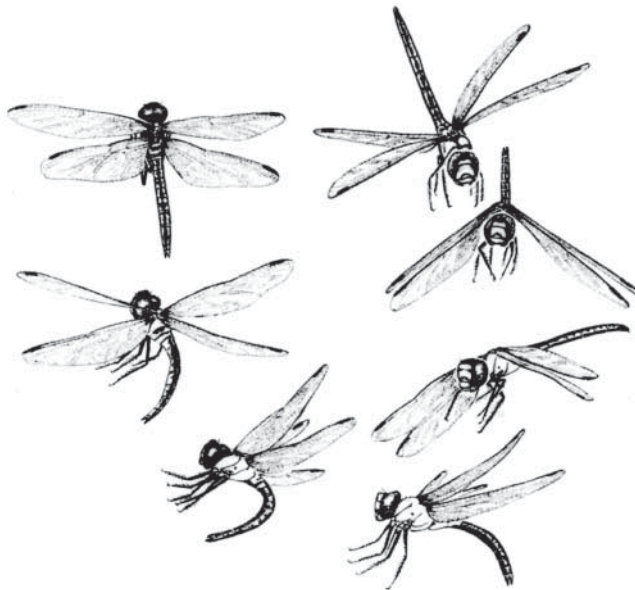


Figure 7. A female of *Anax imperator* pursued by a male of *Libellula quadrimaculata* (not shown) flies a U-turn (from top right downwards to the left). First a turning moment is produced by banking (1st phase) and high angles of attack of the right wings (2nd–3rd phase). This turning moment is enhanced by bending the abdomen very fast (= pirouette effect, 4th and 5th phase). (Drawn from 300 fps film.)

In one case, two non-territorial males approached (maximal flight velocity  $32.4 \text{ cm}\cdot\text{s}^{-1}$ ), both courting the same female for a moment. The beat frequency in courting flight (mean 49.5 Hz,  $n = 31$ ) was much higher than in normal forward flight (mean 15.7 Hz,  $n = 33$ ).

When a *C. xanthostoma* male approached in normal or courting flight, in only one case did an ovipositing female exhibit wing clapping (this lasted 0.18 s at three beats); in all other cases females did not clap wings, but lowered their wings and stayed motionless. They fled at very short distances from males, sometimes when touching them (mean distance  $< 5 \text{ cm}$  to the male ( $N = 5$ ), 5–10 cm ( $N = 6$ ), with four of the males courting before the female's escape. Females

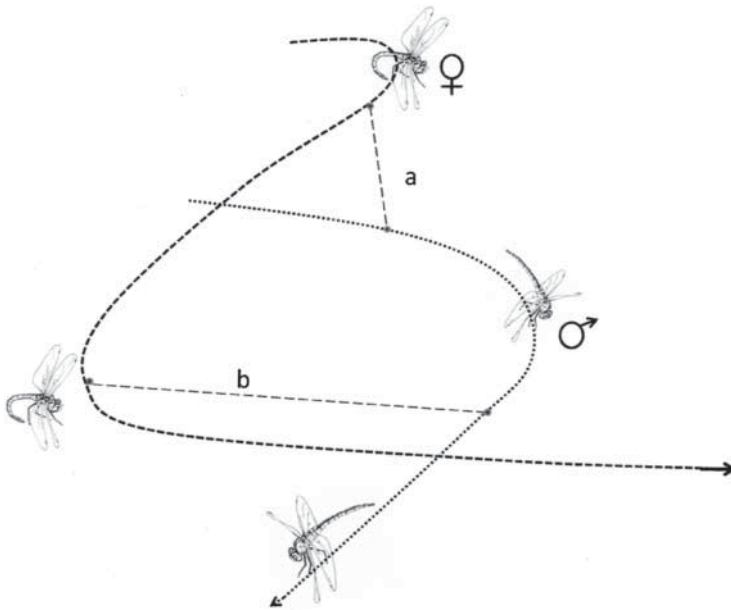


Figure 8. A female *Anax imperator* (solid dashed lines) escaped a pursuing male (short dashed lines) by flying sharp turns accompanied by bending the abdomen downwards. Simultaneous positions of male and female are connected by lines a and b. (Drawn from 300 fps film.)

often struggled and defeated the attacking male. One female attacked a male frontally by flying straight into him, pushing him from his perch (see supplemental material). Another female altered her flight by changing the wing beat pattern from synchronous beating of forewings and hind wings to counter stroking and doubling the beat frequency from 15 Hz to 37 Hz. She passed and returned to the courting male, showing this behaviour twice, in one case for more than 1.3 s (= 40–50 wing beats).

Another female already in tandem position was filmed not cooperating to form the copulation wheel by holding the abdomen stiff before the male broke off. Yet another tandem was broken when the female flew backwards (Figure 9; see supplemental material). This was done by beating the wings forwards in the flight direction with high angles of attack of *ca.*60° (measured in the middle of the wings). Fleeing of the female when two males, heading for the same female, attacked each other occurred three times. On three occasions, a female fled after two males, heading for her, attacked each other. In two of these cases one male was already coupled to her and the other male pulled the tandem male off (Figure 10).

In total, females managed to avoid or fly free from the male 25 times, whereas in only nine cases did the male copulate with the female.

## Discussion

In both suborders we were able to describe and analyse female behaviour for refusing males: wing clapping without and with physical contact, holding to the substratum, flying loops or sharp turns and several behavioural specialities in *Calopteryx*. This mostly led to escapes of the females. The slowed-down films show the kinematics and how precise and fast flight manoeuvres were performed. Anisoptera are stronger fliers (Rüppell, 1989a) and because their males have greater control over tandem formation, they have a greater potential for coercion than

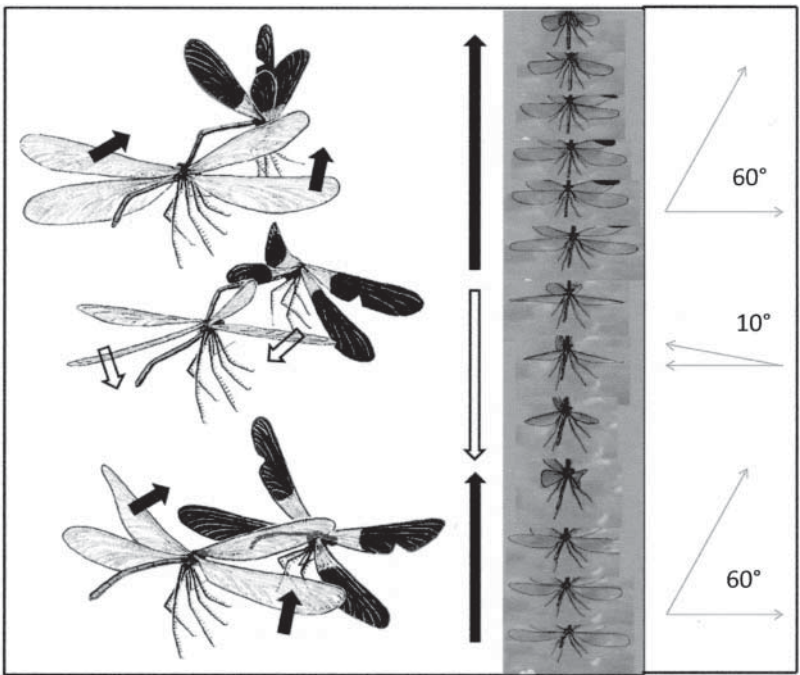


Figure 9. A female of *Calopteryx xanthostoma* flew backwards and separated from the male in tandem. The wings during forward beating (black arrows) with high angles of attack (right: calculated at the middle of the forward stroke at half hind-wing length) generated negative thrust, while in backward beating (middle, open arrows) the wings were only slightly inclined (middle phase, right: angles of hind wings) and generated mainly lift. Black solid and opened arrows indicate the beating direction. (Photographs in the middle are framed phases from the film; sketches drawn from 300 fps film.)

Zygotera males (Fincke, 1997). Moreover, female Anisoptera, in contrast to female Zygotera, are unable to submerge for oviposition, and thereby avoid males' interference. The spread wings and large bodies of Anisoptera do not allow them to cut easily through the water's surface. The described refusal behaviour performed mainly in flight underline the more powerful flight ability of Anisoptera. Together this may select for females either acquiescing or effectively evading harassing males. Avoiding physical battles is probably favoured, they can cause serious injury (Rüppell & Hilfert-Rüppell, 2013).

### *Wing clapping with signal function*

Wing clapping can be used as a physical resistance behaviour (Corbet, 2004, p. 471) or as a distance communicative signal (Günther et al., 2014). Males may have evolved non-aggressive sexual signals because direct, physical sexual coercion entails the risk of injury and, owing to evasion, resistance, and physical repulsion by females, sexual conflict often is resolved in the females' favor (Fincke, 1997). In this investigation wing clapping used as a signal lasted for only a short period and was done in bursts of 1–4 claps, in contrast to multiple wing clapping used to repel males physically. An *Anax imperator* female needed 15 claps to push a male of *Brachytron pratense* away (Figure 2). In *Neurobasis chinensis* the physical wing clapping was also long lasting, in contrast to the signalling ones (Günther et al., 2014). We also filmed refusal behaviour by wing clapping in *Calopteryx splendens*. Interacting by wing clapping of the female and by courting flight of the approaching male seems to have a communicative function. Every time he approached the female showed wing clapping or took off (Figure 1). When he then retreated

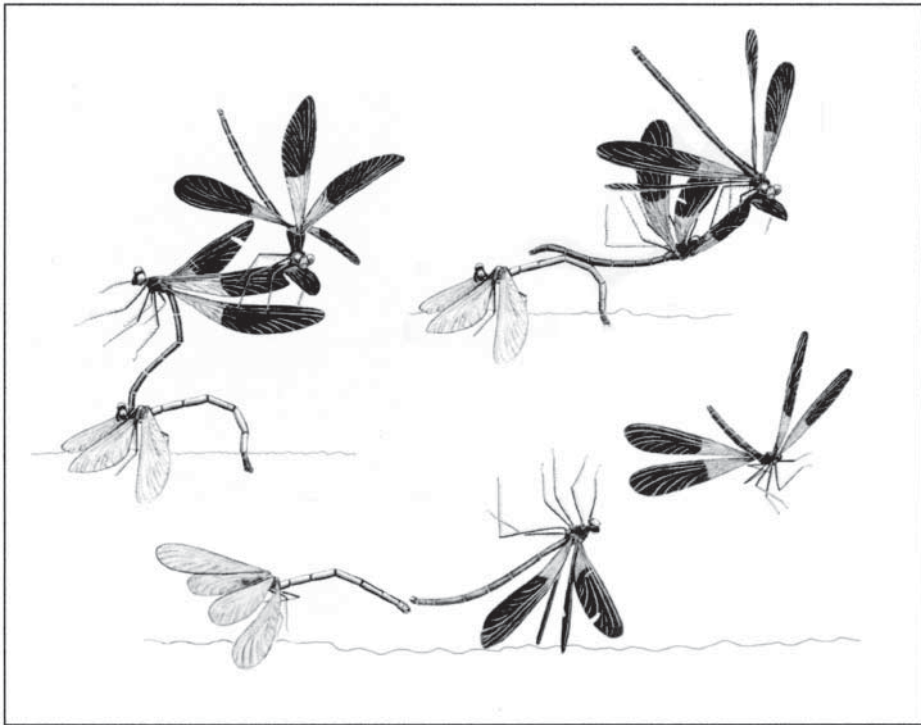


Figure 10. A female *Calopteryx xanthostoma* fled while another male pulled the tandem male away with his mouth-parts. The tandem male already had three injuries to the wings before that. (Beginning at top left; then top right and ending at bottom; drawn from 300 fps film from river Célé in SW France.)

she rested perched and finally accepted mating. Such signalling, also described in *Calopteryx maculata* by Waage (1984b), could be interpreted as testing the male.

#### ***Fleeing or holding onto the substratum when harassed by a male while egg-laying***

*Anax imperator* females prefer open spaces with floating leaves or dead stems of reed to oviposit (Corbet, 1957). There, complex flight manoeuvres to escape harassing males can take place successfully. Another Aeshnidae (*Aeshna cyanea*) prefers bank substrata for oviposition, often within dense vegetation (Sternberg & Buchwald, 2000). Males probably recognized ovipositing females when hovering near to them, but mostly did not approach. The force with which females hold onto the plants exceeds the force that hovering males can exert to pull her off. If she does not need new sperm or a new spot for oviposition she repels the male by wing-clapping and body-shaking, holding on and going on egg-laying. This holding was different in Zygoptera and Anisoptera. From the broadness of the legs of Aeshnidae, their muscle layout and therefore their power outcome should be much larger than that of Zygoptera, which possess thinner legs. While females of *A. cyanea* and *Leucorrhinia rubicunda* (Rüppell, 1989b) can hold onto the substrate for long periods, *Calopteryx splendens* and *C. xanthostoma* females could not and were broken away by males after a few moments.

#### ***Refusal behaviour sometimes generates new costs***

Holding onto the substratum means being exposed to attacks by other males. Two, or even four males coupled together with one female of *Leucorrhinia rubicunda* have been filmed



(Rüppell, 1989b). In *Calopteryx* at high densities, territorial behaviour is replaced by alternative reproductive behaviour (Cordero, 1999; Córdoba-Aguilar & Cordero Rivera, 2005; Higashi & Nomakuchi, 1997; Hilfert-Rüppell, 2004, Hilfert-Rüppell & Rüppell, 2008). Then males often pursued females. The pursuing males often impeded each other by demonstrating their coloured wings, so that females escaped easily (this study and Rüppell & Hilfert-Rüppell, 2009). Only when the density was so high that females could not retreat, were they seized and brought to copulation, as also reported by Cordero Rivera and Andrés (2002). Very fast plunge diving (amidst several males) led to an escape under water to lay eggs.

*Calopteryx* females may submerge for oviposition, as is the case in many Zygoptera species (see Corbet, 2004). Their folded wings can cut the surface of the water and their well-developed wax-layer around body and wings (Gorb, 2009) allows them to emerge very effectively. When surfacing, they typically are immediately seized by males, as in *Enallagma* species or *Erythromma lindenii* (Fincke, 1984; Sternberg & Buchwald 2000), with little or no opportunity to evade being taken in tandem. Furthermore, to lay maximal numbers of eggs undisturbed, submerged females need to stay long under water, even though submerging is risky and energy-intensive; it may even be fatal through fish predation (Hilfert-Rüppell, unpublished observations) or by drowning (Fincke, 1986) especially at low water temperatures. Thus, in the river Oker, in early summer with a water temperature of 14–16°C, females of *C. splendens* often drowned, not being able to take off from the water's surface. Having seen this several times over a few days in 2010 on a stretch of 30 m, the total number of drowned females on the population's area of > 8 km river should be high.

In fleeing by flying, females might have the opportunity to test following males, as reported of *Onychogomphus forcipatus unguiculatus* (Miller & Miller, 1985). Such testing could be the case in *C. splendens* and in *L. quadrimaculata* as well, as our slow-motion films suggest (see supplemental material).

### ***Special flight manoeuvres to escape***

*Libellula quadrimaculata* females at high male densities flew loops to escape, but those of *Anax imperator* flew sharp turns. Perhaps there exists a relation between their different refusal manoeuvres and their body shapes and flight types. The shape of *L. quadrimaculata*, as a member of Libellulidae with a short body and large wing span, allows loops easily. Libellulids are perchers (Corbet & May, 2008) and therefore spend less time on the wing than flyers. Aeshnidae are flyers and possess a longer abdomen, serving as a balancing pole for flight stability. So females of *A. imperator* should be able to fly loops only with effort (but they can). Flying sharp turns by bending the abdomen very quickly accelerates the turning already generated by the wings before. This bending displaces the mass of the abdomen near to the turning axis and lowers the centrifugal force, thus accelerating the turning comparable with an ice dancer performing a pirouette. Even in forward flight, females of *A. imperator* show abdomen bending downwards when harassed by males (Corbet, 1957), decreasing the unprotected body area. Males obviously did not evolve this trait. This might explain why males are not able to fly such turns with arched abdomen and have difficulties following fleeing females.

### ***Water current enhances refusal behaviour***

At the river Célé in south-west France, where there were high densities of *C. xanthostoma* and a strong water current which did not allow submerged oviposition, very intense male aggressive behaviour (for *C. virgo* see Pajunen, 1966) and intense female refusal behaviour was observed: ovipositing females were continually harassed by males and kept rejecting them intensively.

Obviously crucial for the frequent coercion of the males was the extreme water current, hindering the females from ovipositing under water. These types of refusal behaviour, e.g. ramming a male, separating from a tandem by backwards flight, or performing a special high-frequency, counterstroking flight, have not previously been reported and may be due to the extreme conditions. The latter flight type probably has a threatening function, as it was also found in females of *C. splendens* competing for food (Hilfert-Rüppell, in preparation). In other populations of *C. xanthostoma* no refusal behaviour was reported (Plaiستow & Siva-Jothy, 1996; Siva-Jothy, Gibbons, & Pain, 1995).

We should not only expect to find differences in refusal behaviour in different related species of odonates (Heymer, 1972; Waage, 1984b) but also in different populations of the same species living under different ecological conditions.

## Conclusion

Dragonfly behaviour occurs mostly in flight and is very fast. Details of it, such as wing or leg movements, are not visible to the naked eye. Females in particular, being inconspicuous, have rarely been studied. This slow-motion analysis shows how variable female behaviour occurs and offers new insight in the behavioural capacity of these insects. Females are not passive in reproductive behaviour. Their role in sexual conflict (Arnqvist & Rowe, 2005) is more like that of males, deciding to a large part their fitness (Fincke, 1997). So, not only is male coercion at work, but also successful female evasion and physical resistance to copulation, and communication of readiness to mate. The density of males and the accessibility and availability of females affects these interactions: the higher the male–male competition the more coercion and female evasion and resistance are likely to be dominant, whereas at lower density communication is more likely to solve the conflict. The latter includes the possibility of sexual selection. This slow-motion analysis showed that, even at high densities, females can control their reproduction. Further time-transformed filming could reveal more of these possibilities.

## Acknowledgements

We thank Georg Seifert for first corrections of the English, Hansruedi Wildermuth for important comments, Frank Suhling for statistical help, Peter Mill and Michael May for polishing our English and Ola Fincke for helpful corrections and remarks, as well as two anonymous reviewers.

## Supplemental data

Supplemental data for this article can be accessed via the online version [<http://dx.doi.org/10.1080/13887890.2014.972893>].

## References

- Alcock, J. (1993). *Animal behaviour – An evolutionary approach*. Sunderland, MA: Sinauer Associates.
- Andersson, M. (1994). *Sexual selection*. Princeton: Princeton University Press.
- Arnqvist, G., & Rowe, L. (2005). *Sexual conflict*. Princeton and Oxford: Princeton University Press.
- Bick, G. H., & Bick, J. C. (1978). The significance of wing clapping in Zygoptera. *Odonatologica*, 7, 5–9.
- Clutton-Brock, T. H., & Parker, G. A. (1995). Sexual coercion in animal societies. *Animal Behaviour*, 49, 1345–1365. doi:10.1006/anbe.1995.0166
- Conrad, K. F., & Hermann, T. B. (1990). Seasonal dynamics, movements and the effects of experimental increased female densities on a population of imaginal *Calopteryx aequabilis* (Odonata: Calopterygidae). *Ecological Entomology*, 15, 119–129. doi:10.1111/j.1365-2311.1990.tb00792.x
- Cooper, G., Holland, P. W. H., & Miller, P. L. (1996). Captive breeding of *Ischnura elegans* (Van der Linden): observations on longevity, copulation and oviposition (Zygoptera: Coenagrionidae). *Odonatologica*, 25, 261–273.

- Corbet, P. S. (1957). The life-history of the Emperor-Dragonfly *Anax imperator* Leach (Odonata: Aeshnidae). *Journal Animal Ecology* 26, 1–69. Retrieved from <http://www.jstor.org/discover/10.2307/1781?uid=3737864&uid=2&uid=4&sid=21105158967913>
- Corbet, P. S. (2004). *Dragonflies, behaviour and ecology of Odonata*. Colchester, UK: Harley Books.
- Corbet, P. S., & May, M. L. (2008). Fliers and perchers among Odonata: dichotomy or multidimensional continuum? A provisional reappraisal. *International Journal of Odonatology*, 11, 155–171. doi:10.1080/13887890.2008.9748320
- Cordero, A. (1999). Forced copulations and female contact guarding at a high male density in a calopterygid damselfly. *Journal of Insect Behavior*, 12, 27–37. Retrieved from <http://link.springer.com/article/10.1023%2FA%3A1020972913683>
- Cordero Rivera, A., & Andrés, J. A. (2002). Male coercion and convenience polyandry in a Calopterygid damselfly (Odonata). *Journal of Insect Science*, 2, 14. doi:10.1093/jis/2.1.14
- Córdoba-Aguilar, A., & Cordero Rivera, A. (2005). Evolution and ecology of Calopterygidae (Zygoptera: Odonata): status of knowledge and research perspectives. *Neotropical Entomology*, 34, 861–879. doi:10.1590/S1519-566X200500600001
- Debano, S. J. (1996). Male mate searching and female availability in the dragonfly, *Libellula saturata*: relationships in time and space. *The Southwestern Naturalist*, 41(3), 293–298. Retrieved from <http://www.jstor.org/discover/10.2307/30055127?uid=3737864&uid=2129&uid=2&uid=70&uid=4&uid=21104285879333>
- Dijkstra, K. D., & Lewington, R. (2006). *Field guide to the dragonflies of Britain and Europe*. Gillingham, UK: British Wildlife Publishing.
- Fincke, O. M. (1984). Sperm competition in the damselfly *Enallagma hageni* Walsh (Odonata: Coenagrionidae): benefits of multiple matings to males and females. *Behavioral Ecology and Sociobiology*, 14, 235–240. Retrieved from <http://link.springer.com/article/10.1007/BF00299623#>
- Fincke, O. M. (1986). Underwater oviposition in a damselfly (Odonata: Coenagrionidae) favors male vigilance, and multiple mating by females. *Behavioral Ecology and Sociobiology*, 18, 405–412. Retrieved from <http://link.springer.com/article/10.1007/BF00300514>
- Fincke, O. M. (1997). Conflict resolution in the Odonata: implications for understanding female mating patterns and female choice. *Biological Journal of the Linnean Society*, 60, 201–220. doi:10.1111/j.1095-8312.1997.tb01492.x
- Gorb, S. N. (2009). *Functional surfaces in biology – adhesion related phenomena*. Dordrecht, Heidelberg, London, New York: Springer.
- Günther, A., Hilfert-Rüppell, D., & Rüppell, G. (2014). Reproductive behaviour and the system of signalling in *Neurobasis chinensis* (Odonata, Calopterygidae) – a kinematic analysis. *International Journal of Odonatology*, 17, 31–52. doi:10.1080/13887890.2014.881305
- Heymer, A. (1972). *Verhaltensstudien an Prachtlibellen. Beiträge zur Ethologie und Evolution der Calopterygidae Selys, 1850 (Odonata: Zygoptera)*. Parey: Berlin.
- Higashi, K., and Nomakuchi, S. (1997). Alternative mating tactics and aggressive male interactions in *Mnais nawai* Yamamoto Zygoptera: Calopterygidae. *Odonatologica*, 26, 159–169.
- Hilfert, D. (1997). Early morning oviposition of dragonflies with low temperature for male-avoidance (Odonata: Aeshnidae, Libellulidae). *Entomologia Generalis*, 21(3), 177–188. Retrieved from <http://cat.inist.fr/?aModele=afficheN&cpsidt=2545890>
- Hilfert-Rüppell, D. (2004). *Optimierung des Fortpflanzungsverhaltens: wichtige Einflussgrößen auf Territorialität und auf Paarungen von europäischen Prachtlibellenmännchen (Odonata: Zygoptera)* (PhD thesis). Technical University of Braunschweig, Germany. Retrieved from <http://www.digibib.tu-bs.de/?docid=00001567>
- Hilfert-Rüppell, D., & Rüppell, G. (2008). Alternative Taktiken im Fortpflanzungsverhalten von *Calopteryx splendens* in einem geographischen Vergleich (Odonata: Calopterygidae). *Entomologie heute*, 20, 93–103.
- Hilfert-Rüppell, D., & Rüppell, G. (2013). Do coloured-winged damselflies and dragonflies have flight kinematics different from those with clear wings? *International Journal of Odonatology*, 16, 119–134. doi:10.1080/13887890.2013.763332
- Kaiser, H. (1974). Verhaltensgefüge und Temporalverhalten der Libelle *Aeschna cyanea* (Odonata). *Zeitschrift für Tierpsychologie*, 34, 398–429; (2010) Epub: *Ethology*. doi:10.1111/j.1439-0310.1974.tb01811.x
- Kaiser, H. (1985). Availability of receptive females and mating chances of males in the dragonfly *Aeschna cyanea*. *Behavioural Ecology and Sociobiology*, 18, 1–7. Retrieved from <http://link.springer.com/article/10.1007/BF00299231#page-1>
- Koenig, W. D. (1991). Levels of female choice in the White-Tailed Skimmer *Plathemis lydia* (Odonata: Libellulidae). *Behaviour*, 119, 193–224.
- Miller, A. K., & Miller, P. L. (1985). Flight style, sexual identity and male interactions in a non-territorial dragonfly, *Onychogomphus forcipatus unguiculatus* (Van der Linden); (Odonata: Gomphidae). *The Entomologist's Monthly Magazine* 121, 127–132.
- Miller, P. L. (1991). Notes on the reproductive biology of *Zyxomma petiolatum* Rambur in India (Anisoptera: Libellulidae). *Odonatologica*, 20, 433–440.
- Moore, N. W. (1960). The behaviour of the adult dragonfly. In P. Corbet, C. Longfield, N. W. Moore (Eds.), *Dragonflies* (p. 110). London: Collins.
- Pajunen, V. I. (1966). Aggressive behavior and territoriality in a population of *Calopteryx virgo* L. (Odonata: Calopterygidae). *Annales Zoologici Fennici*, 3, 201–214. Retrieved from <http://www.jstor.org/discover/10.2307/23731277?uid=3737864&uid=2&uid=4&sid=21105158967913>

- Plaisiow, S. J., & Siva-Jothy, M. T. (1996). Energetic constraints and male mate securing tactic in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). *Proceedings of the Royal Society London B*, 263, 1233–1238. Retrieved from <http://www.jstor.org/discover/10.2307/50526?uid=3737864&uid=2129&uid=2&uid=70&uid=4&uid=sid=21104285879333>
- Rehfeldt, G. E. (1989). The influence of male interference on female perching behaviour before and during oviposition in libellulid dragonflies (Anisoptera). *Odonatologica*, 18, 365–372.
- Rehfeldt, G. E. (1991). Site-specific mate-finding strategies and oviposition behaviour in *Crocothemis erythrea* (Brullé) (Odonata: Libellulidae). *Journal of Insect Behavior*, 4, 293–303.
- Rowe, R. J. (1988). Alternative oviposition behaviors in three New Zealand cordulid dragonflies their adaptive significance and implications for male mating tactics. *Zoological Journal of the Linnean Society*, 92(1), 43–66. doi:10.1111/j.1096-3642.1988.tb01526.x
- Rüppell, G. (1989a). Kinematic analysis of symmetrical flight maneuvers of Odonata. *Journal of Experimental Biology*, 144, 13–42. Retrieved from <http://jeb.biologists.org/content/144/1/13>
- Rüppell, G. (1989b). Fore legs of dragonflies used to repel males. *Odonatologica*, 18, 391–396.
- Rüppell, G., & Hilfert-Rüppell, D. (2009). Males do not catch up with females in pursuing flights in *Calopteryx splendens* (Odonata: Calopterygidae). *International Journal of Odonatology*, 12, 195–203. doi:10.1080/13887890.2009.9748339
- Rüppell, G., & Hilfert-Rüppell, D. (2013). Biting in dragonfly fights. *International Journal of Odonatology*, 16(3), 219–229. doi:10.1080/13887890.2013.804364
- Siva-Jothy, M. T., Gibbons, D. W., & Pain, D. (1995). Female oviposition- site preference and egg-hatching success in the damselfly *Calopteryx splendens xanthostoma*. *Behavioral Ecology and Sociobiology*, 37, 39–44.
- Stange, G., Chal, J., & Mizutani, A. (2001). *Target tracking in dragonflies: the role of compound eyes and ocelli*. Abstract in the Second WDA International Symposium of Odonatology, Gällivare, Sweden, p. 18.
- Sternberg, K., & Buchwald, R. (2000). *Die Libellen Baden-Württenbergs. 2: Großlibellen (Anisoptera)* Ulmer Verlag: Stuttgart.
- Ubukata, H. (1973). Life history and behaviour of a cordulid dragonfly, *Cordulia aenea amurensis* Selys. 1. Emergence and pre-reproductive periods. *Journal of the Faculty of Science, Hokkaido University. Series 6, Zoology*, 191, 251–269.
- Ubukata, H. (1984). Oviposition site selection and avoidance of additional matings by females of the dragonfly, *Cordulia aenea* SELYS (Cordulidae). *Researches on Population Ecology*, 26, 285–301. Retrieved from <http://link.springer.com/article/10.1007/BF02515495>
- Waage, J. K. (1984a). Sperm competition and the evolution of odonate mating systems. In R. L. Smith (Ed.), *Sperm competition and the evolution of animal mating systems* (pp. 251–290). Academic Press: New York.
- Waage, J. K. (1984b). Female and male interactions during courtship in *Calopteryx maculata* and *C. dimidiata* (Odonata: Calopterygidae). Influence of oviposition behaviour. *Animal Behaviour*, 32, 400–404.
- Wildermuth, H. (2008). Die Falkenlibellen. *Die Neue Brehm-Bücherei*, 653, p. 496.
- Xu, M., & Fincke, O. M. (2011). Tests of the harassment-reduction function and frequency-dependent maintenance of a female-specific color polymorphism in a damselfly. *Behavioral Ecology and Sociobiology*, 65, 1215–1227. Retrieved from <http://link.springer.com/article/10.1007/s00265-010-1134-6>