POSTCOPULATORY GUARDING BEHAVIOUR IN A TERRITORIAL DAMSELFLY, *PSEUDAGRION P. PILIDORSUM* (BRAUER), FOR SUBMERGED OVIPOSITING FEMALES (ZYGOPTERA: COENAGRIONIDAE)

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The postcopulatory mate guarding behaviours by territorial and non-territorial ♂ ♂ for submerged ovipositing ♀ ♀ were investigated in the field. After copulations, ♀ ♀ in tandem began to oviposit at the water surface and thereafter they usually submerged completely underwater. The ♀ often repeated the submergence and emergence at several oviposition sites. When the ♀ submerged completely, the ♂ released her without submergence and rested above the water surface during oviposition (non-submerged guarding), or the ♂ submerged completely and remained in tandem, whether only at first or for the duration of the oviposition (submerged guarding). Territorial ♂ ♂ always performed non-submerged guarding when the ♀ oviposited inside their territories. The non-submerged guarding inside the territory might allow the territorial ♂ both to guard the ovipositing ♀ and to maintain his territory. On the other hand, when the ♀ oviposited inside another ♂'s territories, territorial and non-territorial ♂ ♂ exhibited both non-submerged guarding and submerged guarding. Thus, *P. p. pilidorsum* ♂ ♂ may adopt either submerged guarding or non-submerged guarding in response to change in the probability of a takeover of the emerged ♀ by rival ♂ ♂ inside another ♂'s territory.

INTRODUCTION

In many insects, the male prolongs its relationship with its mate beyond the time it takes to transfer a sufficient number of gametes to fertilize all of the fe-

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male's eggs (ALCOCK, 1994). Most prolonged associations with mates have developed to assure paternity under the condition that the last male to inseminate a female is more likely than previous males to fertilize the eggs. Postcopulatory mate guarding, seen in many species of dragonflies and damselflies, is one of the most familiar examples of how a male prevents other males from copulating with his partner (WAAGE, 1984; ALCOCK, 1994; CORBET, 1999). In these species, postcopulatory mate guarding is a particularly effective means, because sperm removal or displacement gives the last male a fertilization advantage (see WAAGE, 1984).

Dragonflies and damselflies exhibit two manners of postcopulatory mate guarding: contact guarding (tandem oviposition), in which the male stays physically attached to the female (e.g. BICK & BICK, 1963), and non-contact guarding, in which the male remains near the female and chases away other males (e.g. WAAGE, 1973). Although contact guarding offers better security against takeover of his mate (SAKAGAMI et al., 1974; WAAGE, 1984), the male may be unable to mate with other females as long as he is attached to the ovipositing female (ALCOCK, 1979; UÉDA, 1979; WAAGE, 1984). On the other hand, non-contact guarding allows the male to acquire new mates while guarding the ovipositing female and to simultaneously defend his territory (ALCOCK, 1979; UÉDA, 1979; SHERMAN, 1983; WAAGE, 1984), but the risk that the female will re-mate may be greater (UÉDA, 1979; ALCOCK, 1982; CONVEY, 1989).

Submerged oviposition by females is found only in the Zygoptera (WAAGE, 1984). When a female commences submerged oviposition, her partner guards her by hovering or perching above the water surface (e.g. *Hetaerina vulnerata*, ALCOCK, 1982; *Enallagma hageni*, FINCKE, 1982). On the other hand, in some genera (e.g. *Paracercion*, ARAI, 1977; *Erythromma*, WINSLAND, 1983; *Lestes*, ANDO, 1969; *Pseudagrion*, FURTADO, 1972, MESKIN, 1986, 1989; and *Xanthocnemis*, CRUMPTON, 1975), the guarding male submerges completely and in tandem with the female. However, the adaptive significance of submerged guarding by males has not been fully analyzed.

In *P. p. pilidorsum*, the male forms a territory near oviposition sites, and the female frequently performs submerged oviposition (AZUMA, 1987; ISHIDA et al., 1988). In order to elucidate the adaptive significance of submerged guarding, we investigated postcopulatory guarding behaviours by territorial and non-territorial males for submerged ovipositing females. We discuss the mating tactics of territorial and non-territorial males associated with postcopulatory mate guarding.

METHODS

STUDY SITE. – The study was carried out at the upper part of a stream on the Senbaru campus of the University of the Ryukyus in Nishihara, the southern part of Okinawa Island, Japan (26°14'N, 127°45'E). At the study site, the river was about 3 m wide and 0.5 m deep, and above its banks stood a grove of leucaena *Leucaena leucocephala* and an evergreen forest. Damselflies rarely appeared at

the study site in the early morning or evening, when the water surface was shaded. The reproductive behaviour was observed at a 15 m length of the river in the study site. In the observation area, there were two sandy sites on the riverbank, one 3 m long and the other 5 m. These sites were covered with grasses, and some of the stems and roots were underwater. Female damselflies used these underwater stems and roots as oviposition substrates.

OBSERVATION OF REPRODUCTIVE BEHAVIOUR. – Although damselflies appeared successively at the site from early April to late November, the observation was conducted in two periods, July and September 1996, when many damselflies appeared. In two days before each observation period, we collected as many male and female damselflies as possible at the study site and surrounding area, and individually marked them with paint markers (Mitsubishi Paint Marker PX-21®) on the thorax and abdomen. Furthermore, if any unmarked individuals were found during observation period, they were marked and released. The behaviour of damselflies did not appear to be affected by marking them on the thorax and abdomen. We carried out continuous observation principally from 0900 to 1500 h, except on rainy or overcast days (observation time: 114 h in July, 69 h in September). We examined the time and location in which reproductive behaviours by individual damselflies were observed. The time and duration of these behaviours were measured with the use of a stopwatch.

In total, 149 males and 46 females (July: 77 males and 31 females; September: 72 males and 15 females) were individually distinguished by marking.

TERRITORIAL BEHAVIOUR

Territories were usually established around oviposition sites that contained two or three locations of oviposition substrates, elongated stems and roots of grasses underwater, and each territory always contained at least one perch on stems or leaves of grasses protruding 10-15 cm above the water surface. Territorial males defended against intruding conspecific males by the chase display and/or the elaborate confrontation-chase display (FURTADO, 1972; MESKIN, 1986, 1989, 1993). Territorial males almost always succeeded in defending their territories; intruders ousted defending males from their own territory only 3 times (1.4%) in 220 observed interactions. Territory size, as defined by the distance at which the owner reacted to an approaching male, was quite variable, ranging from 1-4 m. Territorial males were resident at the same oviposition site from 1-8 days (mean ± SE = 2.33 ± 0.43, n = 21). The social status of males sometimes changed from territorial to non-territorial or vice versa.

MATING BEHAVIOUR

Neither territorial nor non-territorial males courted females. When a territorial male discovered a female approaching an oviposition site inside his territory, he immediately grasped the female with his anal appendages. The non-territorial male, on the other hand, usually stayed around another male's territory until a female approached an oviposition site, whereupon it quickly seized the female. After a territorial or non-territorial male had seized a female, the couple flew immediately in tandem to a nearby plant and perched on it. The male simultaneously raised and curved his abdomen to bring the primary gonopore in con-

tact with the secondary genitalia, presumably translocating sperm. Copulation ensued immediately. The mean (± SE) copulation duration was 17.42 ± 1.75 min (range = 4.08-50.30, N = 29).

OVIPOSITION BEHAVIOUR BY FEMALES AND POSTCOPULATORY BEHAVIOURS BY MALES

In the 85 (91.4%) of 93 tandem pairs that appeared at the observation site, the female performed submerged oviposition. In the other 8 tandem pairs (8.6%), the female oviposited on the water surface and then flew away from the observation site. Thus, when tandem pairs appeared at the observation site, most females performed submerged oviposition at least once.

Figure 1 is a flow chart of the successive postcopulatory behaviours. After copulation, the male and female in tandem flew to an oviposition site near the water's edge. The female landed on the site and bent her abdomen, and her ovipositor contacted the oviposition substrates. The male responded by adopting an "upright tandem posture" in which his body stood vertically on the tip of his abdomen (his anal appendages remaining interlocked with the female's thorax), and flapped and vibrated his wings. Oviposition began at the water surface and usually was completed underwater. In surface oviposition, only the female's ovipositor was immersed, while the male maintained the upright tandem posture. When the female submerged by moving backwards along the site in tandem (n = 107), the male often released the female without submerging with her (nonsubmerged guarding, n = 74), or the male remained in tandem and submerged either for a short moment at first (n = 10) or for the duration of the female's submergence (n = 23) (submerged guarding, n = 33). Males that submerged only at first returned to the surface to rest above the oviposition site. Any individual was able to perform both submerged and nonsubmerged postcopulatory guarding for submerged ovipositing his partner.

After the male released the submerged ovipositing female and perched on vegetation above the female, he usually recaptured the female once she emerged, and the tandem pair began searching for another oviposition site. When the male remained submerged with the female throughout oviposition, their search for another site resumed promptly upon emergence. The female often submerged and emerged at several oviposition sites (the average number of ovipositions (n, mean ± SE.): n = 17, 2.2 ± 0.4, range = 1-7).

The mean (± SE) duration of submerged oviposition by females and the mean (± SE) duration of submerged guarding by males when the male adapted either the submerged guarding or the non-submerged guarding are shown in Table I.

A female with a nonsubmerged male had a similar oviposition time as that of a female with an at-first submerged male or with a fully submerged male (significant post hoc Mann-Whitney U test (P adjusted to 0.0167 to reduce the chance

of committing a type I error), vs. a female with an at-first submerged male, z = -1.400, P = 0.1615; vs. a female with a fully submerged male, z = -1.847, P = 0.0647). The duration of submerged oviposition by a female was shorter when the male remained submerged with her throughout oviposition than when the male was submerged only at first (significant post hoc Mann-Whitney U test (P adjusted to 0.0167 to reduce the chance of committing a type I error), z = -2.663, P = 0.0077). The duration of submerged oviposition by females was significantly longer than that of submerged guarding by males (Wilcoxon signed ranks, a female with an at-first submerged male, n = 7, z = -2.366, P = 0.0180; a female with a fully submerged male, n = 17, z = -3.621, P = 0.0003). The duration of submergence by males did not differ between males that submerged only
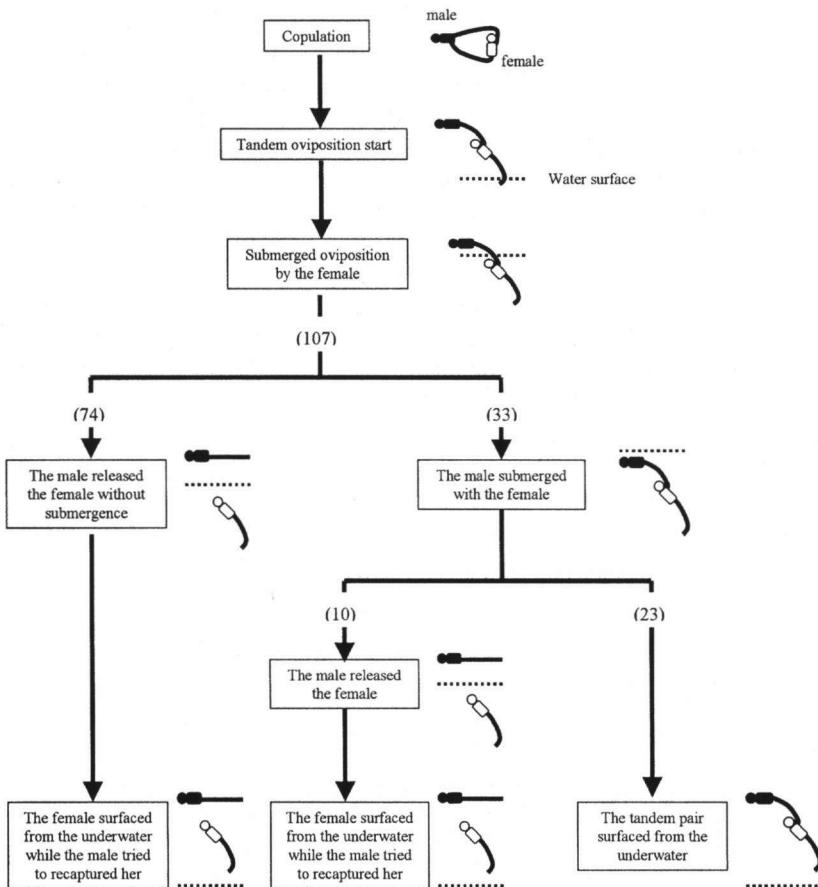

Fig. 1. The flow chart of the successive postcopulatory behaviours in *P. p. pilidorsum*. — [A quadrilateral shows a particular event. Figures in parenthesis indicate sample size]

at first and those that remained submerged throughout (Mann-Whitney U test, $z = -0.896$, $P = 0.3704$).

Tandem pairs were often interfered by alone males. The interference frequently resulted in a noticeable change of flight or oviposition behaviours by the pair.

TERRITORIALITY AND POSTCOPULATORY MATE GUARDING BEHAVIOURS

After copulating with a territorial male, the female oviposited underwater inside his territory or in another male's territory.

Figure 2 shows the proportions of submerged guarding and non-submerged guarding by territorial males when females oviposited inside their territories or another male's territory, and those proportions by non-territorial males when females oviposited inside another male's territory.

When the female performed submerged oviposition inside her partner's territory, the territorial male always released the female and rested on the water surface during oviposition. On the other hand, when the female oviposited underwater inside another male's territory, the male owning the territory exhibited either submerged guarding or non-submerged guarding; the proportion of submerged guarding was higher than that of non-submerged guarding (Fisher's exact test, $P = 0.0021$, Fig. 2a).

Of 12 territorial males performing non-submerged guarding for his partner inside their territories, only one male acquired an additional female that had emerged from underwater, by eliminating the non-territorial male performing non-submerged guarding for his partner.

Non-territorial males also performed both submerged and nonsubmerged postcopulatory guarding when the female oviposited underwater inside another male's territory, and the proportion of submerged guarding was similar to that of nonsubmerged guarding (chi-square goodness-of-fit test, $\chi^2 = 3.13$, df = 1, $P > 0.05$, Fig. 2b).

Table I

The mean ($\pm$ SE) duration (min) of submerged oviposition by females and the mean ($\pm$ SE) duration (min) of submerged guarding by males when the male adapted either the submerged guarding or the non-submerged guarding [Figures in parenthesis indicate sample size]

	Duration of submerged oviposition by females	Duration of submerged guarding by males
Non-submerged guarding	21.23±3.24 (42)	-----
Submerged guarding		
A female with an atfirst submerged male	33.25±8.25 (7)	8.42±2.08 (9)
A female with a fully submerged male	8.58±1.35 (18)	6.14±1.09 (20)

DISCUSSION

Females *P. p. pilidorsum* began oviposition on the surface but usually completed it underwater. Submerged oviposition has been found in *P. perfuscatum, P. microcephalum* (FURTADO, 1972), *P. hageni tropicanum* (MESKIN, 1986), *P. citricola, P. i. inconspicuum* and *P. salisburyense* (MESKIN, 1989). Thus, submerged oviposition might be ubiquitous in the genus *Pseudagrion*.

When the female submerged deeply, the male either released the female without submerging or the male submerged completely and remained in tandem, whether only at first or for the duration of the oviposition. Similar postcopulatory behaviours by males have been found in *P. perfuscatum* and *P. microcephalum*. FURTADO (1972) reported an example in which a male of *P. microcephalum* submerged with an ovipositing female in tandem but then separated after two minutes, floating to the surface to rest on the nearest perch. FURTADO (1972) reported two other examples in males of *P. perfuscatum;* in one, the male submerged with the ovipositing female for 15 minutes, and in the other, the male hovered above the ovipositing female without submerged guarding. In the genus *Pseudagrion*, therefore, the postcopulatory behaviours by males for submerged ovipositing females may be flexible, depending on how long the females are submerged.

Submerged oviposition sometimes lasts more than an hour and may incur considerable energy costs and predation risks for the female (WAAGE, 1984). Furthermore, FINCKE (1988) and MILLER (1990) considered that submerged ovipositing females suffer costs such as waterlogging and impaired vigor upon surfacing. Therefore, it is likely that submerged guarding has the same effects on males, and the cost may be far larger than that of non-submerged guarding. However, submerged guarding may be more advantageous to the male than non-submerged guarding, since there is a higher risk that other males will take over the emergent female if the guarding male did not submerge.

When a female oviposited inside her partner's territory, the male owing that territory always

Fig. 2. The proportions of submerged guarding and non-submerged guarding by territorial males when females oviposited inside their territories or another male's territory (a), and those proportions by non-territorial males when females oviposited inside another male's territory (b). − [Figures in columns indicate sample size]

released the female without submergence and rested above the water surface during oviposition (non-submerged guarding, Fig. 2a). Two hypotheses have been proposed to explain the adaptive significance of non-contact guarding by the territorial male inside his territory (ALCOCK, 1979, UÉDA, 1979, SHERMAN, 1983). ALCOCK (1979) and UÉDA (1979) suggested that non-contact guarding is reproductively advantageous because it allows a male both to guard the ovipositing female and to acquire new mates (multiple mating hypothesis). On the other hand, SHERMAN (1983) and WAAGE (1984) suggested that non-contact guarding is reproductively important because it allows a male both to guard the ovipositing female and to maintain his territory (territory maintenance hypothesis). Of 12 territorial males performing non-submerged guarding for his partner inside their territories, only one male could acquire an additional female. From this result, we could not necessarily discard the multiple mating hypothesis, because of shortage of the data. SHERMAN (1983) posited that a male in tandem would have difficulty fighting off intruders and that tandem mate guarding could reduce a male's ability to maintain his territory. In this study, although the territorial male in tandem did not eliminate intruders from his territory, the territorial male when alone prevailed in most male-male interactions. Therefore, non-submerged guarding allows territorial males both to guard the ovipositing female and to maintain his territory, supporting the territory maintenance hypothesis.

When a female oviposited underwater inside another male's territory, both submerged guarding and non-submerged guarding by the territorial male were found, and the proportion of submerged guarding was higher than that of non-submerged guarding (Fig. 2a). Non-territorial males also exhibited both types of guarding when the female oviposited underwater inside another male's territory (Fig. 2b). UÉDA (1979) showed that *Sympetrum parvulum* males could adopt both contact guarding and non-contact guarding, changing from one to the other in response to changes in the probability of a takeover. In *P. p. pilidorsum*, males also adopt either submerged or non-submerged guarding for the same reason.

The territorial male often left his territory to accompany a mate to an oviposition site elsewhere. Since a male did not always detect the presence of a female ovipositing in his territory, the probability that he would take over a female ovipositing with an intruding male might be low. Therefore, a male might not always engage in submerged guarding even when they are in another male's territory.

CONVEY (1989) reported that *Sympetrum sanguineum* males decided whether or not to switch from contact to non-contact guarding based on their assessment of male harassment early in an oviposition bout. Such harassment by other males may affect the male's choice between submerged and non-submerged guarding in *P. p. pilidorsum*, but this remains to be elucidated.

When a male submerged in tandem for the duration of oviposition, the female completed her oviposition faster than when the male remained submerged only at first. The male remained underwater the same amount of time whether his

submergence was only at first or for the duration (Tab. I). Therefore, males may cease submerging when the female's submergence is prolonged, partially because the male cannot stay underwater as long as the female.

Submerged guarding may have evolved in close association with the manner of oviposition, in which the female frequently repeats submergence and emergence, laying eggs at several sites. If a female oviposits almost all of her mature eggs at a single site, she may then be taken over by another male at no cost to her partner, since she will not produce mature eggs again for several days. On the other hand, if a female oviposits eggs at several sites by repeated submerged ovipositioning, such as *Hetaerina vulnerata* females do (ALCOCK, 1982), her partner's submerged guarding may substantially benefit him by preventing another male from taking her. Therefore, submerged guarding by *P. p. pilidorsum* males may have evolved by the females' frequent, repeated submergence to lay eggs at different sites until almost all of her eggs are gone.

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