Ejaculatory strategies associated with experience of losing

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Modulation of behaviours as a result of fighting experience has been observed in many animals and can influence pre-copulatory sexual selection. This study investigated how fighting experience affects ejaculatory strategies. In male flour beetles, *Gnatocerus cornutus*, experience of losing a fight decreases a male's aggressiveness for up to 4 days. We found that males losing a fight show increased ejaculatory investment, but there was no ejaculatory modulation owing to winning. However, the increase in ejaculate investment following a loss was no longer observed after 5 days. These results indicate that males adjust their investment in sperm competition according to their experience, and that fighting experience can significantly influence pre- and post-copulatory reproductive tactics.

Keywords: alternative tactics; phenotypic plasticity; sperm competition; winner and loser effects

1. INTRODUCTION

Male–male competition is a key component of sexual selection and can influence the evolution of morphology and behaviour (Andersson 1994). However, sexual selection can continue after copulation, and when a female copulates with two or more males, the sperm of those males compete in the female reproductive tract for fertilization of her eggs (Parker 1970). Furthermore, males are predicted to adjust their investment in ejaculatory expenditure to maximize their fertilization success according to the situation in which they are placed (Parker 1990, 1998). Indeed, there is abundant evidence that males adjust investment in response to the risk of sperm competition (e.g. Hosken 1997; Hosken & Ward 2001) and males may need to invest not only in pre-copulatory fighting but also in sperm competition (Simmons & Emlen 2006).

Often males engage in fighting over mates more than once during their lifespan, and previous contest experience often influences current contest behaviours (Hsu *et al.* 2006; Rutte *et al.* 2006). After winning fights, males often establish territories such as a nest and a harem, and guard these territories against intruder males in order to maintain their paternity

(Shuster & Wade 2003; Hsu *et al.* 2006); thus, these males are sometimes at risk of sperm competition with intruders (Simmons *et al.* 1999, 2007). By contrast, because losing males obtain no mating success through fighting, they reduce their investment in physical fighting (e.g. energy and time) across a wide range of taxa (reviews in Hsu *et al.* 2006). Instead, such males may use other behavioural tactics to obtain mates, for example, searching for territories and females, and sneaking behaviour (Moczek & Emlen 2000; Okada & Miyatake 2010). These males are frequently subject to sperm competition with the males guarding females and/or territories (Simmons *et al.* 1999, 2007). Therefore, the risk of sperm competition may differ between males with a history of winning and losing. Because this difference can affect ejaculatory characters (Pizzari & Parker 2009), ejaculatory strategies may be influenced by fighting experience.

Here, we investigated whether males adjusted their investment in ejaculates according to their previous fighting experience, using the broad-horned flour beetle *Gnatocerus cornutus*. These males usually fight for territories, and losing a fight decreases male aggressiveness for 4 days following a fight (Okada & Miyatake 2010). Okada & Miyatake (2010) indicate that males adjust their investment in physical fighting, based on the previous experience. Because females copulate with two or more males (Yamane *et al.* 2010), the resulting sperm competition could also select on ejaculatory characteristics. In this study, we compare the number of sperm within females mated to males that had experienced victory or defeat, and discuss ejaculatory strategies associated with the experience of losing in male *G. cornutus*.

2. MATERIAL AND METHODS

(a) Insects and manipulation of fighting experience

The *G. cornutus* beetle culture used originated from adults collected in Miyazaki City (31°54' N, 131°25' E), Japan, in June 1957. Following Okada & Miyatake (2010), we obtained males with winning (W) and losing (L) experiences. See methods in the electronic supplementary material for details.

(b) Effect of experience on ejaculatory expenditure

To examine the number of sperm transferred by males at 1 day before the manipulation of experience described above, 120 males with no fighting experience—'potential' W and L males—were paired with virgin females collected from the stock culture (first copulation). Having manipulated their experience, we obtained 60 W and L males and divided them into two blocks of 30 W and L males. As shown in our previous study (Okada & Miyatake 2010), the experience of losing lasts 4 days, with no apparent decay in the effect during that period. W and L males were paired individually with virgin females at 1 day after the fight and the other block were paired with virgin females 5 days after the fight (second copulation), to investigate the effect of experience on the sperm number. Following Yamane *et al.* (2010), we assessed the number of sperm transferred by males. See methods in the electronic supplementary material for details.

(c) Effect of experience on fighting behaviour

To investigate whether the effects of experience influenced the outcome of a subsequent fight, we staged 20 contests between an N male (males with no fighting experience) and a male that had experienced either a victory (W) or a defeat (L), in the same manner as the manipulation of experience, at 1 and 5 days after the manipulation of experience.

(d) Statistics

In each block (first and second copulations: 1st day before a fight and 1st day after a fight or 1st day before a fight and 5th day after a fight), copulation duration and sperm number transferred by males were analysed using repeated measures analysis of variance (ANOVA),

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Figure 1. Mean ($\pm$ s.e.) number of sperm transferred by males on first day before fighting and (a) 1 day or (b) 5 days after fight. $n = 30$ in each bar. Before fighting, males have no fighting experience, i.e. 'potential' W (filled bars) and L (white bars) males. Different letters indicate a significant difference at $p < 0.05$ by Student's t -test with the sequential Bonferroni method ($\alpha = 0.05$; Rice 1989).

with experience (W and L) as the between-subject factor and copulation order (first and second copulations) as the within-subject factor. As a post hoc test, a Student's t -test was used to compare the mean sperm number correcting the significance level for multiple comparisons by the sequential Bonferroni method (Rice 1989). All statistical analyses were carried out using JMP 7 (SAS Institute 2007).

3. RESULTS

(a) Effect of fighting experience on ejaculatory expenditure

For the block of males that copulated both 1 day before and 1 day after the manipulation of experience, we detected a significant effect of experience and a significant interaction between experience and copulation order on sperm number (repeated measures ANOVA, experience: $F_{1,58} = 8.498$, $p = 0.005$; copulation order: $F_{1,58} = 0.203$, $p = 0.654$; experience $\times$ copulation order: $F_{1,58} = 7.027$, $p = 0.010$). Sperm number was significantly greater when L males copulated on the first day after the manipulation, compared to other males (figure 1a). This indicates that the losing experience positively influences the number of sperm transferred by the male. By contrast, for males that copulated 1 day before and 5 days after

Figure 2. (a) Frequencies of attacking and (b) winning of W (filled bars) and L (white bars) males 1 day and 5 days after fighting, $n = 20$ in each bar. Asterisk indicates L males attack and win significantly less frequently than opponents as males with no fighting experience ($p < 0.0001$, χ^2 -test).

manipulation, no significant experience effect was found (experience: $F_{1,58} = 0.194$, $p = 0.661$; copulation order: $F_{1,58} = 1.677$, $p = 0.201$; experience $\times$ copulation order: $F_{1,58} = 0.584$, $p = 0.448$); 5 days after a fight, there was no modulation of sperm number owing to fighting experience (figure 1b). There was no modulation of copulation duration owing to fighting experience in either block (see the electronic supplementary material, figure S1, first day after the manipulation, experience: $F_{1,58} = 1.397$, $p = 0.242$; copulation order: $F_{1,58} = 0.0001$, $p = 0.993$; experience $\times$ copulation order: $F_{1,58} = 0.255$, $p = 0.615$; fifth day after the manipulation, experience: $F_{1,58} = 1.689$, $p = 0.199$; copulation order: $F_{1,58} = 0.778$, $p = 0.381$; experience $\times$ copulation order: $F_{1,58} = 0.393$, $p = 0.533$).

(b) Effect of fighting experience on fighting behaviour

Figure 2 shows the frequencies of males attacking the opponent first (a) and winning (b) by W and L males. On the first day after the manipulation of experience, L males never attacked and were always defeated by W males with no fighting experience

(attacking, $\chi^2_1 = 20$, $p < 0.0001$; winning, $\chi^2_1 = 20$, $p < 0.0001$). By contrast, no difference was found between L and N males on the fifth days after the manipulation (attacking, $\chi^2_1 = 0.2$, $p = 0.655$; winning, $\chi^2_1 = 0$, $p > 0.999$). On the other hand, no difference was found in the frequencies of attacks or wins between W and N males, whether tested 1 or 5 days after the manipulation (on the first day, attacking, $\chi^2_1 = 0.2$, $p = 0.655$; winning, $\chi^2_1 = 0.2$, $p = 0.655$; on the fifth day, attacking, $\chi^2_1 = 0$, $p > 0.999$; winning, $\chi^2_1 = 0.2$, $p = 0.655$).

4. DISCUSSION

The experience of losing a fight resulted in an increase in the number of sperm transferred to females by males, but there was no change in sperm number owing to winning in male *G. cornutus*. However, 5 days after fighting, the number of sperm transferred by losing males had returned to standard level. Our previous study showed the experience effect lasted 4 days (Okada & Miyatake 2010), indicating that males can adjust their investment in ejaculates in response to the experience of losing. Additionally, while the losing experience effect lasted, the males never attacked and fought any male, but stopped investing in physical fighting (figure 2a,b). Our results are consistent with our previous study (Okada & Miyatake 2010) and indicate that males adjust their investment in pre- and post-copulatory reproductive tactics based on their previous fight experience.

Several studies have indicated a short-term switch in ejaculatory expenditure in response to proximate experiences, sperm competition conditions or risk states (e.g. Parker *et al.* 1997; Pound & Gage 2004). Male *G. cornutus* normally guard territories and mates (Okada & Miyatake 2010). Winning males have higher remating rates when mating with the same female, and these males thus face a lower risk of sperm competition (Yamane *et al.* 2010). They are therefore unlikely to need to inseminate each female with more sperm per copulation. After winning fights, they do not adjust their investment in sperm, but instead guard territories in order to maintain their paternity, similar to findings in many animals (review in Hsu *et al.* 2006).

By contrast, losing males disperse to new territories, which may or may not contain other males, instead of fighting for mates (Okada & Miyatake 2010). These males are always subject to sperm competition with the males guarding females and territories (Yamane *et al.* 2010). In this case, high fitness will be related to the ability to succeed in sperm competition (Simmons *et al.* 1999, 2007). Indeed, there is strong theoretical and empirical evidence that sperm competition selects for males that invest heavily in sperm production (e.g. Parker 1998; Hosken & Ward 2001; Simmons 2001). Thus selection may favour mechanisms in which the experience of losing increases investment in spermatogenesis in male *G. cornutus*. As result, males may acquire the ability to adjust their investment in ejaculation in response to proximate experience, similar to findings in the rat *Rattus norvegicus* (Pound & Gage 2004). Increased ejaculatory expenditure is predicted to be positively

associated with fertilization success when the outcome of sperm competition is based on a lottery (Simmons 2001); we actually think further investigation is necessary to determine whether increased ejaculatory expenditure results in increased fertilization success under conditions of sperm competition, in *G. cornutus*. However the current study shows that experience effects, which are a widespread phenomena in animals (Hsu *et al.* 2006), can influence not only pre-copulatory but also post-copulatory sexual selection.

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- Andersson, M. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.
- Hosken, D. J. 1997 Sperm competition in bats. *Proc. R. Soc. Lond. B* **264**, 385–392. (doi:10.1098/rspb.1997.0055)
- Hosken, D. J. & Ward, P. J. 2001 Experimental evidence for testis size evolution via sperm competition. *Ecol. Lett.* **4**, 10–13. (doi:10.1046/j.1461-0248.2001.00198.x)
- Hsu, Y., Earley, R. L. & Wolf, L. L. 2006 Modulation of aggressive behavior by fighting experience: mechanisms and contest outcomes. *Biol. Rev.* **81**, 33–74. (doi:10.1017/S146479310500686X)
- Moczek, A. P. & Emlen, D. J. 2000 Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.* **59**, 459–466. (doi:10.1006/anbe.1999.1342)
- Okada, K. & Miyatake 2010 Effect of losing on male fights of broad horned flour beetle, *Gnatocerus cornutus*. *Behav. Ecol. Sociobiol.* **64**, 361–369. (doi:10.1007/s00265-009-0852-0)
- Parker, G. A. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567. (doi:10.1111/j.1469-185X.1970.tb01176.x)
- Parker, G. A. 1990 Sperm competition games: raffles and roles. *Proc. R. Soc. Lond. B* **242**, 120–126. (doi:10.1098/rspb.1990.0114)
- Parker, G. A. 1998 Sperm competition and the evolution of ejaculates: towards a theory base. In *Sperm competition and sexual selection* (eds T. R. Birkhead & A. P. Møller), pp. 3–54. London, UK: Academic Press.
- Parker, G. A., Ball, M. A., Stockley, P. & Gage, M. J. G. 1997 Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. Lond. B* **264**, 1793–1802. (doi:10.1098/rspb.1997.0249)
- Pizzari, T. & Parker, G. A. 2009 Sperm competition and sperm phenotype. In *Sperm biology* (eds T. R. Birkhead, D. J. Hosken & S. Pitnick), pp. 207–245. Oxford, UK: Academic Press.
- Pound, N. & Gage, M. J. G. 2004 Prudent sperm allocation in Norway rats, *Rattus norvegicus*: a mammalian model of adaptive ejaculate adjustment. *Anim. Behav.* **68**, 819–823. (doi:10.1016/j.anbehav.2004.02.004)
- Rice, W. R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225. (doi:10.2307/2409177)
- Rutte, C., Taborsky, M. & Brinkhof, M. W. 2006 What sets the odds of winning and losing? *Trends. Ecol. Evol.* **21**, 16–21. (doi:10.1016/j.tree.2005.10.014)
- SAS Institute. 2007 *JMP release 7*. Cary, NC: SAS Institute Inc.

- Shuster, S. M. & Wade, M. J. 2003 *Mating system and strategies*. Princeton, NJ: Princeton University Press.
- Simmons, L. W. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton, NJ: Princeton University Press.
- Simmons, L. W. & Emlen, D. J. 2006 Evolutionary trade-off between weapons and testes. *Proc. Natl Acad. Sci. USA* **103**, 16 346–16 351. (doi:10.1073/pnas.0603474103)
- Simmons, L. W., Tomkins, J. L. & Hunt, J. 1999 Sperm competition games played by dimorphic male beetles. *Proc. R. Soc. Lond. B* **266**, 145–150. (doi:10.1098/rspb.1999.0614)
- Simmons, L. W., Emlen, D. J. & Tomkins, J. L. 2007 Sperm competition games between sneaks and guards: a comparative analysis using male beetles. *Evolution* **61**, 2684–2692. (doi:10.1111/j.1558-5646.2007.00243.x)
- Yamane, T., Okada, K., Nakayama, S. & Miyatake, T. 2010 Dispersal and ejaculatory strategies associated with exaggeration of weapon in an armed beetle. *Proc. R. Soc. B* **277**. (doi:10.1098/rspb.2009.2017)