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NOTES ON THE REPRODUCTIVE BIOLOGY OF *ZYXOMMA PETIOLATUM* RAMBUR IN INDIA (ANISOPTERA: LIBELLULIDAE)

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Some aspects of the reproductive behaviour and genital structures of *Z. petiolatum* are described from observations made at Madurai in southern India. Sexually active males patrolled continuously over small permanent pools from about 45 min before to 10-15 min after sunset, interacting aggressively with other males. Copulation was brief and aerial, and it was immediately followed by guarded epiphytic oviposition. A few immature individuals commonly appeared in flight at the water from about 10 min after sunset, remaining there until after the departure of all sexually active dragonflies. — Females possess extended and cleft sub-genital plates and males have correspondingly elongated hamules. Internally females are equipped with a relatively small bursa and a pair of spermathecae supplied through a T-shaped duct. The penis bears a pair of partially inflatable cornua and a bifurcated flagellum. Each branch of the flagellum terminates in a large hook and has a helically twisted membrane running along its outer margin. Some functional interpretations of these structures are discussed. The behaviour and morphology resemble those of *Tholymis tillarga* which occurs in the same region, but the species do not normally share the same habitats.

INTRODUCTION

Zyxomma petiolatum is a common insect in India and in parts of SE Asia, and it is sexually active at small pools during crepuscular periods (FRASER, 1924; LIEFTINCK, 1954; KUMAR, 1972, 1985; RAM et al., 1983). Its reproductive behaviour and genital structures share a number of features in common with those of *Tholymis tillarga* (Fabr.): for example sexually active males patrol continuously without perching, aerial copulations are brief and females oviposit epiphytically. The morphology of the secondary genitalia of the males is similar in each species (MILLER, 1988) and marked resemblances occur also in the genital structures of the females. Although both species may occur in the same regions they do not normally share the same habitats and it is therefore of interest to see if their differing ecological preferences are reflected in other aspects of their biology.

LOCALITY AND MATERIAL

Z. petiolatum was observed during October-December, 1987, and also in August-September, 1990, at several small permanent ponds varying in size from 1 to 10 m diameter, and also over deep storage tanks where the water level was about 7 m below ground level, on the campus of Madurai Kamaraj University (9°58'N, 78°07'E). Its behaviour was closely examined on 10 evenings during late August, 1990 (sunset at approximately 18:30 h) at a round ornamental pond 10 m in diameter, surrounded by trees and in which waterlilies and submerged vegetation were abundant. Specimens were caught and stored in 2% formaldehyde for later dissection. They were prepared for scanning electron-microscopy as previously described (MILLER, 1982). The species shows little sexual dimorphism, the thorax and abdomen of both sexes being dull brown in colour, the wings tinged with brown and the eyes very large and green. The abdomen is exceptionally thin except for the swollen second and third segments, a feature which may perhaps enhance manoeuvrability. In contrast, *T. tillarga* which was abundant at a large temporary pond on the campus, shows marked sexual dimorphism and the abdomen is stouter (HAMADA & INOUE, 1985).

REPRODUCTIVE BEHAVIOUR

Males of *Z. petiolatum* usually started to patrol the pond ca 45 min before sunset though they have been seen to do so at other more densely shaded sites as much as 2 h beforehand. Up to three males at one time appeared, one being continuously present and the others being seen intermittently. They flew rapidly and continuously 10-20 cm above the water with many brief bouts of hovering, and they made occasional short excursions over land, quickly returning to the water. Intense male-male conflicts were common but they usually lasted only a few seconds. Male activity ceased 10-18 min after sunset, by when individuals were difficult to see without a torch.

Though females might arrive at any time during the period of male activity, most copulations were observed shortly after sunset. Aerial copulations lasted 10-20 s and were usually followed immediately by oviposition, with the male guarding closely. Ovipositing females hovered above a piece of floating vegetation, commonly a reed or a waterlily stem, periodically dipping down to the vegetation and releasing a batch of eggs. They did so either continually from one direction or alternately from opposite directions, as in *T. tillarga* (MILLER & MILLER, 1985). Collection of the vegetation enabled the numbers of eggs per batch to be counted. In some cases, egg batches had been piled on top of one another and no count was possible, but usually the batches remained discrete. Eggs were arranged irregularly within a batch and there was a mean of 9.48 ± 1.31 (s.e.; n = 29) per batch.

On several evenings 10-20 min after sunset, a few immature males and females were observed to hover and fly very slowly about 1 m above the water surface.

Their identity was confirmed after capture. Patrolling males ignored them or made only very brief investigative flights towards them. Some immatures remained at the water for several min after the departure of all sexually active individuals.

MORPHOLOGICAL FEATURES

The eggs of *Z. petiolatum* and *T. tillarga* are unusually elongate, as was noted by LIEFTINCK (1931), being about 730x300 μm and 610x290 μm respectively. In both species the shell has a complex trabeculate structure and the eggs are firmly glued to vegetation at the water surface by spumaline.

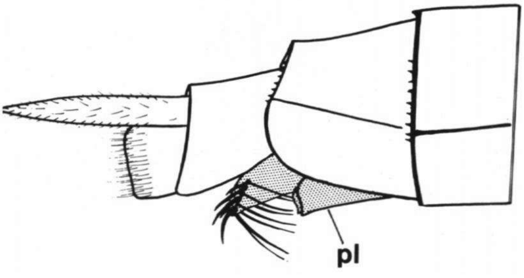

Fig. 1. Lateral view of the posterior abdominal segments of a female *Z. petiolatum*, showing the 9th sternite with its posterior bristles and the sub-genital plate (pl), hatched.

Female *Z. petiolatum* possess an extended sub-genital plate (valvula vulvae) which is about three-quarters of the length of the 9th sternite. It is divided by a medial slot or cleft ca 800 μm long within which the penis can ridge during copulation. The posterior border of the 9th sternite bears a set of stout bristles which enclose a space between the sub-genital plate and the 9th sternite within which the egg batch accumulates after the eggs leave the genital opening (Fig. 1). Six to eight bristles, 200-250 μm long and projecting postero-ventrally, alternate with another 8 bristles which are directed antero-ventrally and are up to 450 μm in length. In addition about 8 small, possibly sensory, bristles are set among the large ones. The bristles trap the egg batch until the female dips down and makes contact with a piece of floating vegetation, whereupon the 9th segment is bent upwards and the eggs are released.

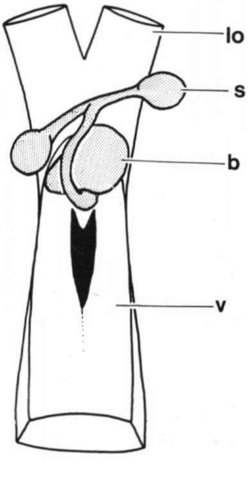

Fig. 2. Dorsal view of the internal reproductive organs of a female *Z. petiolatum*, showing the bursa (b) and the spermathecae (s), both hatched, and the lateral oviduct (lo) and vagina (v). Note that the common spermathecal duct passes to the left of the bursa.

Internally the female genitalia include a bursa and a pair of muscular spermathecae joined to the bursa copulatrix via a T-shaped duct, structures similar in size and shape to those of *T. tillarga* (Fig. 2). However in *Z. petiolatum* the common duct runs to the left of the bursa, joining the branch to the left spermatheca at an acute angle but running smoothly into the right branch (confirmed in mature and immature individuals), very much as described in *Brachythemis lacustris* (MILLER, 1982).

The male genitalia differ from those of *T. tillarga* principally in the structure of the flagellum. The 4th segment of the penis is about 1200 μm long and it contains a pair of cornua and a bifurcated flagellum with each branch being about 660 μm long and ending in a stout hook. Scanning electron-micrographs show a complex membrane, apparently helically twisted and in places double, attached to each

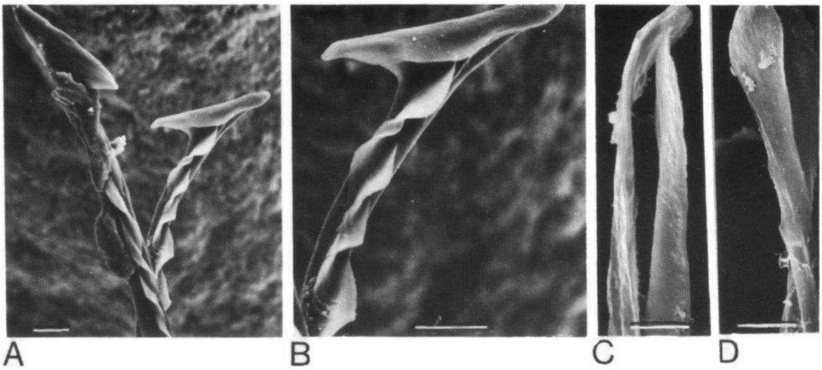

Fig. 3. Scanning electron-micrographs of the tips of the flagellar branches of: (A-B) *Z. petiolatum* [scale bars = 10 μm], and (C-D) *T. tillarga* [C, scale bar = 10 μm; — D, scale bar = 20 μm].

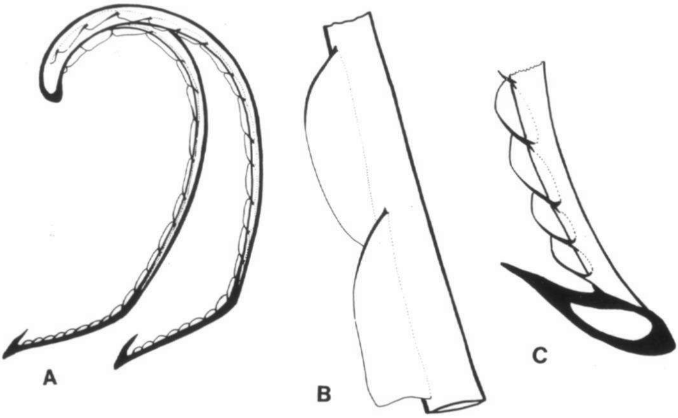

Fig. 4. The bifurcated flagellum from the penis of a male *Z. petiolatum*: (A) the whole flagellum; — (B) part of a branch near its base; — (C) the terminal region.

flagellar branch along its outer margin. In the proximal region the membrane extends laterally by up to 30 μm but in the distal third the membrane is narrower and more tightly twisted (Fig. 3). Light microscopy suggests that each twist of the membrane is held out laterally on a thickened rib (Fig. 4).

The partially inflatable cornua are slightly broadened distally and are covered with spines about 10 μm long. Most spines point distally but in the most distal region they are directed proximally (Fig. 5).

The hamules have a long inner branch and the total length of each is over 1 mm — sufficient to allow them to engage with a pair of proximally placed knotches on the dorsal side of the female's sub-genital plate. The hamuli of *T. tillarga* are maximally only about 500 μm long, which corresponds to the much smaller sub-genital plate of the female in that species.

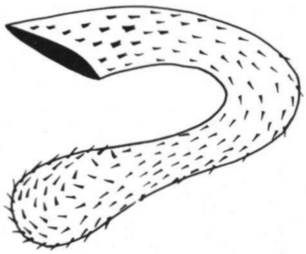

Fig. 5. One cornu from the penis of *Z. petiolatum*.

DISCUSSION

CLASSIFICATION

Z. petiolatum belongs to the tribe Zyxommatini which also includes the genera *Tholymis, Parazyxomma* and *Zyxommoides*. Similarities of *Tholymis* with *Parazyxomma*, and also with *Brachythemis* have previously been discussed (MILLER, 1988).

BEHAVIOURAL AND ECOLOGICAL CONSIDERATIONS

Z. petiolatum and *T. tillarga* are both active during crepuscular periods until well after sunset and they spend much of the remainder of the day resting among dense vegetation. They may become more localised at the water as the light intensity wains and when the density increases (MILLER & MILLER, 1985). Crepuscular activity may be advantageous in that it allows some dragonflies to be active at reproductive sites when few other species are present, and it may permit males to patrol continuously and thereby command extensive search areas with less risk of overheating than would occur in sunlight. It may however be harder for males to detect females in dim light, and previously mated females can choose to oviposit at other times when males are absent.

Both species make aerial copulations of similar duration and females oviposit epiphytically in the same way. Males guard ovipositing females closely, suggesting that sperm competition is a feature of the species and that males gain sperm precedence atleast for a short period after mating, as in some other libellulids

(WAAGE, 1986). Inspite of behavioural similarities, however, the two species exploited different habitats at Madurai with little or no overlap. *Z. petiolatum* was active only at small, permanent pools which possessed much surface vegetation. In contrast *T. tillarga* was active mainly at a much larger temporary pond which was formed during the monsoon, although it occasionally also appeared at the smaller pools. Elsewhere in India and in southern and eastern Africa I have seen it to be active in a broad range of habitat types including lakes, reservoirs, ponds and streams. Thus *Z. petiolatum* appears to have a much more restricted habitat preference and at Madurai it depended on small permanent waters.

An interesting feature of the behaviour of both species is the appearance of immature individuals 10-20 min after sunset, hovering and flying slowly about 1 m above the water and remaining there for several minutes after the departure of all sexually active individuals. Such behaviour may represent feeding and it may also assist immatures to learn about the location of potential oviposition sites by observing the presence and activity of mature individuals. It contrasts with the more usual behaviour of libellulids in which immature forms avoid water.

MORPHOLOGICAL CONSIDERATIONS

The mean egg number per batch in *Z. petiolatum* was 9.48, whereas in *T. tillarga* it was 7.2 but the modal value was 9 (MILLER & MILLER, 1985). The eggs in a batch of *T. tillarga* are usually arranged in 2 orderly rows as a result of the presence of a median ridge and lateral bristles on the 9th sternite where the batch is assembled. The egg batches of *Z. petiolatum* are less well organised and correspondingly the 9th sternite possesses no median ridge and bristles occur only along the posterior border. In other respects oviposition is very similar in the two species. The long and cleft sub-genital plate of *Z. petiolatum* has probably made necessary a lengthening of the hamuli, as in other species with cleft sub-genital plates (MILLER, 1989).

Interpretations of function based on structure must necessarily be tentative, but some aspects of the genital anatomy can be considered in the context of sperm competition. Internally the females of both species possess bursae of similar size and a pair of muscular spermathecae supplied through a T-shaped duct. The eccentric nature of the common spermathecal duct and the acute angle with which it meets the duct to the left spermatheca in *Z. petiolatum* may make it impossible for the male's flagellum to reach the left spermatheca, as was also suggested for *Brachythemis lacustris* (MILLER, 1982), and one or both flagellar branches perhaps therefore enter the right spermatheca, being of an appropriate length to reach into its far end. The flagellum of *B. lacustris* is undivided, though it bears a double hook, and it too may enter only the right spermatheca.

The penis morphology is similar in both species except that the flagellar branches of *T. tillarga* end in flattened spatulate structures whereas those of *Z.*

petiolatum terminate in large hooks. In the brief 10-20-second copulations observed in *Z. petiolatum,* males may gain precedence by releasing new sperm into the dorsal region of the bursa while simultaneously encircling bundles of rival sperm in the lower part of the bursa with the extended cornua which are rotated outwards and downwards, finally pulling the sperm bundles out as the penis is withdrawn (Fig. 6). At the same time the flagellum may be pushed along the spermathecal duct to lock into the right spermatheca, thereby holding the penis in place against the force resulting from the rapid injection of fresh sperm into the confined space of the bursa. The flagellum is also equipped with complex twisted membranes forming a series of supported scoops which perhaps assist in the removal of rival sperm from the duct on withdrawal. Thus some degree of sperm removal is postulated, which, together with a last-in-first-out advantage, would give a current male atleast temporary sperm precedence. More complete sperm removal might occur if longer copulations sometimes took place away from the water, as in some other species (cf. MILLER, 1983; SIVA-JOTHY, 1987).

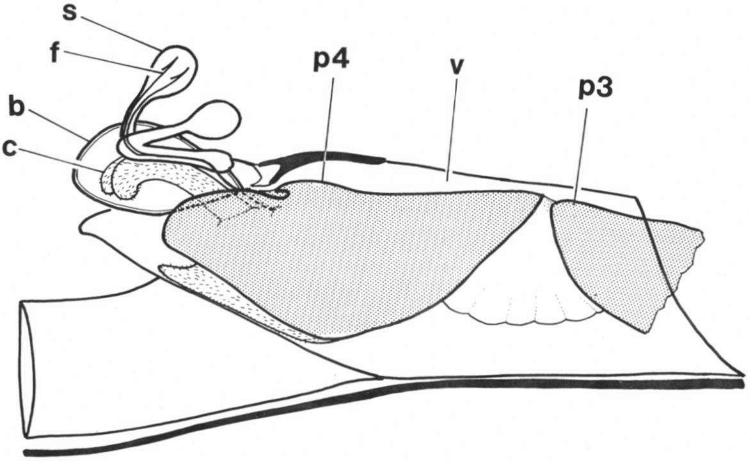

Fig. 6. Postulated position of the penis during copulation of *Z. petiolatum*. The 4th penis segment (p4, hatched) and part of 3rd segment (p3, hatched) are shown within the vagina (v). Both branches of the flagellum (f) are within the right-hand spermatheca (s). The everted cornua (c), which have entered the bursa (b), are turned downwards encircling clumps of rival sperm, while at the same time fresh sperm is injected into the dorsal part of the bursa.

In conclusion the reproductive behaviour and mechanisms of the two species appear to be closely similar. At Madurai *T. tillarga* exploited larger habitats which were of a temporary nature, whereas *Z. petiolatum* was found only at small, permanent pools. Habitat size rather than the degree of permanence was probably the distinguishing feature since elsewhere *T. tillarga* has been observed at a wide variety of permanent and temporary habitats over a certain size. *Z. petiolatum* favours small habitats but is apparently not adapted to exploit the numerous temporary pools which appear only during the monsoon season. Its range may therefore have been considerably extended as a result of the construction of the many permanent artificial dams, pools and ponds which are used to conserve water during prolonged dry seasons.

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REFERENCES

FRASER, F.C., 1924. A survey of the Odonata (dragonfly) fauna of Western India with special remarks on the genera Macromia and Idionyx and descriptions of thirty new species. *Rec. Indian Mus.* 26: 423-522.

HAMADA, K. & K. INOUE, 1985. *The dragonflies of Japan in colour.* Kodansha, Tokyo.

KUMAR, A., 1972. The phenology of dragonflies in the Dehra Dun valley (India). *Odonatologica* 1: 199-207.

KUMAR, A., 1985. A review of the bio-ecology of Indian dragonflies. *Proc. 1st Indian Symp. Odonatol., Madurai*, pp. 73-92.

LIEFTINCK, M.A., 1931. A revision of the genus Epophthalmia Burm. (Odon. Corduliinae). *Treubia* 13: 21-80.

LIEFTINCK, M.A., 1954. Handlist of Malaysian Odonata. A catalogue of the dragonflies of the Malay Peninsula, Java and Borneo, including adjacent islands. *Treubia* 22 (Suppl.): xii + 202 pp.

MILLER, P.L., 1982. Genital structure, sperm competition and reproductive behaviour in some African libellulid dragonflies. *Adv. Odonatol.* 1: 175-192.

MILLER, P.L., 1983. The duration of copulation correlates with other aspects of mating behaviour in Orthetrum chrysostigma (Burmeister) (Anisoptera: Libellulidae). *Odonatologica* 12: 227--238.

MILLER, P.L., 1988. Similarities in the genitalia and reproductive behaviour of male and female Tholymis tillarga (Fabr.), Parazyxomma flavicans (Martin), Brachythemis lacustris Kirby and B. leucosticta (Burm.) (Anisoptera: Libellulidae). *Odonatologica* 17: 59-64.

MILLER, P.L., 1989. Possible functions of the sub-genital plates of female libellulid dragonflies (Anisoptera: Libellulidae). *Adv. Odonatol.* 4: 57-71.

MILLER, P.L. & A.K. MILLER, 1985. Rates of oviposition and some other aspects of reproductive behaviour in Tholymis tillarga (Fabricius) in Kenya (Anisoptera: Libellulidae). *Odonatologica* 14: 287-299.

RAM, R., V.D. SRIVASTAVA & M. PRASAD, 1983. A note on a collection of Odonata from Eastern Uttar Pradesh, India. *Notul. odonatol.* 2: 15-16.

SIVA-JOTHY, M.T., 1987. Variation in copulation duration and the resultant degree of sperm removal in Orthetrum cancellatum (L.) (Libellulidae: Odonata). *Behav. Ecol. Sociobiol.* 20: 147-151.

WAAGE, J.K., 1986. Sperm displacement by two libellulid dragonflies (Anisoptera) with disparate copulation durations. *Odonatologica* 15: 429-444.