REPRODUCTIVE BEHAVIOUR AND TWO MODES OF OVIPOSITION IN *PHAON IRIDIPENNIS* (BURMEISTER) (ZYGOPTERA: CALOPTERYGIDAE)

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P. iridipennis was observed to oviposit into the stems of *Cyperus involucratus* by making long slits through which up to 90 eggs were packed in a single row, 0.5 mm below the surface and at 60° to the long axis of the stem. In contrast, when ovipositing into the leaflike bracts of the same plant, females inserted each egg through a separate slit in a manner resembling that used by other calopterygids. Females more commonly oviposited into bracts than into stems, and bracts were found to contain up to 9 times more eggs mm^{-3} than stems. When offered the subcrescentic leaves of *Typha domingensis* as abnormal oviposition sites, eggs were laid using either of the two modes, and it is suggested that stems and bracts of *C. involucratus* are normally distinguished by tactile receptors on the legs. The stems of *Cyperus* contain many more sclerenchyma strands than the bracts, and the force required to drive in a pin by a set amount was 2.38 times greater than that needed for bracts. The unusual mode of oviposition used by *P. iridipennis* when ovipositing into stems may therefore be an adaptation for penetrating tough plant tissues.

INTRODUCTION

Most calopterygid dragonflies are endophytic ovipositors, inserting their eggs into a variety of plants (ROBERT, 1958; CORBET, 1962, 1980; HEYMER, 1967, 1973; MIYAKAWA, 1982). Oviposition has been extensively studied in *Calopteryx maculata* (JOHNSON, 1962; WAAGE, 1978, 1979, 1984, 1987) but less is known about it in African calopterygids (PINHEY, 1961; LEGRAND, 1985). Most ovipositing Zygoptera make a small incision into a leaf or stem and insert usually a single egg through it. In contrast, *Phaon iridipennis,* a forest species common in much of tropical Africa (Pinhey, 1961), sometimes makes long incisions into the stems of *Cyperus,* inserting up to 90 eggs into each so that they

lie in a closely packed row within the stem (MILLER, 1985). It is of interest to know if this is the only type of oviposition in this species, or if the substrate influences the manner of ovipositing, and also to know what adaptive significance such a method may have. We report here that the method of oviposition in *P. iridipennis* varies according to the part of the plant utilised. When laid into the bracts of *Cyperus,* eggs are placed singly through separate slits as in *Calopteryx* spp., but when stems are used, eggs are always arranged in closely packed rows, as previously reported. We have examined a few of the factors which may determine the type of oviposition which occurs.

MATERIAL AND METHODS

Phaon iridipennis (Burmeister) was studied in the vicinity of Hunter's Lodge, near Simba, in Kenya (2° 15′S, 37° 32′E) for a total of 9 days in April, 1986. The males and females of this species are similarly coloured and the wings of both are transparent but they give glittering, purple reflections in flight. The study site was a 5 m length of a stream which was densely shaded throughout the day by trees (*Ficus* sp. and *Acacia* sp.). The stream was 1-3 m wide and its banks were covered in *Balsam* sp. and other herbaceous plants. A stand of *Cyperus involucratus* Rottb., about 1 m wide at its base, occupied the centre of the stream in the study area, and it formed a focus for the reproductive activity of *P. iridipennis.* Its numerous stems (culms) grew to 1.5-2 m in height and each supported a rosette of 20-30 flat, leaflike bracts, the bracts each being about 25-30 cm long and 2 cm at their widest point. Outer stems tended to fall into the water and lie at the surface providing attractive oviposition sites whose number we could experimentally vary. Stems and bracts into which oviposition had been observed were collected and preserved in 2% formaldehyde for subsequent examination. Stem and bract anatomy and the location of eggs were examined in thin, hand-cut sections.

The hardness of the plant material was measured using a fine steel pin, similar in width to the ovipositor, harnessed to an electronic strain gauge and mounted on a micromanipulator. Under a binocular microscope, the pin was driven against the surface of a piece of stem or bract preserved in 2% formaldehyde and supported in a bracket until it penetrated to a set point, the strain at the moment of penetration being recorded on a Washington pen recorder.

We individually marked 38 males (including 5 tenerals) with numbers, and 53 females (including 4 tenerals) with letters on the wings using an indelible felt-pen (Staedtler, Lumocolor). Insects to be marked were caught at or within 10 m of the stream near the study site, and some were marked at night as they roosted on bushes nearby. Marked individuals could be identified without capture by using a close-focus monocular (Microtec, Oxford).

Values are given as means ± standard deviations.

RESULTS

FEMALE REPRODUCTIVE BEHAVIOUR

The use of marked individuals enabled us to confirm that males established and defended territories (Miller, 1985). The study area usually contained one territory on the downstream side of the *Cyperus* stand, and a second on the upstream side. The downstream territory was occupied for most of the day between 10:00 and 17:00 h during 7 days of observations by one of 11 different

males (sunset being at about 18:25 h, E.A.S.T.), each male staying for a relatively short time (Miller, in prep.).

Many females were seen to arrive and oviposit between 10:00 and 17:00 h. A newly arrived female usually perched on the marginal vegetation for some time before flying down towards the ovipositing sites in a male's territory. Sometimes a resident male approached a perched female and then flew backwards towards the centre of his territory with the female following. As she inspected the site, the male hovered in the close vicinity and then followed the female as she flew back to the margin and again perched. The male then hovered over her, gradually descending and aligning himself with the female: in response the female flapped all 4 wings and then either flew to a fresh perch whereupon the male repeated the behaviour, or she remained perched allowing the male to land on her, grasp her in tandem and then to copulate.

Copulation, which usually took place 1-2 m from the oviposition site on marginal vegetation, lasted a mean of 88 ± 25 s (± standard deviation; n=21; min= 45 s; max = 135 s) after which the female usually remained perched and the male returned to his perch; alternatively she immediately flew down and started to oviposit with the male perched nearby guarding her (cf. MILLER, 1985). In five marked females, oviposition in a resident's territory was maintained with breaks of <3 min for 50, 56, 64, 64 and 194 min, and in a further 6 unmarked females it lasted for 36, 49, 66, 70, 75 and 111 min (mean, 64 ± 44 min; n=11). As females moved slowly backwards they inserted eggs into those bracts and stems of *Cyperus* which lay horizontally at or close to the water surface; no female oviposited entirely submerged or more than 10 cm above the surface.

Ovipositing females attracted other females to oviposit close to them, a behaviour pattern well known in *Calopteryx* spp. (WAAGE, 1979; ALCOCK, 1983) and in libellulids, which results in males guarding non-mates (JACOBS, 1955; WAAGE, 1973, 1978, 1979, 1984). Up to 5 ovipositing female *P. iridipennis* sometimes oviposited within 20 cm of each other, all guarded by a male which might have mated with no more than one of them. Likewise females could be attracted to oviposit close to dead males or females pinned in an ovipositing position, and by this means the number of ovipositing females available for study was conveniently increased.

When a new male took over a territory, he responded very actively towards females already ovipositing there. In 11% of 71 observed encounters of this type the male succeeded in copulating with a female. In 15% of the interactions he drove a female off but, in the other 74%, females resisted the male and remained ovipositing. Of the 53 cases in which males approached females, the females made no response in 63%, but in the remaining 37% they gave strong rejection signals, spreading their wings and elevating the abdomen vertically (cf. CORBET, 1962; PAJUNEN, 1966; BICK, 1972; WAAGE, 1984). Similarly dead and pinned females evoked persistent approaches from new residents, some of which lasted

several minutes. In 8% of 62 observed encounters of this type, the male hovered over a dead female but made no contact, in 24% of them he grappled with her, and in 68% he succeeded in forming a tandem and then attempted to fly off with her. Thus in the absence of rejection signals from dead females, males were able to form tandems on a greater number of occasions than when females were alive.

OVIPOSITION SITES AND EGG-LAYING BEHAVIOUR

All undisturbed oviposition that was observed took place into the bracts and stems of *Cyperus involucratus*. The anatomy of *Cyperus* spp. has been described
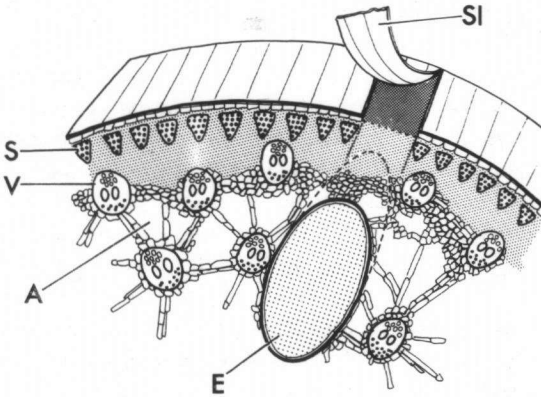

Fig. 1. Diagram of the structure of the stem of *Cyperus involucratus* showing the position of an egg (E), below the sclerenchyma (S), among the air spaces (A) and vascular bundles (V). The slit (Sl), cut by the females, is also shown.
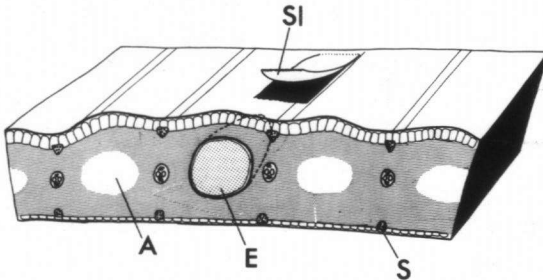

Fig. 2. Diagram of the structure of a bract of *Cyperus involucratus* showing the position of an egg lying in an air-space. Adaxial surface is upwards. Symbols as in Fig. 1.

by METCALFE (1971), but some further points of detail are added here. The stems taper slightly to a diameter of 7-9 mm and in each the vascular bundles are arranged round the periphery and also scattered throughout the parenchyma. About 300 peripheral strands or girders of sclerenchyma, each 50-70 μm wide (circumferentially) and 80-200 μm deep (radially) run longitudinally just under the epidermis. They are separated by only 25-35 μm from each other and provide strong support for the stem (Fig. 1). An extensive system of air channels permeates the more central region of the stem.

Females ovipositing into stems made two long cuts, 170-200 μm apart, parallel to the long axis, and usually passing either side of a sclerenchyma strand. They thus separated a strip of epidermis up

to 40 mm long. A closely packed row of eggs was placed under the slits, the eggs standing at about 60° to the long axis, with 2.3 eggs mm^{-1}. The anterior poles of the eggs were about 0.5 mm below the surface and the posterior poles 1.5 mm below, well within the region of air cavities. The chlorenchyma and parenchyma closed over the eggs concealing them from view, and eggs were thus provided with protection and moist air. Sometimes three parallel rows of eggs were inserted into a stem with little apparent damage, giving up to 7 eggs mm^{-1}. No stem contained eggs inserted individually through separate slits.

The bracts of *C. involucratus* are 350–400 μm thick and about 2 cm wide (Fig. 2). The adaxial (upper) surface is covered by an epidermis 40-60 μm thick, while the abaxial epidermis is 22-28 μm thick and includes large numbers of stomata (not shown in Fig. 2). Pairs of sclerenchyma girders, one on the adaxial and one on the abaxial surface, run longitudinally at intervals of 250-300 μm, and between them a vascular bundle is placed centrally within the mesophyll. Large air spaces separated neighbouring vascular bundles. Ovipositing females cut two parallel slits of mean length 1244 ±412 μm (n=32), separated as in stems by 170-200μm. A single egg was inserted through each incision to lie within an air cavity, horizontal to the bract surface and beyond the ends of the slits, thus preventing it from falling out. Occasionally slits 2-3 mm in length were found but they contained no egg. In 6 bracts examined all had abundant egg-slits on their abaxial surfaces, and two in addition had them on the adaxial surfaces.

Some bracts contained more than 2000 eggs representing the contributions of several females, and densities of 0.68 eggs mm^{-2} of abaxial surface (0.34 eggs mm^{-2} of total surface) or 0.97 eggs mm^{-3} of bract tissue were found. These figures compare with 0.24 eggs mm^{-2} of stem surface, or 0.107 eggs mm^{-3} of stem tissue, and show that bracts carry 1.5 times more eggs mm^{-2}, or 9 times more mm^{-3}, compared with stems. Our observations showed that more females spent more time ovipositing on bracts than on stems, but this has not been quantified. This was probably because bracts were much commoner and presented a greater surface area than stems, but it may also be that bracts were preferred by females.

The different distribution and density of sclerenchyma strands suggested that stems and bracts differed in their hardness and ease of penetration. In order to measure the force required to stab preserved plant material, a small steel pin was slowly driven against a supported bract or a stem. A force of 18.3 ±4.1 mN (n=10) was needed to penetrate a stem, but only one of 7.7±2.55 mN (n=16) was required for a bract. Thus a female might have to exert a force 2.38 times greater in order to penetrate a stem than a bract. We have not measured possible differences in the force required to make cuts parallel to the surface of the two tissues.

We then made a preliminary examination of the nature of the visual or tactile clues which might be used by females to distinguish stems from bracts. We offered the leaves of *Typha domingensis,* a plant which would not normally be en-

countered by *P. iridipennis,* as substitute oviposition sites. These were thickly crescentic, about 17 mm wide and 5-6 mm thick, and they were readily accepted by females. For example in one leaf, 125 mm long, 71 eggs were inserted singly through short incisions of mean length 416 ±58 μm (n=19) and also 40 eggs were placed in 2 rows through slits up to 34 mm in length with the eggs at a density of from 0.67 to 0.97 eggs mm^{-1}. In another leaf the short slits containing single eggs had a mean length of 773 ±439 μm (n=38), the larger means and standard deviations here being caused by the occurrence of a few slits 2-3 mm in length which each contained 2-3 eggs. Thus females laying in *Typha* leaves used the two modes of oviposition which were seen separately in *Cyperus* bracts and stems, but it is not known if individual females used both modes. Females may therefore have received conflicting information from the *Typha* leaves whose rounded abaxial surfaces perhaps resembled *Cyperus* stems, but whose flat adaxial surfaces were more like *Cyperus* bracts. The low density of eggs placed in the long slits (0.67-0.97 compared to 2.3 eggs mm^{-1} in *Cyperus* stems) was due to the eggs being placed nearly horizontally. These preliminary observations suggest that tactile stimuli, perhaps detected by the legs, play a role in switching on the appropriate oviposition behaviour, but this requires further examination.

In a previous examination of the eggs of *P. iridipennis,* one batch was found to contain many hymenopterous parasites (MILLER, 1985), but no comparable parasitism was found during the present study. However some *P. iridipennis* eggs may have been prevented from hatching by the clutches of eggs of *Brachythemis lacustris* which were laid on the surface of some of the bracts and leaves previously used by *P. iridipennis* (cf. MILLER, 1982). Such clutches covered about 150 mm^2 and might therefore have prevented the hatching of about 50 prolarvae of *P. iridipennis.*

DISCUSSION

Many of the features of the reproductive behaviour of *P. iridipennis* resemble those well known in *Calopteryx* spp. (HEYMER, 1967, 1973; MIYAKAWA, 1982; WAAGE, 1973 [summary of earlier work], 1978, 1979, 1984, 1987) and it is unnecessary to discuss them further. However, the occurrence of two types of oviposition within one species has not been previously reported in calopterygids (except for submerged and surface oviposition within a species), although it is known in a few libellulids such as *Micrathyria* spp. (PAULSON, 1969).

When a female *P. iridipennis* oviposits into a stem of *C. involucratus* the eggs are placed in a closely packed row through long continuous incisions, whereas when ovipositing into bracts, the eggs are inserted separately through small slits, the latter being the usual calopterygid method of oviposition. As a check, we observed females of *Calopteryx virgo* in Britain ovipositing into stems of *Mentha* sp. and into petioles of *Potamogeton polygonifolius* Pourr (cf. ROBERT, 1958).

They made separate oval-shaped slits through which single eggs were placed to lie parallel to the surface at a depth of 200-300 μm. During oviposition a female made gimlet-like rotating movements of her ovipositor by twisting the abdomen, an action not seen in *P. iridipennis,* and she gripped the leaf at the water surface working her ovipositor down the petiole by slowly straightening the abdomen. In contrast, throughout oviposition *P. iridipennis* kept her abdomen bent at a right angle between segments 3 and 4, and also between 4 and 5, a position adopted by lestids when ovipositing into wood (ROBERT, 1958; JURZITZA, 1969), and one which keeps the ovipositor close to the hind legs, thereby enabling the female to exert a strong downward pressure which the hard nature of the substrate probably necessitates.

Most of the oviposition observed in *P. iridipennis* was into bracts, which are more abundant and offer more surface area than stems. We do not at present know about the rates of oviposition into bracts and stems or the relative costs they impose on females. Since eggs are stacked nearly vertically in stems, as many as 2.3 eggs mm^{-1} of incision can be inserted, whereas in bracts where a separate incision is made for each egg, only 0.8 egg mm^{-1} of incision is inserted. Moreover, egg densities in stems were found not to exceed 0.11 egg mm^{-3} of tissue, whereas in bracts they reached 0.97 egg mm^{-3} of tissue, and in consequence there was a greater amount of damage to bracts which might in turn jeopardise the eggs. These apparent advantages of stems, however, may be offset because of the greater amounts of sclerenchyma they contain compared to bracts, which may necessitate a different type of oviposition behaviour, as previously suggested (MILLER, 1985). Our measurements indicated that preserved stems might be 2-3 times harder to penetrate than bracts. Since each tissue seems to offer some advantages and some disadvantages for oviposition, a good strategy for a female might be for her to divide her clutch between stems and bracts, but we do not yet know if individuals do this.

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