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SEXUAL BEHAVIOUR IN *PARAPHLEBIA QUINTA* CALVERT: MALE DIMORPHISM AND A POSSIBLE EXAMPLE OF FEMALE CONTROL (ZYGOPTERA: MEGAPODAGRIONIDAE)

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The sexual behaviour and a case of male phenotypic dimorphism in *P. quinta* are described: black-winged (BW) ♂♂ and hyaline-winged (HW) ♂♂. Similar to other territorial odon. spp., some ♂♂ defended a space that ♀♀ used for oviposition while other ♂♂ acted as satellites. Copulation took place in 2 stages which differed in abdominal movement orientation and duration. Copulation duration varied between morphs and was frequently disrupted. During disruption, the genitalia of both sexes disengaged although the tandem position (the ♂'s abdominal appendices grasping the ♀'s prothorax) was maintained. Disruptions, which took place during the first stage (a stage during which displacement of rival sperm occurs in most odon. spp.), were sometimes followed by emissions of sperm from the vagina. Male morphs exhibit striking behavioural differences: HW ♂♂ do not defend territories, but BW ♂♂ do, and the former copulate for longer and show more copulatory disruptions. Some stages of ♀ behaviour are described and suggested as instances during which ♀♀ may be exerting mate choice: ♀♀ copulated on fewer occasions with HW ♂♂, copulations with this morph were longer but ovipositions were not, and sperm emissions (possibly, sperm from previous mates) and copulatory disruptions of BW ♂♂ were less frequent. Because of these differences, it is suggested this is a unique sp. to test current ideas of ♀ control in an insect order in which the idea of ♂ "control" has been traditional.

INTRODUCTION

Alternative mating strategies have been studied in a number of animals (for some examples, see ANDERSSON, 1994). In several species, male mating behaviour consists of a territorial tactic and one or more alternatives – usually, with submissive and non-dominant males. In some species, no morphological differences are associated to each behavioural alternative and males adopt them on the basis of their condition (CARRANZA, 2000). In a few species, however, the tactics involve phenotypic characteristics which do not change ontogenetically and, therefore, individuals are not flexible in adopting more than one tactic. One classical example of the latter was shown by HAMILTON (1979) in his study on fig wasps (*Idarnes* spp.). He described two different male morphs in which some males are wingless and allocate their resources into fighting while others are winged and allocate their resources into dispersal. GADGIL (1972) pointed out that male dimorphism represents alternative mating strategies that are genetically determined.

Male dimorphism in Odonata has been described in a few species. It was first reported by CALVERT (1914) in the genus *Thaumatoneura* (Megapodagrionidae). Recently, male dimorphism and its associated sexual behaviour have been studied in detail in some species of the oriental genus *Mnais* (Calopterygidae) (e.g. UBUKATA, 1979; HIGASHI, 1981; WATANABE & TAGUCHI, 1990; HIGASHI & NOMAKUCHI, 1997). In *M. p. pruinosa and M. p. costalis*, for example, two male morphs are recognised in some populations, one corresponding to territorial individuals and the other to non-territorial individuals whose wing patterns are orange and hyaline (or pale orange in *M. p. costalis*) respectively (HIGASHI, 1981; NOMAKUCHI et al., 1984; WATANABE & TAGUCHI, 1990). These differences are further accompanied by differences in sexual behaviour: orange-winged males are territorial while hyaline-winged males are not. In *M. p. costalis*, females copulated less frequently and for longer with non-territorial males (WATANABE & TAGUCHI, 1990). SIVA-JOTHY & TSUBAKI (1989a) showed that in a population of *M. p. pruinosa* non-territorial males are able to displace more of the female's stored sperm obtained from previous copulations than territorial males. However, in *M. p. costalis*, ovipositions followed by a mating with a territorial male were longer than with a non-territorial male (WATANABE & TAGUCHI, 1990). Given these conditions, possibly both morphs are maintained by the similar reproductive success each morph achieves.

In this paper, we present some observations carried out in the tropical damselfly species *Paraphlebia quinta* which indicate that, similar to *Mnais*, two male morphs are using different mating tactics. These observations are intended to further extend the data presented by GONZÁLEZ-SORIANO (1997) on this species.

MATERIAL AND METHODS

The study was carried out at a small seepage located within the boundaries of the Estación de Biología Tropical de Los Tuxtlas in the state of Veracruz, Mexico (95°04'-95°09'W and 18°34'-18°36'N). The area is mostly covered by tropical rainforest and the study site is located on inclined terrain (ca. 30°) of a hill locally known as Cerro del Vigía. The study was done on the following dates: 14-20 July and 22-26 August, 1984; 14-19 June and 2-9 July, 1985; 19 July-6 August, 1986; 18-23 June and 8-11 July, 1988; and 19-23 August, 1990.

The study site was divided into 36 quadrats of 2×2 m. Individuals of both sexes found in the site were individually marked on a daily basis by writing a number on their wings using a felt-tipped Pilot pen. During marking and using a caliper, the size (in cm) of both male morphs and females was determined by measuring the total length of their left hind wing and body. To facilitate observation during poor forest light conditions, a small dot of fluorescent yellow or red enamel paint was put on the dorsum of the pterothorax of each marked individual. The position of each individual was determined daily by using a map of the study site. Finally, the sexual behaviour of focal individuals was recorded daily using a tape recorder. These observations were mainly on males but some data on females were also obtained.

Time of day is provided as local time. Where stated, data were log transformed for the use of parametrical tests. Data are presented as mean ± STD unless stated otherwise.

DIFFERENCES IN MORPHOLOGY AND COLOUR AMONG INDIVIDUAL MALES AND BETWEEN THE SEXES

P. quinta has two clearly distinguished male types with different colouration: black-winged (BW males) and hyaline-winged males (HW males; Fig. 1). For females, no apparent colour differences were found, and their wings appear to be hyaline (Fig. 1).

Additionally, differences between male forms and between males and females were found in the colouration of face and dorsum of abdominal segments 8-10. From 55 individuals, the following face colour patterns were detected: (a) 6 (30%) out of 20 BW males had the clypeus blue while the remaining 14 (70%) had the clypeus black; (b) 25 (96%) out of 26 HW males had the clypeus blue and one had the clypeus black; and, (c) all females (N = 9) had the clypeus blue.

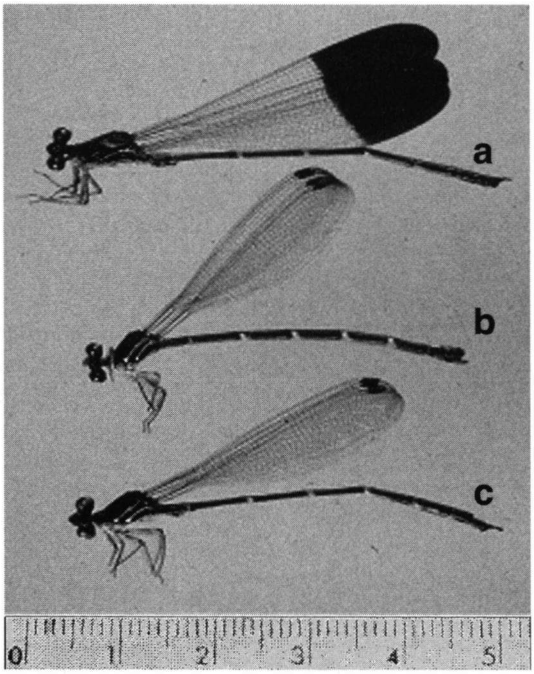

Fig. 1. *Paraphlebia quinta*: (a) BW males; (b) females; (c) HW males. – [Scale is in cm]

As for the colouration of the dorsum of the last three abdominal segments, the following differences were found in 19 individuals: (a) 1 (16.6%) out of 6 BW males had a brilliant pollinosity; (b) 6 (85.7%) out of 7 HW males had a whitish brilliant pruinosity; and, (c) females always had a distinctive whitish area on the dorsum of segments 8-10 (N = 6).

BW males were larger than HW males in body ($F_{2,60} = 37.9$, $P < 0.0001$) and wing size ($F_{2,60}$

= 48.2, P < 0.0001; Tab. I). Females appeared to be intermediate between the male forms (Tab. I).

SEXUAL BEHAVIOUR

The time during which individuals were present at the mating site was from 06:47 to 17:14 h and 06:44 and 17:14 h for males and females respectively. Copulatory activity occurred from 09:17 to 14:40 h.

BW males behaved as territorial, dominant individuals in the sense that they: (a) aggresively defended an area against conspecific males (both BW and HW males); and, (b) returned to the defended area during several consecutive days. HW males behaved as subordinate males showing submissive behaviour in the presence of BW males, however, they acted aggressively towards males of their own colour and some returned to the same general area for successive days. A particular characteristic of *P. quinta* behaviour is that HW males remained unusually close to the perching site of a territorial male even after an "attack" and chasing carried out by the BW male.

Males perched on different substrates such as rocks, twigs and leaves, among others, but generally very close to the ground level. Perching site heights differed between the two male colours: BW males' perching sites (16.26 ± 12.87 cm, N = 39) were lower than those of HW males (32.89 ± 21.11 cm, N = 19; t-test on log transformed data = 4.27, P < 0.0001, d.f. = 56).

Pair formation took place in two ways: (1) when a female comes out from the forest vegetation, flies into the territory and is taken by a male (N = 4); or (2) when an ovipositing female is taken by another male when the male that the female has most recently mated with is interacting with other males or trying to obtain additional copulations (N = 11). Tandem formation was not apparently preceded by any male courtship behaviour.

Two copulatory stages were recorded. The first consisted of a number of regular pumping abdominal movements which were similar to MILLER & MILLER's (1981) description of the sperm transfer stage II. During the first stage, two behavioural series, based on the abdominal movements, were observed: a series of 11-15 pumps per minute (during the first 3 to 6 min of its initiation, N = 3) followed by 7-10 pumps towards the end of the stage (during the last 16 to 49 min, N = 2). The second stage consisted of an inactive phase during which the pair remained motionless. This behaviour resembled the inactive stage III of MILLER & MILLER (1981). Signi-

Table I
Morphometric features (mean ± STD in cm) of female and male *P. quinta*. Numbers in parentheses reflect sample sizes

Sex/male morphs	Body size	Wing length
BW males (20)	4.66 ± 0.26	3.21 ± 0.17
HW males (26)	4.33 ± 0.18	2.88 ± 0.09
Females (15)	4.03 ± 0.21	2.87 ± 0.11
Differences	$F_{2,60} = 37.9$, P < 0.0001	$F_{2,60} = 48.2$, P < 0.0001

ficant differences between male types were found in copulatory duration in both the first (775 ± 373 s, N = 8, for BW males; 2059 ± 575 s, N = 5, for HW males; t test on log transformed data = -4.99, P = 0.001, d.f. = 11) and second stage (50.65 ± 14.99 s, N = 6 for BW males; 87.3 ± 25.6, N = 4, for HW males; t test on log transformed data = -2.81, P = 0.03, d.f. = 8). Thus, HW males spent longer in the first and second stage than BW males.

During the first copulatory stage, the pair repeatedly interrupted genital contact. HW males had a significantly higher number of interruptions (2.5 ± 2.03, N = 15) than BW males (0.95 ± 0.99, N = 24; t-test on log transformed data = 2.8, P < 0.01, d.f. = 37). The time in which the pair was not making genital contact differed between BW (171 ± 112.3 sec, N = 20) and HW males (291.2 ± 152.0, N = 38): HW males had longer interruptions (t-test on log transformed data = -3.14, P = 0.003, d.f. = 56). During these interruptions, females ejected drops of sperm (as checked using a microscope) from their genital pores. A close inspection during ejection revealed active movements by the ovipositor valves. Ejection occurred at any genital intromission break.

Sperm emissions were more frequent in females that were copulating with HW males (3 ± 2.16, N = 13) than with BW males (0.73 ± 0.93, N = 23; t test on log transformed data = 4.06, P = 0.001, d.f. = 34). When the pair separated and before females started oviposition, some females with hanging drops tried to clean-up the sperm mass by rubbing their abdomen against the substrate. In one case, a female was observed using a hind leg to clean-up her genital pore. Copulation time (excluding copulatory breaks) differed significatively between both male types: HW males (2397± 748 sec, N = 13) copulated for longer than BW males (927 ± 284 sec, N = 23; t test on log transformed data = 9.35, P < 0.0001, d.f. = 34).

Soon after the pair finished copulation, females went directly to the oviposition substrates. However, oviposition sometimes did not immediately take place as it was normally delayed due to several factors such as the sexual advances of conspecific males and, to a lesser extent, interferences by other animals including odonates (e.g. *Palaemnema paulitaba*), ants, *Anolis* lizards and bad weather such as rain. Females spent longer in effective egg-laying behaviour when recently paired with BW males (4793.7 ± 2609 sec, N = 18) than with HW males (2209 ± 1236 sec, N = 9; t test = 3.49, P = 0.002, d.f. = 25).

Females laid eggs predominantly in decayed material (rotten wood and leaves), but also used a variety of substrates such as living plants (liverworts, secondary roots, palm trunks, mosses) and moist soil. Males of both colours guarded their mates. However, HW males were frequently driven off by conspecific BW and HW males. Females clapped their wings frequently during oviposition. This was followed by the clapping of BW guarding males but not by HW guarding males. In a few occasions, some females arrived at a territory and started oviposition without having copulated with the territorial male.

MALE MATING SUCCESS

The maximum number of matings recorded for a single BW and a single HW male was 10 and 5 respectively (followed during a period of 17 consecutive days). The maximum number of copulations recorded for a single female during the same period was 5. The highest number of matings during a single day for a male and female was 2, except one female who obtained three copulations during the same day, two of them carried out with the same male.

The number of copulations obtained by both male types differed significantly: BW males (2.36 ± 2.27, N = 36) had a higher mating success than HW males (0.71 ± 0.72, N = 49; t test on log transformed data = 4.04, P = 0.0002, d.f. = 83). Since both types tended to remain in the same site during several days, we investigated whether this time was related to the number of copulations obtained for each type. For both, the longer the period remaining in the territory, the higher the number of copulations (using log transformed data; for BW males, Pearson correlation, r = 0.69, N = 36; for HW males, Pearson correlation r = 0.63, N = 49; both P < 0.001).

DISCUSSION

Our results clearly show two discrete male sets which exhibit both morphological and behavioural differences. Similar results have been observed in the asiatic genus *Mnais* (e.g. WATANABE & TAGUCHI, 1990). Given these differences, we believe this is a case of male dimorphism in which each morph is possibly using distinct mate--seeking strategies.

In *P. quinta*, BW males act as dominant, territorial individuals which behave aggressively toward conspecific males. HW males behave submissively toward BW males, acting as satellite individuals. HW males were also aggressive but only towards HW males, not against BW males. Interestingly, HW males perched very close to BW males. WATANABE & TAGUCHI (1990) have suggested that, given the morphological similarities between HW males and females in the male dimorphic *Mnais pruinosa*, hyaline males may mimic females. *P. quinta* HW males also look similar to females in body colour and even in size so that the female mimic hypothesis may well apply to this species.

Similar to other male dimorphic species, such as those of *Mnais* (HIGASHI, 1981; SIVA-JOTHY & TSUBAKI, 1989a, 1989b; WATANABE & TAGUCHI, 1990; HIGASHI & NOMAKUCHI, 1997), male morphs show striking differences in mating behaviour. BW males are territorial and have a shorter copulation compared to HW males. In *Mnais pruinosa costalis*, it has been postulated that both morphs coexist given the similar reproductive pay-offs that both morphs achieve (WATANABE & TAGUCHI, 1990). Despite the intrinsic limitations of our observational-based study, similar conclusions can be derived. Because of the differences in the duration of the first stage of copulation, we suggest that BW males may be displacing less sperm than

HW males, a hypothesis that is compatible with studies in other species in which copulation duration inversely correlates with sperm displacement rate (see CÓRDOBA-AGUILAR et al., in press). HW males may obtain a higher displacement rate, but since females copulated more frequently with BW males and remained in oviposition for longer periods after pairing with these males, immediate fertilisation success for HW males would be lower. Currently, however, we have no explanation for the differences in the second stage of copulation in which HW males spent longer times.

Another interesting behavioural aspect of mating in *P. quinta* is that copulatory breaks were common during the first stage. A similar behaviour has been observed in *Megaloprepus coerulatus* (FINCKE, 1984) and *Coenagrion scitulum* (CORDERO et al., 1995). In Odonata, copulatory control is apparently exerted by the male (MILLER & MILLER, 1981; MILLER, 1987), which include duration and copulatory breaks. Research in *C. scitulum* suggests that males have a poor sperm removal ability so that they need to inseminate repeatedly and transfer sperm to the female after each break (CORDERO et al., 1995). Males of this species may compensate for the inability to displace sperm by outnumbering the rivals' sperm within the female sperm storage organs. In *P. quinta*, unlike *C. scitulum*, males did not translocate sperm after each break so that the multiple insemination hypothesis seems unlikely (although still males may be passing sperm during the first stage). Interestingly, females ejected sperm drops after each break. Sperm ejection has been observed in other species (e.g. *Calopteryx splendens*, LINDEBOOM, 1998), a behaviour that is common at the end but not during copulation. Currently, we do not know whose sperm was that that was expelled but two possibilities can be outlined: (a) it is sperm received from previous partners; and (b) assuming that sperm transfer takes place during the first stage, it is a mixture of both past males and the copulating male. Current research is being carried out to investigate the patterns of sperm displacement, the male origin of ejected sperm, and male or female control of copulatory breaks.

Increasing attention has been recently paid to odonate female sexual behaviour as a potential selective force (FINCKE, 1997; CÓRDOBA-AGUILAR, 1999; ANDRÉS & CORDERO, 2000). Female behaviour has been a neglected study topic in this order but recent results suggest that females may play a determining role in the evolution of male sexual behaviour and morphology (CÓRDOBA-AGUILAR et al., in press). *P. quinta* is a unique species for addressing this issue as there are different instances at which females may exert control: (a) in choosing particular male phenotypes to mate with; (b) in inducing copulatory breaks thereby making difficult some internal processes (such as sperm transfer); (c) in ejecting sperm, affecting potentially that of the copulating male; and, (d) in varying oviposition duration and, therefore, the numbers of eggs laid.

A TAXONOMIC NOTE

The discovery of male dimorphism in the genus *Paraphlebia* raises some questions about its current taxonomic status. The only key to separate *Paraphlebia* species is based mainly on the male wing tip colouration (CALVERT, 1901). *P. abrogata* males show a hyaline wing membrane whilst *P. quinta* males have a dark band on the wing tips. However, a recent revision of the types of both species shows that *P. abrogata* males are in fact the hyaline morph of the dark-tipped *P. quinta* males and hence, *P. abrogata* should be synonymized with *P. quinta* (E. González & V. García, unpublished data). Male dimorphism has also been observed in other species of this genus such as *P. zoe* (GONZÁLEZ, 1997). Interestingly, male dimorphism is not extended to all *Paraphlebia* species: all males of *P. hyalina* Brauer collected from a small population in the state of Veracruz seemed to be of a hyaline morph (E. González, unpublished data), another striking similarity to what occurs in *Mnais* species (Y. Tsubaki, pers. comm.).

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