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Reduced female mating receptivity and activation of oviposition in two *Callosobruchus* species due to injection of biogenic amines

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Analyses of proximate mechanisms that control mating and oviposition behaviours in insects are important because they link behavioural ecology and physiology. Recently, seed beetles have been used as models to study evolution of female multiple mating and cost of reproduction including mating. In the present study, we investigated the effects of biogenic amines into the abdomens of females of two *Callosobruchus* species, *Callosobruchus chinensis* and *Callosobruchus maculatus*, on mating receptivity and oviposition behaviour. In *C. chinensis*, injection of octopamine and tyramine reduced receptivity to mating and tyramine and serotonin increased the number of eggs laid. Similarly, injection of tyramine reduced the receptivity of females and increased the number of eggs laid by females of *C. maculatus*. These results show the possibility that biogenic amines control mating receptivity and oviposition behaviour in females of two *Callosobruchus* species.

1. Introduction

Females usually lay eggs after receiving seminal fluids including sperm during mating. Females of some insect species exhibit reduced receptivity after mating for a period of time, in extreme cases until death (Chen, 1984; Gillott, 1988; Eberhard, 1996; Chapman et al., 1998). These changes in oviposition behaviour and reduction of receptivity of females are caused by components of seminal fluids, the sperm itself, and mechanical stimulation (Eberhard, 1996; Chapman et al., 1998; Miyatake et al., 1999; Simmons, 2001; Wedell, 2005).

Biogenic amines are physiologically neuroactive substances that affect behavioural and physiological traits in vertebrate and invertebrate animals, and they act as neurotransmitters and endocrine disruptors in the central and peripheral nervous systems (Evans, 1980; Blenau and Baumann, 2001). Several roles of monoamines in mating and oviposition in insects have been reported: octopamine and tyramine regulate egg-laying in *Drosophila melanogaster* of Diptera (Monastrioti et al., 1996; Monastirioti, 2003; Cole et al., 2005), octopamine, tyramine and serotoinin regulate the muscle contractions in female reproductive organs in *Locusta migratoria* (Clark and Lange, 2002, 2003; Donini and Lange, 2004; da Silva and Lange, 2008); and dopamine and tyramine accelerate ovarian development in reproductive workers in Hymenoptera (Dombroski et al., 2003; Sasaki and Harano, 2007; Sasaki et al., 2009). Few studies, however, have reported the effects on female mating receptivity in insects, except for the report that the production of bombykol, the sexual hormone of *Bombyx mori* females, seemed to be regulated by tyramine (Hirashima, 2008).

In Coleoptera, reduction of female receptivity after mating is reported in some species of Bruchidae, including *Callosobruchus maculatus* (Eady, 1995) and *Callosobruchus chinensis* (Miyatake and Matsumura, 2004). In these two *Callosobruchus* species, costs and benefit of females in reproduction are complex, and the interests of each sex in mating are considered from the viewpoint of sexual conflict between sexes (Crudgington and Siva-Jothy, 2000; Eady et al., 2004, 2007; Edvardsson and Tregenza, 2005; Rönn et al., 2006; Harano et al., 2006; Sakurai and Kasuya, 2008).

Unlike the above evolutionary ecological studies, in the present study we investigated the possible effects of monoamines on female mating behaviour and oviposition in *Callosobruchus*, by injecting them into the female abdomen and examining the effect on reductions in receptivity to mating and oviposition behaviour, from a physiological standpoint.

2. Materials and methods

2.1. Insects and culture

We used a strain of *C. chinensis*, referred to as the jC strain, that was established in 1936 and is maintained in our laboratories (Utida, 1941a,b, see also Harano and Miyatake, 2005) and the strain of *C. maculatus*, referred to as the hQ strain (Miyatake and

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Matsumura, 2004). These cultures were maintained in a chamber (Sanyo, Tokyo, Japan) kept at 25 °C, 60% RH, and 16L8D conditions at the density about 100 adults per plastic Petri dish (1.5 cm height, 9.1 cm diameter). All beetles used for the experiments were reared in adzuki beans (*Vigna angularis* 'Dainagon') from eggs laid by parents randomly collected from the stock culture. Mated females were allowed to lay one egg per adzuki bean. If more than one egg was laid on a bean, excess eggs were scraped off with forceps. Each bean was transferred to a separate 48-well tissue culture plate (Greiner Bio-One, Frickenhausen, Germany) and kept in the chamber described above. Virgin males and females emerging from these beans were collected.

2.2. Injection of biogenic amines

Virgin females (body weight, *C. chinensis*: 5.87 ± 0.10 mg (*N* = 30); *C. maculatus*: 5.95 ± 0.12 mg (*N* = 25)) 1–4 days old were chilled on ice for a few minutes and fixed to agarose medium by using fine forceps. A hole was made between the second and fifth segments of the ventral abdomen with forceps, and an amine solution was injected using a fine glass capillary whose tip was as thin as possible connected to an oil pressure injection machine (Nanoject Auto-nanoliter injector, Drummond Scientific Company, Broomall, PA, USA) under a microscope. Each amine, dopamine (Nacarai, Kyoto, Japan), octopamine (Nacalai Tesque, Kyoto, Japan), tyramine (Sigma–Aldrich, Tokyo, Japan), or serotonin (Sigma–Aldrich, Tokyo, Japan), was dissolved in Milli-Q water, respectively, at a concentration of 10% (dopamine, octopamine: 0.53 M, tyramine: 0.58 M) except for serotonin (2%: 0.05 M). Serotonin dissolves with difficulty in water and more than 2% serotonin solution could not be made. To examine the dose-dependency of their effects, octopamine and tyramine solutions were also injected at 10%, 5%, and 1%. An amine solution (0.05 μl) was injected into each female, and the same amount of Milli-Q water was injected into females as control. Injection was conducted in a laboratory kept at 25 °C. A few hours after the injection, the female and a virgin male were placed in a small plastic Petri dish (1.5 cm height, 3.0 cm diameter) kept in the chamber described above, and whether female mates or not for 1 h. Females have mature eggs in their oviducts and bursa copulatrix when they emerged from bean, and 3–6-day-old virgin females have enough mature eggs in both *C. maculatus* (Wang and Horng, 2004) and *C. chinensis* (Yamane, personal observation). Therefore, they should already develop ovaries at mating. In addition, they lay immediately after mating with males. So it is thought that they enhance egg-laying after mating. To measure the number of eggs laid by females, virgin female injected with biogenic amine and an adzuki bean were immediately transferred after injection to a separate 24-well tissue culture plate (Nalge Nunc International K.K., Tokyo, Japan), and the numbers of eggs laid on a bean in each 24-h period was counted for 5 days. If eggs were laid on a bean, the bean was replaced with a new one.

2.3. Statistics

To compare female mating receptivity among treatments, the sequential Bonferroni methods (Rice, 1989) were applied after the χ^2-test at the 5% significance level. StatView Version 5.0 (SAS Institute, 1998) was used for χ^2-tests.

Numbers of eggs were compared using repeated-measures one-way ANOVA with "amines" as the between-subject factor and "days after injection" as the within-subject factor. Because Mauchly's test indicated a significant violation of the assumption of sphericity ($P < 0.001$), significant levels for within-subject effects were calculated using a Greenhouse-Geisser test for the degrees of freedom (Quinn and Keough, 2002). Separate one-way ANOVA tests were applied to the differences among amines. When a significant interaction effect was encountered between-subject and within-subject, separate one-way ANOVA tests were applied to the differences among amines on each day. Tukey–Kramer tests with sequential Bonferroni corrections were performed to assess differences among amines when significant effects were detected in the separate one-way ANOVA. The level of statistical significance was set at $P < 0.05$. JMP Version 6.0.3 (SAS Institute, 2005) was used for a series of analyses.

3. Results

Injection of octopamine or tyramine solutions significantly reduced female receptivity compared to controls in *C. chinensis*, whereas no differences were found between injections of control and dopamine or serotonin solutions and the control (Fig. 1). Injection of 5% or 10% octopamine solution significantly reduced female receptivity compared to 1% solution or control, but no difference in receptivity was found between the control and 1% solution or between 5% and 10% solutions (Fig. 2a). Injection of 5% or 10% tyramine solutions significantly reduced female receptivity compared to the control or 1% solution, whereas no difference in receptivity was found between the control or 1% solution or between 5% and 10% solutions (Fig. 2b). These results suggest that the effects of these amines on reduction of female receptivity are dose-dependent in *C. chinensis*.

Fig. 3 shows the total number of eggs laid by *C. chinensis* females injected with Milli-Q water (control), dopamine, octopamine, tyramine, or serotonin solutions. A repeated-measures one-way ANOVA for the eggs laid by injected females revealed that "amines" as between-subject, and "days after injection" and by "amine interaction" effects were all significant factors affecting the number of eggs laid (Table 1). Therefore, separate one-way ANOVA tests were applied to the differences among amines in each day. The number of eggs laid differed significantly among amine

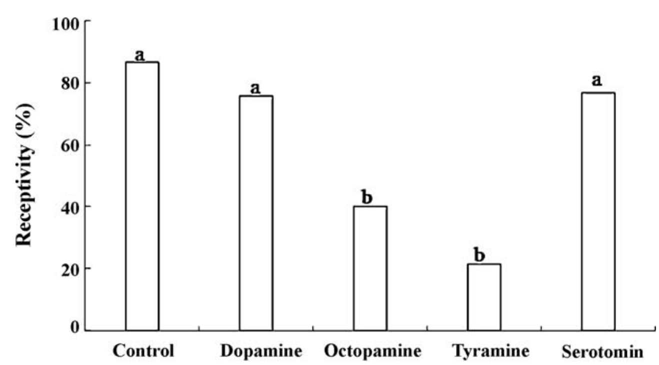

Fig. 1. Receptivity of *Callosobruchus chinensis* females to Milli-Q water as the control (*N* = 30), or solution of dopamine (*N* = 29), octopamine (*N* = 30), tyramine (*N* = 30), or serotonin (*N* = 30) at 3–4 h after injection. The same letters on each bar indicate no significant difference by the sequential Bonferroni method (Rice, 1989) at 5% significant level after χ^2-tests.

Table 1
Repeated-measures one-way ANOVA of the eggs laid by *Callosobruchus chinensis* females after amine injection.

Source	*df*	Mean squares	*F*	*P*
Between-subject				
Amine	4	105.24	14.30	<0.001
Error	486	22.81		
Within-subject				
Day	1.19	10.21	10.21	0.001*
Day x amine	4.78	1.42	1.42	0.012*
Error	90.76	1.71		

* Corrected *P*, Greenhouse-Geisser ε = 0.2388.