DISTURBANCE BY UNPAIRED MALES PROLONGS POSTCOPULATORY GUARDING DURATION IN THE DAMSELFLY LESTES VIRENS (CHARPENTIER) (ZYGOPTERA: LESTIDAE)

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In L. virens, the tandem post-copulatory guarding varies from some minutes to more than 4 hours and appears correlated to the time of the day and disturbance by unpaired ♂ ♂. Using a multiple regression analysis, with guarding duration as the dependent variable and time of day, temperature and disturbance as the independent variables, it is shown that only disturbance significantly explains the model. An experimental test, in which early-occurring tandem males were not disturbed, while late-occurring ones were disturbed (a reverse situation of what happens in the field), showed that the latter kept their ovipositing ♀ ♀ for significantly longer times than the former. The capability of ♂ ♂ of varying guarding duration accordingly to the density of solitary ♂ ♂ allows them to invest more or less time for guarding, according to the actual risk of losing sperm precedence.

INTRODUCTION

During copulation, males of many dragonfly species remove or displace stored sperm from the female’s storage organs and then place their own sperm to gain sperm precedence (CORBET, 1999). To make sperm precedence more effective, males perform non-contact or in-tandem post-copulatory guarding, in the latter case by holding females by their abdominal claspers, as in L. virens (UTZERI et al., 1987). Tandem guarding is particularly effective, since clasped females can hardly be taken over by rivals (CORBET, 1999), and males can inseminate the majority of their eggs. However this behaviour can carry heavy costs for males, because guarding can last several hours, during which the males cannot feed or remate and are probably more vulnerable to predators (WAAGE, 1984; CONVEY, 1989). Furthermore, the in-tandem flight may be energetically more expensive (SINGER, 1987). Since tandem guarding bears important benefits
and costs, it is probable that natural selection has favoured some mechanisms by which, in contingent situations, the benefits balance the sexual costs.

In many dragonflies, post-copulatory guarding duration is highly variable. For some libellulids there is a statistical (McMILLAN, 1991; CONVEY, 1989) or observational (MARTENS, 1991) evidence that guarding is more intense or prolonged if the guarding male interacts aggressively with other males. For the Zygoptera there are some analogous observations (THOMPSON, 1990; UTZERI et al., 1987; UTZERI & SORCE, 1988; CORDERO et al., 1995; review by SIMMONS, 2001). In particular, in *Lestes virens* (and *L. barbarus*) a negative correlation was recorded between post-copulatory guarding duration and time of the day, so that the function of the prolonged guarding was suggested as the way to release females at times when most of the unpaired males have left the mating place, so reducing the risk for guarders of losing sperm precedence due to female’s remating (UTZERI & SORCE, 1988). However so far, in the Zygoptera, evidence that male disturbance can affect guarding (and copulation) duration has been demonstrated only for *Coenagrion scitulum* (CORDERO et al., 1995).

In *L. virens*, the possible effect of male disturbance can also be experimentally investigated: males use a post-copulatory guarding which is highly variable in time (up to 261 min; UTZERI et al. 1987) and males and females can be induced to perform “normal” sexual behaviour while in insectaries. Here we present observations and experiments on a population of this damselfly (subsp. *vestalis* Rambur, 1842) with particular reference to the relationships between male density, disturbance to tandem males by unpaired males, ambient temperature and time of the day.

**METHODS**

The research was carried out during August-September 1989, 1991, 1993 and 1994, at Castel Porziano, Rome, Italy, at a permanent pond of about 30 m in diameter, when fully flooded. The peripheral belt of the pond was richly covered by rushes (*Juncus effusus*), which is mainly used by *L. virens* during oviposition (cf. UTZERI et al., 1987). Most emergence took place between the second half of May and the first half of June, and reproductive activities from the beginning of August to late October (cf. UTZERI et al., 1988).

**COLLECTION OF FIELD DATA.** During the reproductive period in 1989, we individually marked 600 males and 188 females, by placing one or more enamel colour spots on their wings. We then recorded almost daily all successive sightings and matings of these individuals in the field.

Twenty-seven field matings and post-copulatory tands were continuously timed, of which 20 were ended by males. We considered the tandem as being ended by the male when the female never showed any movement which could be interpreted by the observer as attempts to break free from the male (e.g. eyes cleaning, turning the head from side to side, holding on to the perch while the male was attempting to shift, attempts to fly while the male was still perching, and wriggling). We recorded the starting time, guarding duration, male density at the pond and all disturbance actions to guarding males by unpaired males. Male density was recorded as low (i.e. less than 10 males all over the pond, which was relative to early reproductive season or cloudy days) or high (i.e. more than 10 males, actually more than 30, which was relative to sunny days in full reproductive season). To assess the degree of disturbance to tandem males relative to the abundance of solitary males, we recorded all solitary males that were present, or flew, within 1 m from in-tandem pairs, of which we also recorded any eventual disturbance. Disturbance was recorded when tandem males reacted to approaching unpaired males by either displaying their wings (cf. below) or moving to another site (dragging their female
mating). Ambient temperature was recorded at the beginning and at the end of each guarding bout.

EXPERIMENTS IN THE INSECTARY. — In 1991 and 1993 we carried out experiments in a 2x2x2 m nylon net insectary, that was mounted daily in the field over and around some rushes, in order to supply damselflies with a suitable oviposition substrate.

Experiment I — Several males and females were put into the insectary and then, after one or more tandems were formed, all unpaired individuals were removed. During oviposition, the observer(s) disturbed these tandem males one or more times at regular intervals of about 5 min apart, by means of approaching to, and then retracting from, them a conspecific flying male tied at its abdomen by a thin cotton thread, a modified ST. QUENTIN's (1934) "fishing line technique". For each tandem male, the number of reactions to the tethered male and guarding duration were recorded. Temperature was also recorded. Tandem males in the insectary reacted to the tethered male in the same way as tandem males in the field to solitary males, except that the former could not flee. After tandem release, both members of each pair were marked and released, to avoid using the same individuals more than once.

Experiment II — Since in this species, males that start mating earlier guard their females for longer, and vice versa (UTZERI et al., 1987; UTZERI & SORCE, 1988), and guarding duration is positively correlated with disturbance (cf. below), we set up the following experiment, to separate the effect of disturbance from that of other factors depending on time of the day (as e.g. temperature). For this, 8 tandem males whose females began ovipositing between 11:30 hr and 11:53 hr were allowed to guard their females undisturbed, while 8 tandem males whose females began ovipositing between 12:01 hr and 12:51 hr were disturbed 6-33 times by the preceding technique. This experiment is the reverse of what happens under normal field conditions, where earlier pairs probably undergo more disturbance (and vice versa) (cf. below). If disturbance causes a prolonged guarding, it should produce the same effect at any time in the day.

To assess the degree of disturbance to tandem males in relation to the abundance of solitary males, in the insectary, in different sessions, we put 5 pairs either alone or together with 10 or 15 unpaired males and then we recorded all disturbance actions and guarding durations. All these sessions were set up between 10:38 hr and 11:35 hr.

RESULTS

DEMOGRAPHIC NOTES

In 1989, the first mating was observed on 7 August. Males arrived at the pond some days earlier than females. 285 out of 600 males (47.5%) and 63 out of 188 females (33.5%) were recorded at the pond on at least another day besides the marking day ($\chi^2 = 10.77; P < 0.01$). This much lower recapture rate of females during the reproductive period brought the male-to-female ratio from 3.2:1 (at marking) to 4.5:1 (at resighting), and the ratio between those males and females that attended the pond on a given day was always over 2:1.

A sample of 90 males, which were marked between July 21 and August 6, 1989, and recorded at the pond at least once after the marking day, averaged 19.1 days of mature adult life, from the first mating recorded (August 7) and the last recording day of each male.

The breeding population was at the pond roughly between 10 hrs and 15 hrs, the individual numbers increasing from 10:00 hr to 13:30 hr and thereafter decreasing, similarly to UTZERI & SORCE's (1988) report. The males usually arrived at the pond before females, and the latter were immediately seized. Normally after 15:00 hr, most individuals had left the pond, but some females went on ovipositing alone.
REPRODUCTIVE BEHAVIOUR

The behaviour of *L. virens* was described by UTZERI et al. (1987). As a reference point, we outline here some behavioural events, as recorded in the field during the present investigation. The unpaired males frequently interacted, chasing or facing each other according to the patterns reported in UTZERI et al. (1987). Males reacted to other males that were as far as 1.5 m away. Individual reactions to approaching or passing males were frequent, since males did not seem to distinguish sexes and attempted to form tandems with any lestid damselfly. After some minutes after seizing a female, the copulation occurred and lasted from between 5-31 min. Then the female, with her male still in tandem (post-copulatory guarding) laid eggs. Both copulation and oviposition could take place in the same site, or tandems could shift one or more times either to choose adequate substrates into which to lay or to escape harassment by solitary males. These males regularly approached pairs, sometimes attempting to seize either males or females (in the latter case, however, without succeeding in taking them over). In most cases, seizure was prevented by the wing display performed by one or both partners (BICK & BICK's [1963] "wing warning"), which induced the disturber(s) to retreat, without interrupting oviposition, but sometimes, the pairs shifted in search of a less disturbed place, in the event interrupting genital contact (when in copula), but never breaking the tandem position. Each shifting did not cause the interruption of oviposition for longer than a few seconds. In the field, disturbance to tandem pairs was directly cor-

![Fig. 1. Correlation between density of unpaired males and amount of disturbance to tandem pairs in the field (N = 27; r = 0.81; P < 0.001) (excluding the outlier: N = 26; r = 0.58; P < 0.01).](image1)

![Fig. 2. Correlation between post-copulatory guarding starting time and duration in the field (N = 8; r = - 0.78; P < 0.02).](image2)
related to the number of nearby (i.e. flying within the distance of 1 m) unpaired males (N = 27; r = 0.81; P < 0.001) (without the outlier: N = 26; r = 0.58; P < 0.01) (Fig. 1).

Eggs were mostly inserted into rush stems. Twenty-seven pairs were observed from mating to post-copulatory guarding. One of these tandems was accidentally interrupted by the observer. In 20 out of the remaining 26 pairs, the male released the female spontaneously (cf methods). Only these 20 observations were used in the correlation test between tandem duration and disturbance by unpaired males. Usually after separation, the females either left or went on ovipositing alone, sometimes up to 18:00 hr. None of the 5 females that laid alone after release remated the same day, even though some mating pairs were recorded late in the day.

In the field, post-copulatory guarding lasted 11-132 min (x = 49.1 ± 7.9; N = 20). At high population density, as in the middle of the reproductive season, the earlier guarding started in the day the longer it lasted (N = 8; r = -0.78; P < 0.02) (Fig. 2). This correlation did not exist at low population density, as early in the reproductive season, or on cloudy days (N = 12; r = 0.13; P > 0.10).

Guarding duration was positively correlated with the amount of disturbance to tandem males by unpaired males (N = 20; r = 0.83; P < 0.001; number of disturbances between 0 - 20) (equation line: y = -0.395x^2 + 13.135x + 27.673; R^2 = 0.79).

A multiple regression analysis, with guarding duration as the dependent variable and time of day, amount of disturbance and average ambient temperature during guarding as the

<table>
<thead>
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<th>Independent variable</th>
<th>Coefficient</th>
<th>Std. error</th>
<th>t-value</th>
<th>P =</th>
</tr>
</thead>
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<tr>
<td>Constant</td>
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<td>5.25</td>
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<td>Temperature</td>
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<td>1.86</td>
<td>-0.67</td>
<td>0.5120</td>
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</table>
independent variables, showed that only disturbance explains the model significantly (P < 0.001) (Tab. I).

OBSERVATIONS IN THE INSECTARY

In the insectary the amount of disturbance also increased with solitary male density (Tab. II). Post-copulatory guarding varied between 15 and 195 min and was positively correlated with disturbance (N = 20; r = 0.76; P < 0.001; no. of disturbances 0-43) (Fig. 4). Guarding duration was also correlated with starting time (r = 0.56; P < 0.02). However, the duration of 15 selected post-copulatory tandems, which were disturbed independent of the time of the day, was not correlated to starting time (r = -0.19; P > 0.10) (Fig. 5).

A multiple regression analysis, with guarding duration as the dependent variable and starting time, amount of disturbance and average ambient temperature during guarding as independent variables, showed that both disturbance and starting time explain the model significantly (P < 0.001 and P < 0.025 respectively) (Tab. III).

In the experiment designed to assess the relationship between male density and amount of disturbance (cf. Tab. III), some tandem males, which were kept together with several unpaired males, experienced an
intense disturbance, and were frequently struck by the latter to such an extent, that they were forced to release their females. Excluding these tandems, that did not end spontaneously, the average duration of undisturbed guarding (N = 15; \( \bar{x} = 35.7 \) min; R = 2-83) was significantly shorter than that of disturbed guarding (N = 5; \( \bar{x} = 80.5 \) min; R = 53-122) (t = 3.98; P < 0.005).

The average duration of earlier undisturbed guarding (oviposition starting time between 10:43-11:53 hr) (N = 13; \( \bar{x} = 35.5 \) min; range = 2-83) was significantly shorter than that of later disturbed guarding (oviposition starting time 12:01-12:37 hr) (N = 5; \( \bar{x} = 80.8 \) min; range = 53-122) (t = 3.69; P < 0.005).

**DISCUSSION**

Sperm displacement is widespread in dragonflies, although among lestids it has only been demonstrated in *Lestes vigilax* (WAAGE, 1982). However, in *L. virens* copulation, the presence of stages (UTZERI et al., 1987) similar to those in *Enallagma cyathigerum* (Coenagrionidae), where sperm removal and insemination respectively occur (MILLER & MILLER, 1981), as well as a prolonged post-copulatory tandem guarding, make it reasonable to assume that sperm competition is also present in *L. virens*. Post-copulatory guarding duration in *L. virens* is widely variable; sometimes the tandem release is promoted by the female that can loosen to the male’s grip, but more often males release their females spontaneously. For several lestids, a further copulation of the same female in the same day was reported by LOIBL (1958) and UTZERI et al. (1987) and it is possible that occasionally it also occurs in *L. virens*, although in our observations, none of the females that were released before ending oviposition mated again in the same

**Table III**

Model fitting results of multiple regression of guarding duration in the insectary (dependent variable) against starting time, disturbance and temperature

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient</th>
<th>Std. error</th>
<th>t-value</th>
<th>P =</th>
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<td>Disturbance</td>
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<td>0.53</td>
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<tr>
<td>Temperature</td>
<td>2.71</td>
<td>6.36</td>
<td>0.43</td>
<td>0.6758</td>
</tr>
</tbody>
</table>
day. For egg fertilisation, this means that either long- or short-lasting guardings might be adaptive.

The earlier the males mate, the longer they guard their females, and guarding duration is positively correlated to disturbance. Such a correlation per se does not prove that either guarding is prolonged as a consequence of disturbance or that males that perform longer guarding undergo more disturbance simply because their guarding is longer. However, the following observations and experimental results let us assume that disturbance is the main cause of longer guarding: (1) in the field, guarding duration is correlated to time of the day only at high population density. Probably, at low density, males are unevenly distributed across the pond throughout the day and disturbance is constantly of a low intensity. (2) In the insectary, guarding duration was correlated to both disturbance and time of day. However, the latter is probably an experimental artefact, since no correlation to time of the day exists for the 15 pairs for which the disturbance pattern, relative to time of the day, was the opposite of the natural pattern (i.e. less disturbance was given to earlier tandems and vice versa). (3) Also in the insectary, the males of earlier tandems, which were not disturbed, guarded their females for significantly shorter times than those of later tandems, which were disturbed, that is again the opposite of what occurs in the field. (4) In a relatively short time span, the undisturbed tandem males in the insectary released their females after a significantly shorter time compared to the tandem males that were disturbed by a double or triple number of solitary males. And (5) neither in the field nor in the insectary, was there any significant correlation between guarding duration and temperature.

The negative correlation of guarding duration to guarding starting time, which is observed in the field (UTZERI et al., 1987; UTZERI & SORCE, 1988; this paper), is consistent with the effect of disturbance on guarding duration. Since at the pond, the population increases from the morning to the early afternoon, the males that mate earlier usually undergo more disturbance, which is in fact correlated to the number of unpaired males (Fig. 1). The reverse is true for males that mate later, when the number of solitary males, and disturbance, decrease. Interestingly, correlation to starting time (negative) and male disturbance (positive), with the latter explaining most of variance, was also evidenced for pre-copulatory guarding and copulation duration in Coenagrion scitulum (Coenagrionidae) (CORDERO et al., 1995; in part observed also by UTZERI & SORCE, 1988).

Guarding males may perceive the presence of unpaired males and tandems around them, as some of their behaviours (wing display, shifting from perches) suggest. ERCOLI & UTZERI (1992) showed that tandem males more frequently react to approaching solitary males (potential rivals) than to tandems (non-rivals). But even though actual disturbance is lowered by means of the wing display or by shifting to other places, apparently the interactions between guarders and their rivals stimulate the former to firmly grab their females for at least a period of time after disturbance has ended. But although the tendency to keep the female is probably activated by disturbance, the regression line which better fits the data set of Figure 3, suggests that an upper limit of
guarding duration might exist.

Relative to the same amount of disturbance, we recorded a wide variation in guarding duration. For instance, in the insectary, undisturbed males kept their females for times between 15 min and 72 min, while two tandems, which were disturbed 11 times, lasted 77 min and 128 min. The same can be seen in the field sample, although differences are not so wide. Also, undisturbed guarders in the insectary (N = 18 in two experimental setups, cf. above) kept their females for an average 65.2 min, which is more than four times the shorter guarding duration of an undisturbed male (15 min). The influence of captivity on guarding duration was sometimes evident, since after copulation, tandem males could spend some time attempting to cross the insectary walls, thus delaying oviposition. Further research might explain whether a longer guarding duration can be determined by disturbance experienced before guarding, as e.g. during copulation or even before mating, and/or if disturbance causes different effects according to whether a longer or shorter time has passed between when a male experienced disturbance and when he started guarding. Also, a different distribution pattern of disturbance (viz. if the same amount is distributed in a shorter or longer time) might cause a different guarding duration. Moreover, while we have assumed as disturbed only those males that performed the wing display or flew away, we cannot rule out that simply the presence of solitary males, as far as guarders can perceive them, can influence guarding duration. Finally, the individual reaction capability, either as genetically determined or age-dependent, as well as other unknown factors, might also play a role. The fact that males which mated earlier in the season, which probably experienced less disturbance, guarded for a shorter time compared to those that mated later, could be just a hint for the possibility that disturbance undergone during the days preceding the mating day may play his role in increasing guarding duration.

THORNHILL & ALCOCK (1983) have pointed out that when females are rare, and intra-male competition is high, it is convenient for males to mate as soon as possible and, in case females are not soon receptive, to pay the cost of a prolonged pre-copulatory guarding. In *L. virens*, the high male:female ratio gave a low probability of a male to finding a female, but females soon accepted copulation and males paid the cost of a prolonged post-copulatory guarding. However, males may balance the guarding costs with the probable risk of losing sperm precedence, by their capability of assessing, through the experience of disturbance, the density of their rivals. This capability is particularly advantageous for the males. In fact, if a male mates late in the day, he will guard his female for a short time. Indeed, if the female has not yet completed oviposition at release, she will probably go on with unguarded egg laying, but the risk for the male that she is recaptured will be low, since at this time most males have left the pond, and his sperm precedence will be probably secured. On the other hand, if that female has already oviposited earlier that day, and preserves only a few eggs to fertilise, the male will inseminate just a few eggs, and he will also invest only a little energy in a short guarding. Females ovipositing early in the morning, having not completed oviposition the preceding day (UTZERI et al., 1987), are expected to preserve a small number of eggs. If a male
happens to mate with one such female, he would probably perform a prolonged post-copulatory guarding. Females however, are quite able to rid themselves of the male and probably do this upon exhaustion of their eggs. This is of course an advantage for the female but also for the male, that avoids the cost of further, useless guarding.

McMILLAN (1991) has shown that in Plathemis lydia, (non-contact) guarding is less successful when there are many rival males nearby. This was not recorded in L. virens, probably because tandem guarding is more efficient than non-contact guarding. In the insectary, when tandems were harassed by many males, some males were forced to release their females in fact, but this was probably due to their confined conditions, which prevented them from retreating from excessive disturbance.

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