

Reproductive behaviour of *Libellago semiopaca* on a Bornean rainforest stream (Odonata: Chlorocyphidae)

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ABSTRACT

The reproductive behaviour of *Libellago semiopaca* was studied on a swift-flowing shallow forest stream in Brunei. Females oviposited just below the water-line, commonly in groups, only on large, firm-textured, semi-submerged logs, usually guarded by males. Both sexes were very sedentary. Suitable sites, with good illumination and deep deposits of fine gravel and leaf mulch in dead water immediately behind the log were scarce. When stream levels were high, no oviposition sites were available. When possible, females generally oviposited every day, arriving between 10:00 and 15:00 h, and usually remaining on site for at least two hours. Males arrived earlier, between 09:00 and 13:00 h, and established small territories along the log. Females apparently began reproductive activity only when all oocytes were mature, and the egg load diminished daily as eggs were laid. Most matings occurred before 12:00 h with early-arriving females. Females mated every 2-3 days, probably to replenish sperm supplies. Male density was at its highest after 11:00 h and males shared territories, spending much of their time flying in low intensity confronting contests. Removal of males from a site, just as it was becoming available by falling water levels, resulted in little use of the site by females. Pinning decoy dead females at a good oviposition site failed to attract females if males had been removed. It is suggested that the prolonged male agonistic display attracts females to the site, and possibly commits them to future matings with the territory holders.

INTRODUCTION

Members of the old world tropical family Chlorocyphidae have among the most complex and varied systems of courtship and mating of all Odonata (Corbet 1999: 479-481). In many species males are characterised by markings on the wings, body, and legs (Paulson 1981; Robertson 1982a, b; Orr 1996; Günther 2005, 2007, 2008), all of which have semiotic functions during territorial defence and/or courtship,

behaviour first noted by Fraser (1934, 1949) and Laidlaw (1950). Most members of the genus *Libellago* have relatively simple displays, in which the main ornamental components are dark, slightly iridescent-blue terminal spots on the fore wing, expressed mainly in confronting head to head agonistic displays, and body markings, especially dorsal abdominal colour and white pruinose flexor surfaces to the tibiae, expressed mainly in courtship (Furtado 1966; Orr 1996; Günther 2008). Males of many species also have a basal pinkish, orange or amber translucent blush at the base of the wings, best developed in fully mature individuals. This may also be significant especially in courtship, the whirring wings creating a bright coloured halo about the body of the male as he courts the female (Günther 2008; AGO unpubl.). In some species of the genus some or all of these features are lacking, or are exaggerated, and in these cases behaviour is usually also atypical (Orr 1996; Günther 2008). There is at present insufficient knowledge to explain these many variations in the context of a general model, especially when species of other chlorocyphid genera, including *Aristocypha*, *Heliocypha*, *Rhinocypha* (Orr 1996; Günther 2008), *Chlorocypha*, *Platycypha* (Robertson 1982a, b; Lempert 1988; Miller & Miller 2003) and several basal genera, are also considered. However a close examination of mating and communication systems in one of the less typical species as documented here will hopefully contribute significantly to our understanding of behaviour and its relation to male ornamentation within the family.

Libellago semiopaca Selys is widely distributed in lowland areas throughout Sundaland, except for Java and Bali (Lieftinck 1954). It occurs locally on both broad, open sluggish rivers and on swift flowing shallow forest streams receiving significant levels of direct sunlight through the day (Orr 2001, 2003, 2006). Males of *L. semiopaca* differ morphologically from most closely related *Libellago* species in two ways which directly affect the signals they send to each other and to females. Firstly, the fore wing bears an extensive dark apical patch, instead of a small dark spot at the tip of the wing. Secondly, the legs do not have white pruinescence on their flexor surfaces, and during courtship are dangled, rather than spread fan-like, in front of the female (Orr 1996; Günther 2008). Males vary considerably in the extent of the dark marking on the fore wing and pale green marking on dorsal surface of the abdomen (Orr 2001). In specimens from broad muddy rivers in Brunei and also those studied in Peninsular Malaysia by Günther (2008), the apical dark wing patch occupied ca 30% of the total fore wing length, whereas on the Belalong River in Brunei, and also in similar situations in north-east Borneo the patch occupied ca 35-40% of the fore wing length. It is still not entirely certain if these forms represent local variations of the same species, but studies on the agonistic and mating behaviour in the different forms by Orr (1996) and Günther (2008) respectively, produced essentially similar results. In particular, males spent long periods in continuous agonistic displays, in the vicinity of female oviposition sites, in which they faced each other, circling slowly back and forth, and periodically presenting the pigmented fore wings held absolutely motionless in what Orr (1996) termed "the stationary wing display". These contests were generally of longer duration than in other, similar *Libellago* species studied by either author, with Orr (1996), reporting contests lasting 30-40 min, and Günther (2008) reporting contests lasting 40-60 min or even longer. After mating females are guarded for a short time by males, and often oviposit in groups. Preferred sites always include fairly intact, semi-submerged logs, with good illumination and deep deposits of fine gravel and leaf mulch, in quiet water

immediately behind the log. The wood into which the females oviposit is hard, and eggs are inserted just on the water line. Such sites are evidently in short supply. When stream levels are high, no oviposition sites may be available.

A major aim of the study was to characterise principal aspects of the mating system, including the extent to which both sexes moved within the habitat during their reproductive phase. Some of the data presented here was the basis for brief summary accounts of *L. semiopaca* given in Orr (2001, 2003). In addition, in November 1995, an experiment was conducted in which males were excluded from the oviposition site. This was initially designed to determine the possible role of male harassment, in forcing females into aggregations, as opposed to females aggregating directly by virtue of attraction to groups of their own sex. The results failed to produce conclusive results on this question, but however, serendipitously suggested an answer to an unasked question. Do male agonistic displays attract females to an oviposition site?

MATERIAL AND METHODS

Study area

All observations were made on the Belalong River, Temburong District, Brunei, in the vicinity of the Kuala Belalong Field Studies Centre of the Universiti Brunei Darussalam, situated at ca 4°32'48"N, 115°09'29"E, at an elevation of 60 m a.s.l. General features of the habitat and the spatial relationships of the two discrete major study sites, site A and site B, are shown in Figure 1. Figure 2 provides a detailed map of the most intensively studied site, designated Site A. Other physical features of the habitat were described by Orr (2006) and a photograph of the area can be found in Orr & Hämäläinen (2007: 34).

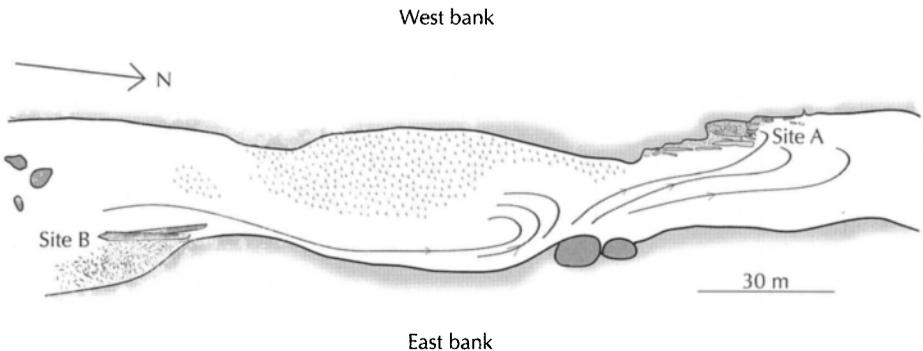


Figure 1: Area where *Libellago semiopaca* was studied — general view showing the relationships of sites A and B.

General features of the habitat and *L. semiopaca* biology

A general understanding of the habitat and the biology of *Libellago semiopaca* (Pl. I) was obtained from numerous casual observations during more than 200 days spent at the centre between November 1990 and March 1996. These casual observations are particularly informative given that the preferred habitat of *L. semiopaca* was in the river directly below the centre where one normally bathed in the afternoon, at a time when the insects were still active, hence some observations were made almost every day. These do not contribute directly to results reported here, but provide a very useful and necessary background to understanding the results. In particular, *L. semiopaca* and most other chlorocyphids which breed on the main stream (see Orr 2001), are generally dependent on water levels being sufficiently low to expose oviposition sites. The river is subject to rapid fluctuations, sometimes rising 5-10 m in a few hours. Depending on rainfall patterns, which are aseasonal and unpredictable, the water level may remain too high for sexual activity for several weeks, or it may fall to very low levels in a few days. The centre was provided with a water level gauge, and it was ascertained that at levels above 2.1 m on this scale, no activity was possible, with high activity when it was between 1.2-1.8 m. During extended periods of drought, when the water level fell to 0.4 m, populations of *L. semiopaca* fell to low levels unsuitable for observation of reproductive behaviour. For the first two study periods (see below) the water level fluctuated between 1.2 and 1.8 m, occasionally rising as high as 2.8 m overnight with an evening thunderstorm, but dropping rapidly the next morning. This had generally been the pattern in the weeks preceding observations on both occasions. At Site A (Fig. 2) the habitat used by the insects during all study periods was defined by the area ca 15 m long, labelled A1. The area labelled A2, was used on other occasions (not reported here) when the water level was very low (0.4-0.8 m).

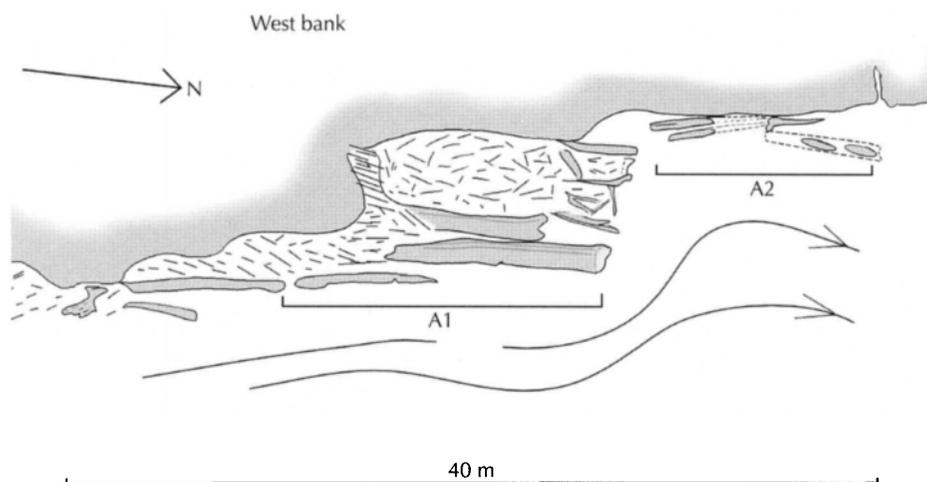


Figure 2: Detailed view of site A, showing areas used by *Libellago semiopaca* depending on water level. Results from this study are all from the region A1.

Detailed observations and experiments

Detailed recording reported here was carried out on three separate occasions between 8-14 October 1994, 18-29 January and 8-12 November 1995. Each of these three exercises followed a different protocol, each designed to reveal different aspects of the insects' biology. Apart from measuring general parameters of reproductive activity, the first sampling period aimed specifically to discover to what extent males and females move between oviposition sites along the river. Between 8 and 14 October 1994, groups of *L. semiopaca* were simultaneously monitored at site A (west bank, downstream), and Site B (east bank, upstream) (Fig. 1), from 09:00 h to 17:00 h solar time; time of sunrise and sunset being very nearly 06:00 h and 18:00 h respectively. The distance separating the two sites was ca 120 m across shallow water, which could be covered by the observer in ca 2 min. The first half of every hour was spent monitoring all agonistic, mating and oviposition behaviour at Site A. In particular duration and time of occurrence of all matings was recorded, and every 5 min the number of males engaged in aerial agonistic duels was noted. Records were made using a pocket dictaphone, augmented with field notes and sketches when time allowed. Close-focus binoculars were sometimes used to examine individuals at a distance. The second half of each hour was spent monitoring site B in the same manner. This methodology meant that on average only one half of all matings could be observed. A subsample of 10 males and 10 females were captured at each site, in the first two days of study, and marked with 1 (day 1) or 2 (day 2) spots on the hind wing, using fine Faber-Castell® OH LUX permanent pens. Individuals at site A were marked with red, those from site B with blue. Unmarked members of mating pairs were always allowed to separate before marking. Mostly it was possible to capture perched insects between the fingers, which avoided the trauma of netting. Insects were gently replaced on their perch immediately after marking. Males were also marked on the dorsum of S2 with a red or blue spot, depending on the site, so that even in flight, it was possible to determine if they were marked and which site they had originally been recorded. On 13 November 1994, the sixth of seven observation days, the river was explored ca 1.5 km upstream and 1.5 km downstream to investigate dispersal of marked insects. This prevented normal observations at Sites A and B for that day.

During the second period of study, from 18 to 29 January 1995 similar observations were made continuously at Site A (Fig. 2) for 12 consecutive days. All *L. semiopaca* present were individually marked using codes of 1-3 spots in red, blue and black. Behaviour was monitored using the same methods of direct observation and recording as before. The time of arrival and departure of every individual each day was noted. Every mating was monitored closely, with the identity of each member of the pair noted, hence it was possible to describe individual mating patterns for both sexes. In addition, the duration of almost every aerial contest between males was recorded, and, as far as possible, every courtship was also recorded, although usually not timed. With the intense general activity at certain times some shorter contests and courtships were probably overlooked, but it is unlikely these omissions significantly bias the results. The following priority was given to recording behavioural events: (1) matings, (2) courtships, and (3) agonistic displays. On the final day of study, seven mating pairs were captured as soon as the wheel was completed, and preserved in KAA fixative. These represented two female individuals each in the classes young presumed virgin, medium mated previously and old mated previously.

In addition, there was one probable virgin female who mated and remated within 10 min. Three females which refused matings after sustained courtship were also captured and preserved. These were later dissected using a Leitz stereomicroscope to assess the condition of the ovaries, the egg load, and the presence or absence of sperm in the bursa copulatrix and spermathecae, the latter being very reduced in this species. In addition the presence or absence of food in the gut was noted. Males from the mating pairs were also retained and dissected to examine the state of the gut and seminal vesicles. Previous dissections of freshly killed teneral females and freshly mated females, in which the ovaries and living spermatozoa were examined under a high power phase contrast transmission microscope served as a reference in interpreting these results.

The third period of study, over five days from 8 to 12 November 1995, was designed to measure the extent to which the presence of males affected female aggregation while ovipositing. This had been attempted before, using controlled artificial oviposition sites and decoy pinned females, but both males and females ignored these sites. Therefore, males were excluded from the natural site B by capture, usually by net, and allowed to behave naturally at site A. The study began at a time when the water had been continuously high for about two weeks, preventing any sexual activity. It dropped rapidly overnight on 17 November 1995, allowing oviposition to begin, and continued to fall slowly for the next five days from 1.8 m to 1.5 m. At each site, three pinned dried decoy females, set in an ovipositing posture, were placed in a group, each ca 5 cm apart, on a favoured oviposition site every morning at 09:00 - 09:10 h. The distribution of females was monitored within each site at half hour intervals, and interactions between males and arriving females at site A were recorded. Individuals were not marked on this occasion.

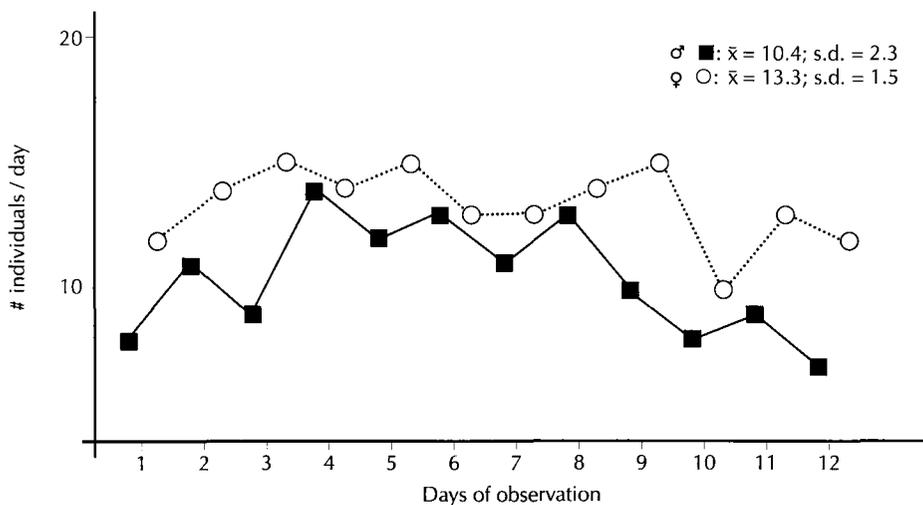


Figure 3: Absolute numbers of males and females of *Libellago semiopaca* visiting site A per day from 18-29 January 1995. Open circles: females; solid squares: males.

RESULTS

Population levels

The numbers of individuals of *Libellago semiopaca* visiting site A during the 12 days of the second period of observation were 10.4 males (s.d. = 2.3) and 13.3 females (s.d. = 1.5) per day (Fig. 3). Because not all individuals were marked during the first period of observation, it was not possible to give absolute abundance for this period, but recorded levels of activity (see below) suggested similar population levels were present at that time. Similar levels of activity were also evident in the third period of study.

Return rates of marked individuals

During the first period of observation high return rates to the site of marking were recorded for the first three days after marking (females 50-70%, males 40%). After six days 20% of females and 15% of males were still present. Figure 4 shows the rates of return, based on marked individuals actually present each day of observation. During the second study period at Site A only, a total of 27 males and 32 females were uniquely marked over the 12 days of study. Because every individual could be recognised, it was possible to calculate the minimum number of days since marking for each insect, even if they failed to appear on earlier days. Figure 5 shows the pattern of returns, with female return rates generally higher than those of males, but two males and one female returning 10 days after marking. More detailed patterns of return are discussed for selected individuals below (see also Fig. 13).

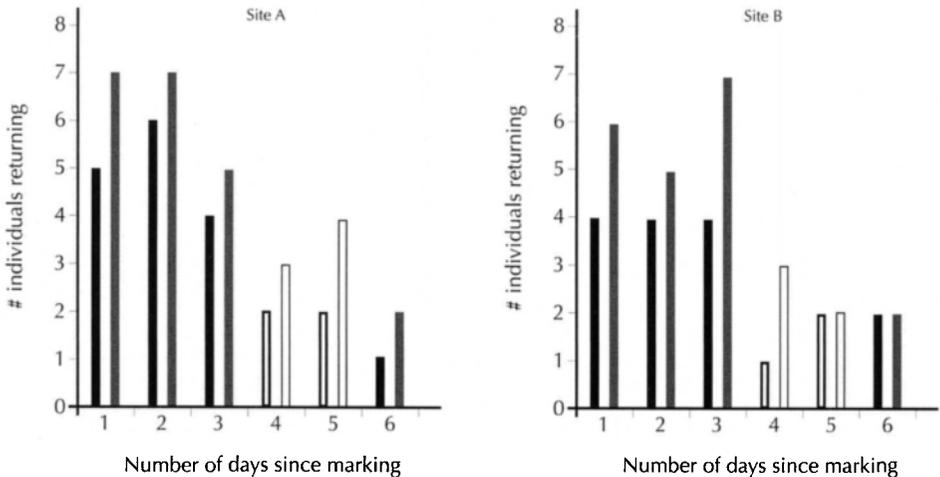


Figure 4: Numbers of marked individuals of *Libellago semiopaca* returning to (a) site A and (b) site B, against time since marking. Males: black bars, females: grey bars. Days 4 and 5 are represented by open bars since these periods represent half the sampling effort of other days.

Movements between sites and longer distance movements

During study period 1 marked insects were very sedentary, especially females. In total only six individuals, four males and two females were recorded switching sites in 5-6 days following marking. On day 3 a female marked at site B was found at Site A, and presumably the same female was also present on days 4 and 5. On day 4 a female marked at Site A, was found at Site B. On days 2, and 5 a total of three males marked at Site A were recorded at Site B and on day 4 a male marked at Site B was recorded at Site A. On day 6, after 12:00 h, a total of three marked males (two red and one blue) were found outside the study area. One was guarding a small log, in a semi-shaded locality, 300 m downstream, and another was perched on the semi-submerged thin branches of a fallen tree, 700 m downstream, in swift water. Both were completely alone and spent no more than 10% of their time in short flights over the stream during the half an hour observation of each. 1.2 km downstream was a larger log sunlit with three males and four females ovipositing, all unmarked. One marked male was found in a group of six males and five females ovipositing in a log in good habitat, 650 m upstream of site B. In addition, upstream from site B, three solitary unmarked males were found at distances of 200 m, 280 m, and 800 m. At 1,200 m upstream there was a group of about eight males and 10 females ovipositing on a log in good habitat. No marked female was found outside the main study area of Sites A and B. These results suggest that immigration/emigration contributed little to return rates reported above (Fig. 5), particularly for females.

Predation

During study period 1, two males were seen taken by *Dolomedes* spiders while perched in their territories on their log near the waterline (see Orr 2003: 48). During study period 2, three males and two females were observed to be taken by *Dolo-*

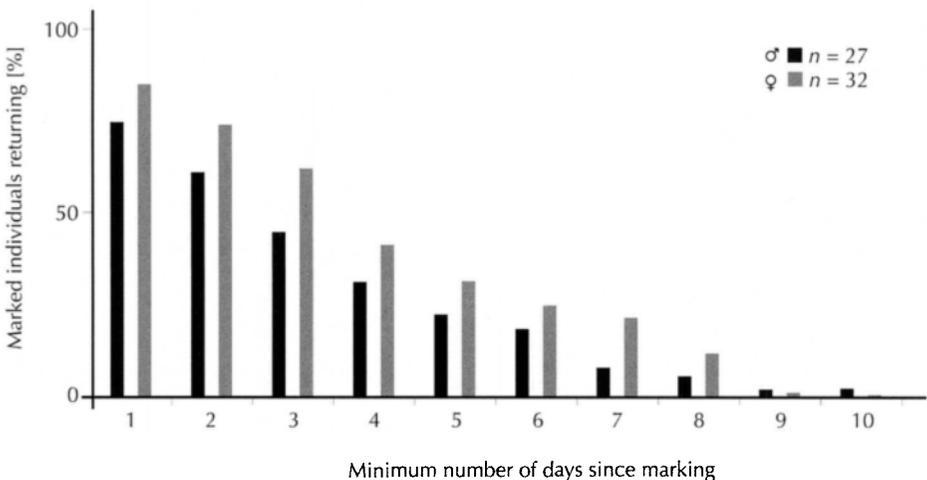


Figure 5: Percent of marked individuals of *Libellago semiopaca* returning plotted against minimum number of days since marking. Each individual is counted on the last day seen and on all previous days, whether it appeared at the site or not.

medes, and one marked female was found in the web of a Golden Orb Spider, *Nephila* sp. These incidental observations probably considerably underestimate the true level of predation at the oviposition site, and, apart from the single chance record of capture by *Nephila*, take no account of predation away from the site when resting, foraging or travelling.

Daily activity patterns

During all study periods the pattern of activity was similar, with the first males arriving at Site A ca 09:00 h, ca 15-20 min later at Site B owing to the sun reaching this site later, and remaining at high density from 11:00 - 15:00 h. Females arrived later, ca 10:00 h, with the greatest numbers between 12:00 and 15:00 h (Fig. 6). Before 12:00 h the numbers of males normally exceeded those of females, whereas after 12:00 h this relationship was reversed.

Territoriality and agonistic behaviour

Males arriving at the sites set up small territories, sitting ca 2-3 m apart along the logs with suitable oviposition substrates. Initially when these sites were established the occupants perched near the centres of their territories. As females began to arrive they become more active, making short exploratory flights of a few metres. As soon as males arrived, they began an agonistic display in which two individuals faced one another and hovered almost motionless or rotating slowly back and forth around a vertical axis. Physical contact never occurred, and the climax of such contests came when one or both of the protagonists signalled with a 'stationary wing display' (Fig. 7; Pl. II), in which the fore wings were held motionless for about half a second canted so that their upper surface was displayed to the opponent, while flying with the hind wings. This display often resulted in the end of a contest, with the loser retreating (Fig. 7a), but sometimes it appeared to be completely ritualised, with two males both signalling in unison (Fig. 7b), then continuing their display without interruption.

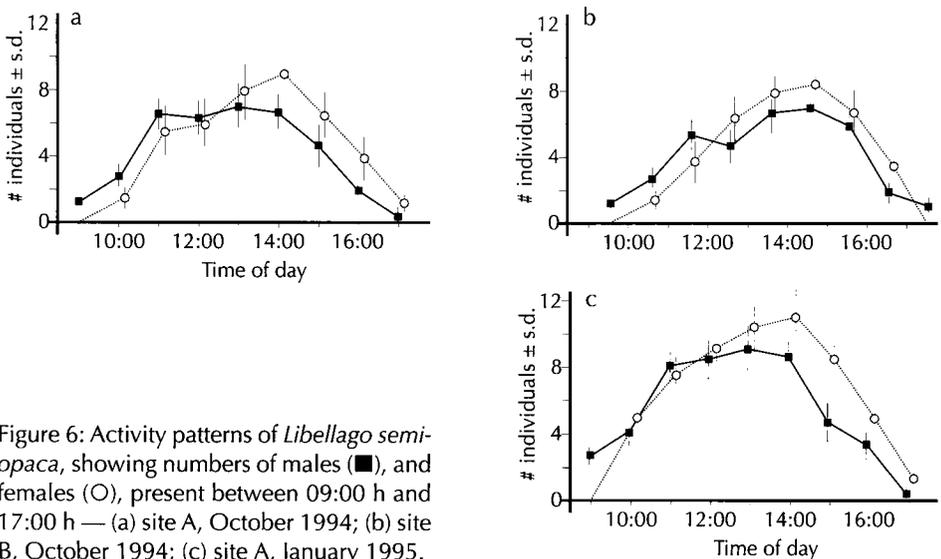


Figure 6: Activity patterns of *Libellago semiopaca*, showing numbers of males (■), and females (○), present between 09:00 h and 17:00 h — (a) site A, October 1994; (b) site B, October 1994; (c) site A, January 1995.

The mean duration of all such contests for study period 2 was 12.6 min ($n = 684$, s.d. = 11.8 min), but many contests were much more prolonged, lasting up to 60 min (Fig. 8). The total time spent in these displays by all individuals increased from less than 5% in the morning, to nearly 90% about 13:00 h (Fig. 9), the time when numbers of females present were at their highest, but after most mating activity had occurred. Nor was the time invested in displays strongly related to the number of males present, since before 12:00 h when male density was already near its peak, the display intensity was only moderate. During these protracted contests, two males were effectively sharing a territory. Their display was most commonly interrupted by a third male entering the fray, which led to a confused chase, and the eviction of one of the three, typically the intruder.

Courtship and mating

During study periods 1 and 2 a total of 547 courtships were observed resulting in 117 matings. The typical sequence of behaviour was as follows: A female arrived in a male's territory and began to test the substrate along the waterline with her ovipositor. The male courted her in a series of semicircular oscillating sweeps centred on the female with legs held straight down and vibrated, and abdomen twisted up to reveal the species-specific green pattern of the upperside (Fig. 10). The heavily pigmented fore wings were also often held nearly still, a gesture undoubtedly integral to the display. The success rate of courtship varied greatly throughout the day, with success rates as high as 90% around 10:00 h with the first arriving females, dropping nearly zero by 15:00 h (Fig. 11). Males persisted in vigorous courtship until ca 16:00 h, but activity diminished after ca 14:30 h. More than 80% of matings took place before 12:00 h (Fig. 11) and the many females arriving after this time were almost all refractory and refused to mate despite persistent courtship. Females unwilling to mate would, if courted, signal reluctance by half opening their wings (Fig. 10). Normally a female harassed by a male or series of males who ignored this signal would

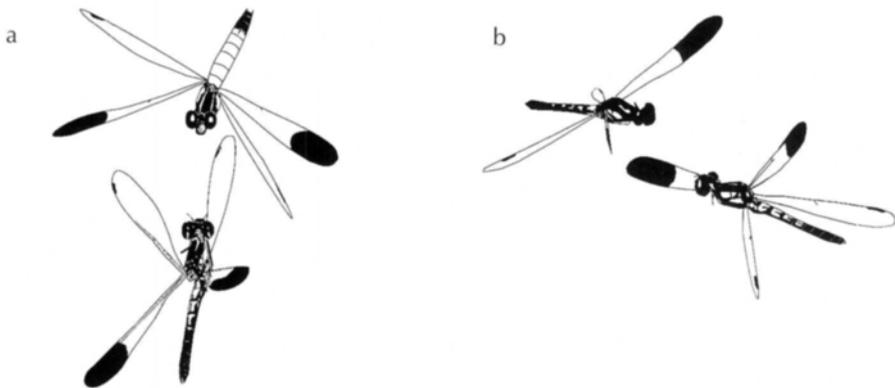


Figure 7: Threat display in *Libellago semiopaca* — (a) stationary wing threat display by upper individual, with lower individual showing an uncoordinated flight pattern at point of withdrawal from contest; (b) mutual stationary wing threat display produced simultaneously by two protagonists in a protracted agonistic display. At this point the two approach exceptionally close for less than 1 s.

move along the log, (downstream at site A), until she joined a group of ovipositing individuals. Courtships after 12:00 h were also often interrupted by other males engaging the male in an agonistic display. Matings were invariably of brief duration, ranging from 48-132 s. After separation the males guarded their mates, perched nearby as they oviposited, for up to 30 min or until interrupted by another female or male. No unguarded ovipositing female was ever seen to mate with another male. It is possible females arriving were intercepted and led back to the male's territory, but if this action occurred, as sometimes appeared to be the case, it was very rapid and difficult to discern with certainty in such small insects.

Mating success

Individual mating success was estimated from 69 matings recorded during study period 2 (Fig. 12). Male mating success was much more variable than that of females. Of the 32 female monitored 27 mated at least once, those not mating presumably having done so before the study period started. Three females mated more than three times although in two cases it is probable that insemination had failed in the first mating which took place less than 15 min before the second. The mean no. of matings per female was 1.77 (s.d. = 1.33). Only 13 of 27 males were seen to mate. Mating success was highly variable, with the maximum number being eight in one individual. The mean number of matings per male was 2.33 (s.d. = 2.83).

Individual patterns of activity and mating

The relationship between general activity patterns and mating patterns can best be appreciated by examining the pattern of residence and mating at Site A, in individuals which returned for at least six days during study period 2. Figure 13 demonstrates this for 13 females and eight males. Owing to the difficulties of individually identifying flying males, it was only possible to record a male's presence or absence at the site and when he mated, so details of courtship activity and agonistic display cannot be provided for individuals.

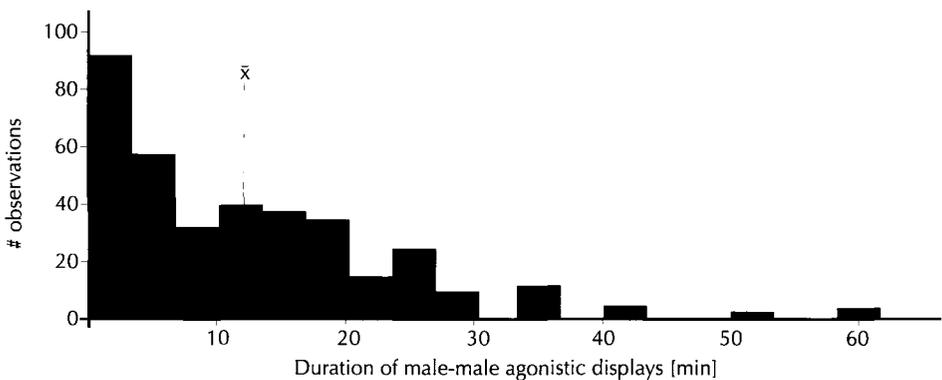


Figure 8: Male agonistic displays in *Libellago semiopaca* — frequency distribution of duration. Mean = 12.6 min, s.d. = 11.8 min. Although long contests up to 60 min occurred, most were briefer than 30 min, but because the period of rest was usually also short, the total time spent in display made up a high proportion of a territorial males' time at certain times of the day.

Table 1. Egg load, sperm load, and food remains present in females of known mating records and behaviour, killed and dissected at point of mating or after refusal of mating. — vF: Virgin females; vFr: Virgin females remated immediately; Fr2: Females remating after 2 or 3 days; vFr5: Females remating for second time after 5 or 6 days; rF: Refractory females, refusing matings; #A-J: dissected individuals.

vF	vFr	Fr2	Fr5	rF
Mature eggs				
#A/620	#C/640	#D/360	#F/120	#H/420
#B/580		#E/280	#G/140	#I/360
				#J/130
Immature eggs				
#A/nil	#C/nil	#D/nil	#F/nil	#H/nil
#B/nil		#E/nil	#G/nil	#I/nil
				#J/nil
Sperm mass in <i>bursa copulatrix</i>				
#A/nil	#C/nil	#D/very depleted	#F/very depleted	#H/bursa full
#B/nil		#E/very depleted	#G/very depleted	#I/bursa full
				#J/bursa full
Food in midgut				
#A/posterior one third full	#C/posterior one half full	#D/posterior one half full	#F/posterior one half full	#H/posterior one half full
#B/empty		#E/posterior one half full	#G/posterior one half full	#I/posterior one half full
				#J/empty

In general, females visited the site every day, with four being absent on single days. It was not known if they were ovipositing at another site, or reproductively inactive. The total length of time spent at the site, mostly ovipositing, ranged from 46-251 min. As a rule females remated every second or third day.

Males were more irregular in their appearance, some visiting the site every day, others on alternate days. Males which remained at the site for several days tended to spend longer at the site than females, ranging from 80-460 min. Every male in this subset of the population achieved at least one mating, and the maximum number of matings on one day was three. One male mated once every one of the seven days he was present. Another achieved the same number of matings in four days.

Patterns of oogenesis and sperm utilization
and feeding indicated from dissected individuals

Table 1 summarises the egg load, sperm load and amount of food in the gut of 10 females (labelled #A-J) dissected either after being separated immediately upon mating (#A-G) or after refusing mating following persistent courtship (#H-J). All individuals carried a load of mature oocytes, each ca 920 μm in length, and elongate, like those of *Calopteryx* species, but larger. No developing oocytes were present in any specimens examined and fat reserves were low. Several had visible remains of prey in the hind half, or less, of the midgut, in the others it was empty. Individuals #A and #B had no trace of sperm in the bursa copulatrix or spermathecae and were believed to be virgin. Individual #C remated almost at once, after her first recorded mating, and also lacked sperm, suggesting she was also a virgin and the male in the first copulation failed to ejaculate. Females remating after 2-3 days always carried a very depleted but obvious sperm mass. The egg counts diminished from over 600, in probable virgins, to less than 150 in older females, suggesting all eggs mature before oviposition commences and the ovaries become steadily depleted, without continuing oogenesis. Of six males collected at the beginning of copulation, all had full seminal vesicles and empty guts. All six males had spent most of the previous day on site. Two had mated the previous day. The average size of the males was given by their hind wing measurement ($\bar{x} = 16.2$ mm, s.d. = 0.2, $n = 6$). This was very similar to a general collection of males from the site ($\bar{x} = 16.0$, s.d. = 0.3, $n = 32$), although the mated individuals were all near or slightly larger than median size. The extent of the fore wing dark marking on mated males ranged from 35% - 40% of the total wing length, and was characteristic of the range of variation within the population.

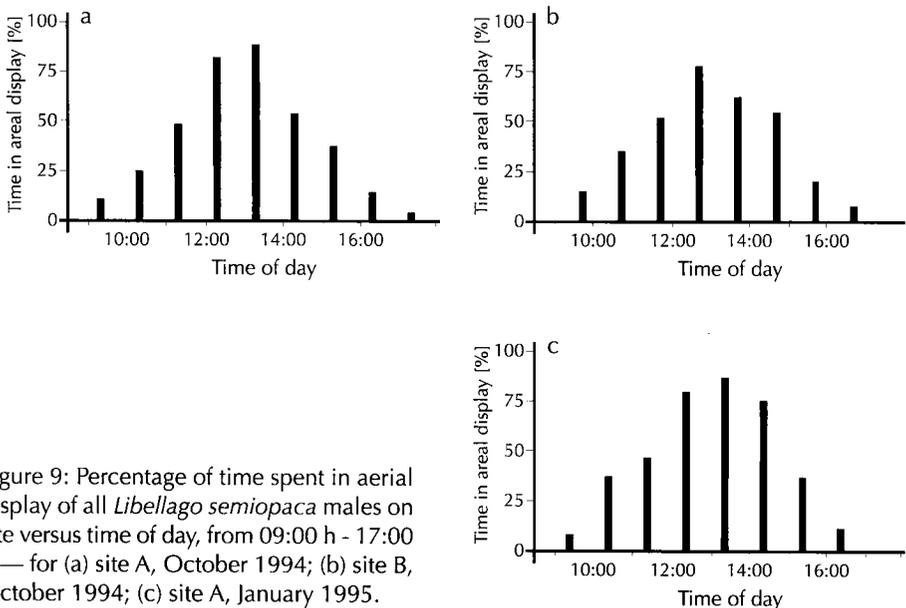


Figure 9: Percentage of time spent in aerial display of all *Libellago semiopaca* males on site versus time of day, from 09:00 h - 17:00 h — for (a) site A, October 1994; (b) site B, October 1994; (c) site A, January 1995.

Oviposition behaviour

Oviposition behaviour was partly monitored during study period 1 at sites A and B, and continually at site A during study period 2. Females arriving to oviposit early most often mated, and oviposited close to the male with which they mated for up to an hour. This was anywhere along ca 15 m length of the log along which males maintained their territories. As the day progressed, females became concentrated; at Site A, this was always at the downstream end of the log, but at site B it was the upstream end. Newly arriving females after 12:00 h typically refused to mate, and as their attempts to oviposit were interrupted by courting males, they tended to be driven along the log to its end where they joined a group of ovipositing females, often including the first females to have arrived and mated, and sometimes still guarded by their original mate perched nearby. In general, at sites A and B before 12:00 h, ovipositing females tended to be dispersed fairly evenly along the streamward side of the log for ca 15 m, whereas after 12:00 h the majority were gathered in a cluster at the end (Fig. 14). About half of all females arriving after 12:00 h were first discovered in the ovipositing aggregation, but there was no direct evidence that they were attracted to the other females or flew directly to the aggregation. Once in the aggregation they were seldom harassed by males, possibly because any courtship attempts were confused by the multiplicity of 'targets'. By 14:00 h almost all females were found within the group. It was in this vicinity also that the most protracted male agonistic displays took place (see above). It was not possible to monitor closely the behaviour of each ovipositing female throughout its bout, but it was observed that oviposition is a protracted process, with the female taking from 1-5 min to insert her ovipositor into the hard wooden substrate. Also many females

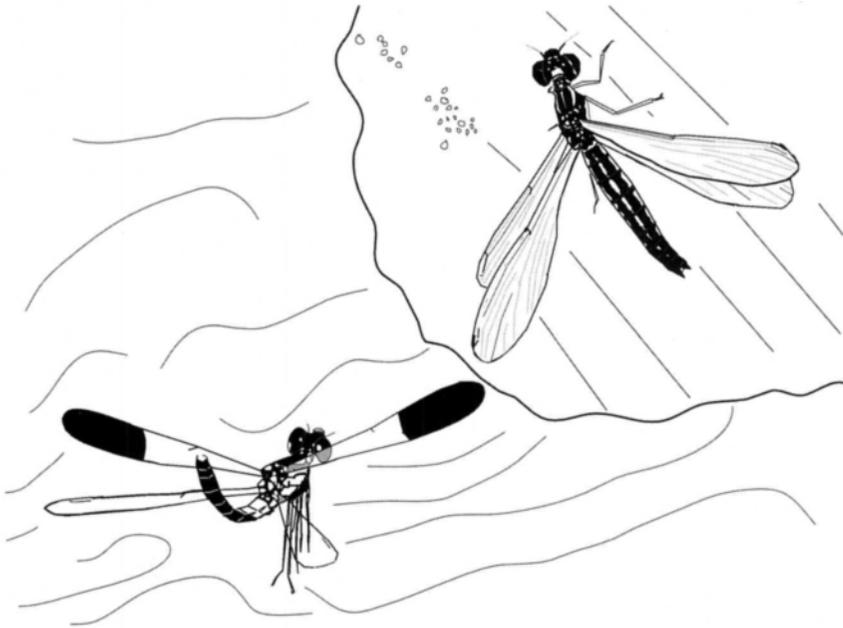


Figure 10: Typical courtship posture of a perched *Libellago semiopaca* female. The male swings to and fro around an arc of ca 180° displaying his fore wing tips, upperside abdomen green colour, and dropped legs. The female here opens her wings partially to indicate refusal.

within aggregations spent periods up to at least 15 min apparently resting, especially towards the end of the day. Of individual females for which records of seven or more consecutive days are available (see Fig. 13), the mean time spent at the site, mainly ovipositing or resting, was 164 min (s.d. = 51 min, $n = 97$).

Female response to presence of males and female decoys

On 18 November 1995, males appeared in substantial numbers following the falling of water levels overnight. By 11:00 h, up to 12 individuals were present at site A, either occupying territories along the log or perched nearby. By this time nine individual males had arrived at site B and had been captured at once. Three females first arrived at Site A at 10:00 h. They did not approach the aggregated decoy females situated at the end of the log, but oviposited within male territories 2-4 m away, guarded by males, suggesting they had mated (unobserved) with territory occupants. By 11:00 h, five females were dispersed along the log, and by 12:00 h, this number had risen to eight, with two females within 10 cm of the decoy females. By 13:00 h, 12 females were present, 10 of which had joined the aggregation, and this pattern continued, with sometimes all females aggregated, and sometimes one or two ovipositing several metres from the group, throughout the afternoon, with activity falling after 14:30 h until 16:30 h when the last female departed. During this period, not a single female was seen to oviposit, or even to be present, at site B, from which males had been removed, despite the presence of decoy females. This pattern continued in a similar way for the next four days (Fig. 14 shows the pattern for 19 November 1995, the second day), with each day two to six males being removed from Site B, and numerous females (up to 15) present at site A. The mean for five days at 12:00 h, 13:00 h, and 14:00h was 12.4 individuals \pm s.d. = 2.6. Only on three occasions were females observed ovipositing at Site B, and on each occasion this was an isolated female 2-4 m from the group of decoys, and only one female stayed more than 30 min, remaining at the site for at least 120 min.

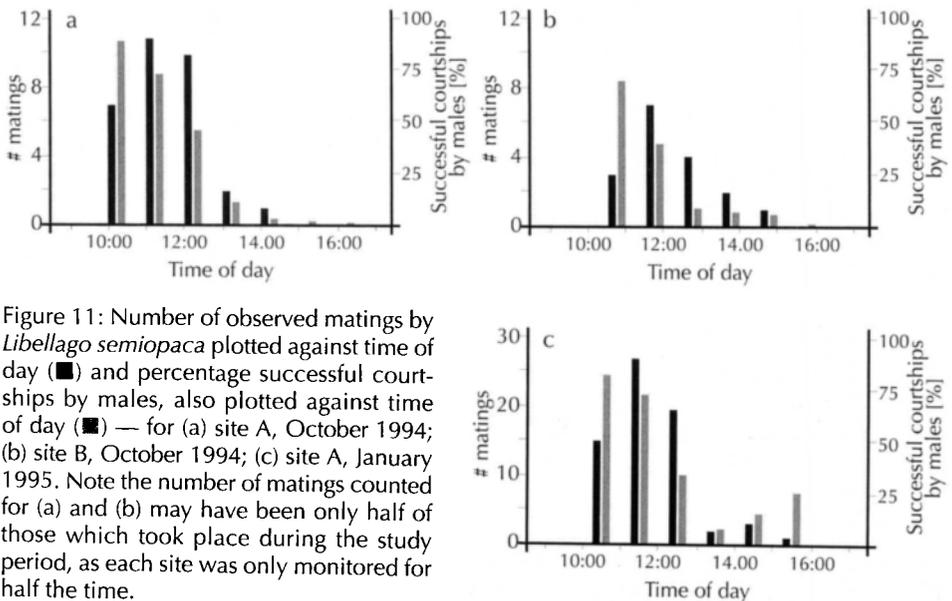


Figure 11: Number of observed matings by *Libellago semiopaca* plotted against time of day (■) and percentage successful courtships by males, also plotted against time of day (■) — for (a) site A, October 1994; (b) site B, October 1994; (c) site A, January 1995. Note the number of matings counted for (a) and (b) may have been only half of those which took place during the study period, as each site was only monitored for half the time.

DISCUSSION

The results from study periods 1 and 2 present a consistent pattern of reproductive behaviour around localized oviposition sites. Males arrive in the morning before females, and establish small territories. Most receptive females arrive before 12:00 h, and most matings take place in the morning. Once the female has mated, she is usually guarded by her mate for 15-30 min, but females normally oviposit for longer periods and, despite intense courtship, mated unguarded females almost never remate for two-three days. Isolated cases of females remating soon after a first mating most probably resulted from inadequate sperm being delivered during the first mating. Although males can easily defend most suitable habitat, mating decisions seem to be largely controlled by the female, and rematings probably result from the need to replenish sperm supplies. Given that the spermatozoa of *Libellago semiopaca* are large (ca 1 mm long; AGO unpubl. data), and the sperm are stored within a relatively small bursa copulatrix, with the spermathecae very reduced, this is plausible, and is supported by dissections of females of known behaviour and mating record. Females arriving at a site occupied by several males can be guaranteed a mating with a proven territory holder, which takes only ca 60 s. In theory, by mating with multiple partners on their own terms, females not only potentially increase the genetic variability of their offspring, but also avoid investment in the long term maintenance of sperm, or in the organs needed to store large quantities of ejaculate. If this is so, it provides an interesting example of female control of male-defended resources, resulting ultimately from the high density achieved by both sexes working in the female's favour, especially as females modulate their behaviour according to their receptivity. Courtship of arriving females becomes increasingly unsuccessful as the day progresses and once a group of ovipositing females is established, it is possible for newly arriving females to join this group, either immediately or as a result of escaping male harassment. In this way they avoid direct attentions from males. On any given day a majority of females do not mate and arrive after 12:00 h.

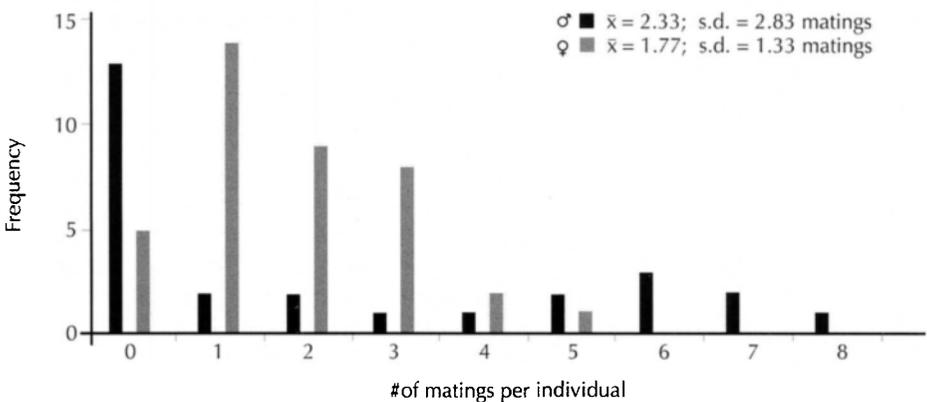


Figure 12: Frequency distribution of number of matings per individual for *Libellago semiopaca* males (■), and females (■), from a total of 69 matings observed during January 1995.

It is evident that the total period of reproductive activity, at least when oviposition sites are continuously accessible, is no more than about 14 days. The data on return rates of marked individuals indicate a maximum figure of 11 days (Fig. 5), but a better estimate must allow some extrapolation beyond this figure. Return rates cannot provide an unbiased estimation of survivorship or even survivorship plus emigration for several reasons. Firstly, for the second study period new individuals were marked throughout the study, creating a bias towards shorter values. Secondly, the insects are very delicate, and it is probable that despite all care, some were damaged or traumatised by capture and marking, in ways that were not immediately obvious. Thirdly the age structure of the population at the commencement of marking was unknown, but if older individuals were marked along with younger individuals, the return rates measured would be skewed towards shorter periods. Immigrating older individuals would also contribute to this bias. Studies on populations of *L. hyalina* Selys (Orr & Cranston 1997) produced comparable estimates for the post reproductive period in that species, suggesting that small equatorial *Libellago* species are generally not long lived. The implication for this study is that in some cases during the second study period, full lifetime records for reproductive activity of individuals of both sexes were probably obtained, but it is generally not possible to be absolutely certain which individuals these were.

Agonistic displays are more protracted than in other species studied by Orr (1996), and distinctly less vigorous, although Günther (2008) reported protracted contests in both *L. semiopaca*, and *L. stigmatizans* (Selys), as well as a pronounced dip in activity in both species around midday, perhaps due to microclimatic factors, which was not evident at Kuala Belalong. Such anomalies emphasise the influence of local conditions on behaviour, and add a note of caution to any adaptive explanation of behaviour within the context of an observed mating system relative to immediate physical characteristics of the environment. Perhaps counter intuitively, the cumulative duration of agonistic displays increases after 12:00 h, at a time when arriving females are less likely to mate. This raises the question of whether these contests really do function solely as agonistic displays, or might they have some other semiotic role within the mating system? In particular it must be asked if such protracted and highly visible displays, with a minimum of overt aggression, are directed towards females, including those already ovipositing at the site and new recruits passing by. It is possible that females ovipositing at the site may be more likely to return there and mate with one of the resident males if males are actively displaying while the females oviposit. This strategy might be beneficial to males if by cooperating in a display, two individuals secure on average more matings each than they would if they were alone in a territory. On the other hand, the rule of "two's company, three's a crowd", may well apply because of the inherent instability of a three cornered contest, but also because in accepting a third protagonist a pair surrender one third of potential matings. The scale of territories defended by *L. semiopaca* meant that up to four such contests could be maintained along the log at Site A at a given time, and such a multiple display might be especially attractive to females, committing them to the site. Given the evidence that *L. semiopaca* is highly sedentary, it is also possible that such a system would be enhanced by kin selection, but kin selection is not essential to maintain the system. There is already good evidence that such a system occurs on some Suluwesi *Rhinocypha* species (Günther 2005, 2007, 2008), and the same principle may also apply in *R. aurofulgens* Laidlaw and *R. cucculata* Selys with their protracted

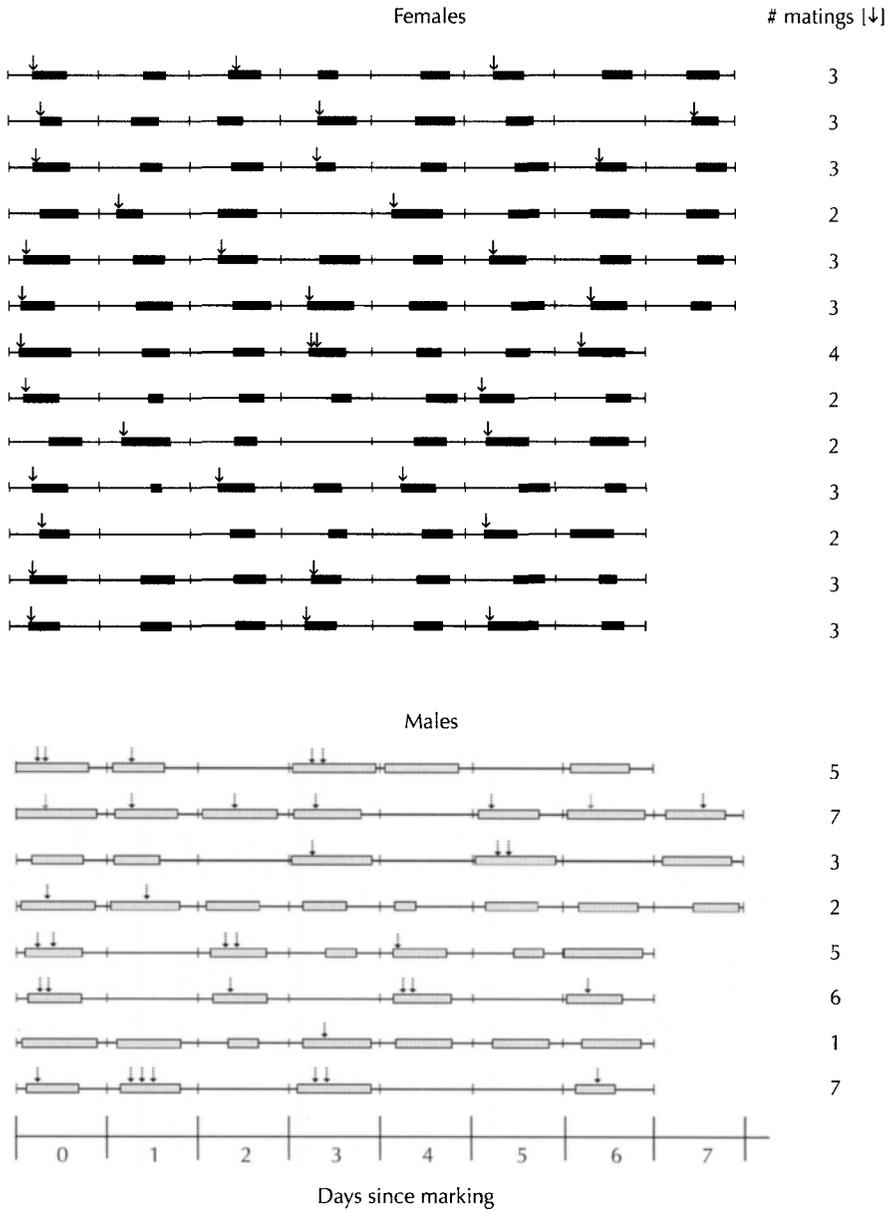


Figure 13: Activity patterns of *Libellago semiopaca* females (■) and males (■) showing time spent at the site on each day for longer residing individuals at site A in January 1995. Each interval represents the observation period for a day, from 09:00 - 17:00 h, and vertical arrows indicate times of mating. Total matings are given in right hand column.

and highly visible displays (Orr 1996). Both species occur at KBFSC, syntopically with, but at much lower densities than *L. semiopaca* (Orr 1996, 2001).

Support for the male display attraction hypothesis is also provided by the fact that once males were removed from Site B, which was known to be normally attractive to females, presumed naïve females virtually ignored the site without males, and those which did visit did not settle and only oviposited briefly, whereas at Site A, no more than 120 m away, females were present in abundance, including many females which almost certainly did not mate before settling to oviposit. Evidently females of *L. semiopaca* respond strongly to the presence of males in assessing the suitability of an oviposition site, and there seems little doubt that males that spend much of their time airborne, displaying to one another, are also highly visible to females arriving after foraging in nearby streamside vegetation. It does of course remain possible that females arriving early in search of matings before oviposition and thus attracted to males, establish groups on a log, and that these all female groups are intrinsically attractive to other later arriving females which do not wish to mate. The fact that static decoys did not attract females may not be significant, if the slight movements of the jostling members of the oviposition pack provide an additional cue necessary to attract other females.

If the male 'agonistic' displays are directed at passing or ovipositing females, as much as at their protagonists, this may partly explain why the fore wings are so heavily pigmented. Such markings would be easily visible to females from any position. It is probable that the fore wings are used in courtship, seemingly more so than in other *Libellago*. If female mating decisions are determined mainly by their long distance attraction to displaying males it might explain why tibial pruinescence, presumably secreted at a high metabolic cost as an indicator of male quality, has been lost in this species. Most other *Libellago* species have just the tips of the fore wings with a dark patch, which is sufficient for two males to gauge each others size, but the small spot probably does not broadcast effectively their presence to passing females. Conversely in most such species pruinose tibia are displayed during courtship. As a note of caution however, when seeking such explanations it should be considered that even though some *Rhinocypha* species which have elaborate wing ornamentation, used in courtship and possibly to attract females from afar by their mutual displays, retain heavily pruinose tibia. On the other hand in *R. aurofulgens*, which courts from a standing position using a vivid wing and body display (Orr 1996), tibial pruinescence is also lost. In the context of the argument advanced above for the loss of pruinescence in *L. semiopaca*, information on encounter rates and male lifetime mating frequencies in such ornamented *Rhinocypha* species, with and without tibial pruinescence, would be of interest.

The mechanism by which females converge to form an aggregation throughout the day is not resolved, although the behaviour is well known in the genus (Orr 1996; Günther 2005, 2008). Martens & Rehfeldt (1989) and Rehfeldt (1989), showed that females of the African chlorocyphid *Platycypha caligata* (Selys) are probably protected from male attentions by joining aggregations. However they also demonstrated that females are directly attracted to aggregations of other females. Female *P. caligata* are however also known to submit to matings by males soon after previous matings, evidently to gain access to high quality oviposition substrate, to which they may be led by males (Robertson 1982a). In *L. semiopaca*, females would seem to have more control over mating decisions, and the pattern of aggregation of females

in this study is consistent with the explanation that they are mainly driven toward these groups of other females, passively by courting males. However the data do not rule out direct attraction to other females, although dead decoy females evidently are ineffective lures. Functionally, the mechanism by which females come to be aggregated is unimportant. If it has the effect of protecting them from disturbance by males, which cannot be disputed (Orr 1996), it will probably be selected for regardless of the mechanism.

The examination of the physiological condition of mating males and females was of necessity perfunctory, and the results are only reported here because they are unusual and showed consistent and clear patterns which may shed light on, and are at least consistent with, observed behaviour, especially that of females.

It is not known at what age females become sexually mature, but in the similarly sized *L. hyalina* experiencing similar climatic conditions this takes place in ca 7-10 days (Orr 2004, unpubl.). It is known that females of both *L. hyalina* and *L. semiopaca* emerge as adults with very immature ovaries (AGO unpubl.), with vitellogenesis just beginning and numerous oocytes present at approximately the same, very underdeveloped, stage. The germaria are probably at this stage still active. It is not certain how ovarian development progresses in *L. hyalina* but immature individuals of both sexes are known to feed several hundred metres from breeding sites, and mature reproductively active females sometimes also return to these sites (Orr 2004). Feeding is hardly ever observed in *L. semiopaca*, and teneral individuals are rarely encountered, so the duration of the prereproductive period and the location of foraging sites must remain open to speculation. However it is evident that females, upon beginning oviposition, evidently do so with a full complement of eggs already matured, unlike most Odonata (Corbet 1999: 37). The egg is relatively large, and the total output is around 600-700 eggs. Bearing in mind the individual investment per egg, this is well within the range of lifetime reproductive output reported for other Odonata by Corbet (1999: 37-41). There was no evidence to suggest females sampled had been prevented from egg laying by high water levels. It is also evident that females feed little during the reproductive period, but they do not abstain entirely. Absences from the oviposition site either in the mornings or in a few cases, for whole days, may enable them to forage. Males are more frequently absent for entire days, and during these periods they are probably feeding to replenish energy supplies following exhausting days spent in agonistic displays. The mated males dissected evidently had not fed for at least a day, which was consistent with their record of attendance at the mating site.

Deferring oviposition until all oocytes are mature is an interesting strategy, particularly for a species which inhabits what is normally regarded as an equitable and predictable environment. However the violent fluctuations in the level of the river may make the availability of oviposition sites less predictable than otherwise. Moreover, given the very hard oviposition substrate, rate of oviposition rather than oogenesis becomes a rate limiting process, and thus females need not only access to exposed logs, but access for several days. These days might be either continuous or broken by periods of bad weather and elevated water levels, in which latter case the longevity of relatively inactive individuals might be prolonged. In such circumstances it may be a better strategy to forage intensively while developing all eggs, and then oviposit intensively when circumstances allow. This would also have the effect of concentrating females, thus allowing them to form the aggregations observed, which

clearly are relevant to their success in determining mating decisions. Further insight into this phenomenon in *L. semiopaca*, which certainly requires verification by larger sample sizes, might be provided by a study of the ovarian dynamics of *L. hyalina*. Females of this swamp-dwelling species generally oviposit in root masses or soft floating debris, and their activities are not curtailed by water level fluctuations. Their egg and body sizes are comparable to those of *L. semiopaca*, but they generally oviposit alone, if possible avoiding males (Orr 1996). A similar pattern of oogenesis in *L. hyalina* to that reported here for *L. semiopaca* would indicate a process likely to be widespread in the genus, and difficult to explain in terms of proximate influences. However, if *L. hyalina* females mature eggs steadily through their oviposition cycles, proximate explanations of the apparently aberrant condition in *L. semiopaca* could reasonably be advanced.

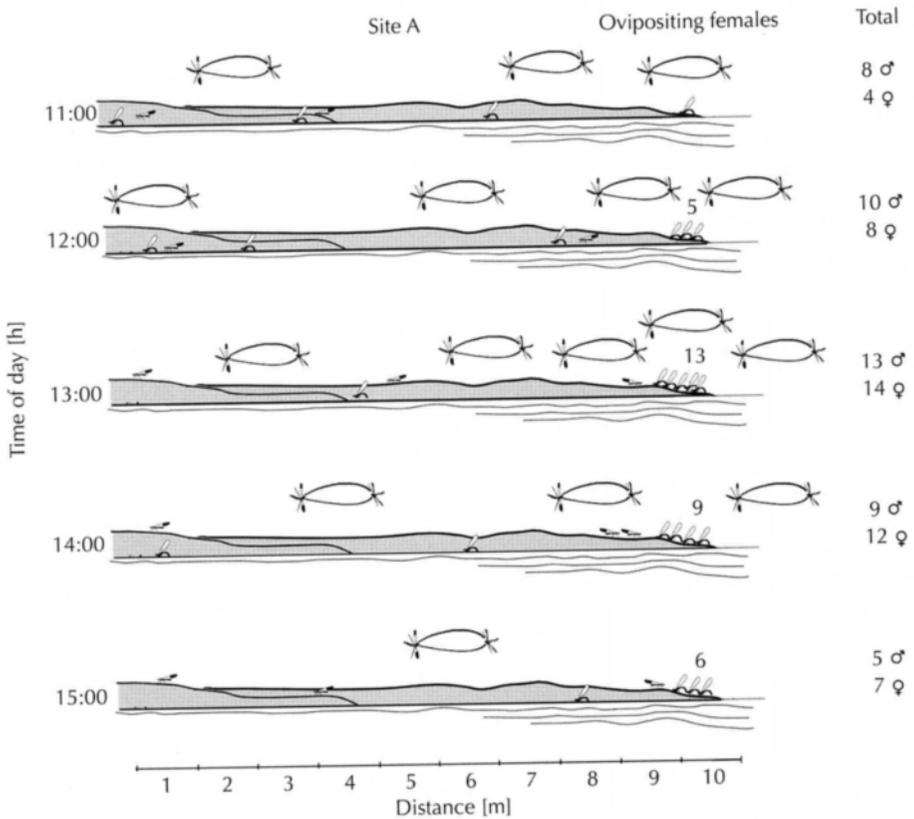


Figure 14: Distribution of *Libellago semiopaca* males and ovipositing females along the log at site A on the hour from 11:00 - 15:00 h on 19 November 1995. Pictographs with curled abdomens represent ovipositing females. Pictographs with straight abdomens or flying represent males. Pictographs are X15 life size relative to habitat. Numbers above female groups indicate number of individuals present in a group. The less favoured end of the log, to the left has been truncated by 5 m for illustrative purposes: total numbers of individuals of males and females refer to total number visible, not including stray individuals outside the area.

On a final note, it should be noted that although male reproductive success in *L. semiopaca* studied here is more variable than that of females, it is perhaps less so than in many other chlorocyphid species. Firm comparative data are not yet available, but it is known that females, even of *L. semiopaca* in some cases (Günther 2008), may mate much more frequently, and this is likely to be associated with greater variation in mating success among males unless they are limited by their capacity to produce sperm. Such information would give the most meaningful comparisons since, apart from being under similar phylogenetic constraints, many chlorocyphids have similar ecologies and mating systems, which are directly comparable with the results presented here. It should be noted however, that the probably reduced male reproductive variance of *L. semiopaca* studied here, together with behavioural data discussed above, suggests greater female control over mating decisions, which may be a key element of this mating system. Therefore if the results are considered in a more general context, *L. semiopaca*, as studied here, probably falls into the category of a 'female control' mating system, as defined by Conrad & Prichard (1992). But it is questionable if it is useful to thus 'shoehorn' the data to fit a general system derived from analyses of systems which have little in common with the present one. By the same criteria, *L. semiopaca* in Peninsula Malaysia studied by Günther (2008) appears to fall into Conrad & Prichard's (1992) category of 'resource control', an entirely different strategy. Similarly Corbet's (1999: 550-554) scheme is of limited heuristic value in this case. Although it is certain that there are morphological and behavioural differences between the *L. semiopaca* population studied here and that studied by Günther (2008), it is also evident that their similarities are more striking than their differences, suggesting that new approaches are needed if we wish to understand these 'exotic' systems in the context of a generalized model odonate mating system.

ACKNOWLEDGEMENTS

It is with particular pleasure that I am able to dedicate this work to the late Philip Corbet. I first met Philip in Essen in August 1995, when I was at work on this project. The stimulating conversations we had, covering the many complexities of chlorocyphid biology, undoubtedly strongly influenced my final experiment, carried out soon after in November 1995. I owe a similar debt to the late Peter Miller, with whom I also discussed the problems of female avoidance on the same occasion, and who opened my eyes to the parallel world of African Chlorocyphidae. Others who have contributed greatly by discussion and/or help with identification and improvement of the text include André Günther, Dagmar Hilfert-Rüppell, Matti Hämäläinen, and Jan van Tol. Joe Pan kindly allowed me to copy his splendid photograph of courtship and rejection in *Libellago semiopaca* taken in Sabah. I owe special thanks to Graham Reels, who provided me with a series of photographs of males in agonistic display, from which several drawings were prepared, as well as offering stunning portraits of male and female.

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