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The influence of sex, line, and fight experience on aggressiveness of the Siamese fighting fish in intrasexual competition

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Abstract

We examined the influence of sex, line, i.e., broods from different parents, and previous fight experience on the aggressiveness of the Siamese fighting fish *Betta splendens* in intrasexual competition. The innate aggressiveness of the fish against their mirror images was measured on the day prior to the direct fight with other individuals, and it was found to be influenced by the line type but not by the sex. In the direct fight with other individuals, the males invested more effort in the fight than the females. In addition, the individuals of a particular line that exhibited a lower innate aggressiveness spent less time in the direct fight and were often losers when compared with those of other lines. After the direct fight with other individuals, the aggressiveness of the fish against their mirror images was remarkably influenced by the outcome of the direct fight, i.e., the winners exhibited more aggressive behavior, whereas the losers exhibited a lesser degree of aggressive behavior. This influence of the previous fight experience on subsequent aggressiveness was the greatest in the individuals of the line that have exhibited the lowest innate aggressiveness. However, the positive effect of the winning experience or the negative effect of the losing experience on subsequent aggressiveness decreased following several days after the previous fight increased.

Keywords: Aggressive behavior; *Betta splendens*; Fight outcome; Intrasexual competition; Sexual difference

1. Introduction

Animals often compete with individuals of the same species to access resources. In intraspecific competition in particular, the individuals of the same sex fight intensely with each other for the same resources. For example, males of many species frequently fight with other males to acquire females as their mates (Andersson, 1994). Females of some species also compete with other females to obtain resources such as territories, high social status, or mates (Smith et al., 1994; Karino, 1999; McDonald et al., 2005; Shibata and Kohda, 2006). However, competition often results in the contestants incurring costs such as injuries, stress, and exhaustion. Therefore, the degree of investment of effort in a competition by an individual may be influenced by many factors, e.g., body conditions, fight opponents, and rewards of the competition (Rohwer and Rohwer, 1978; Austad, 1983; Moczek and Emlen, 2000). In addition, genetic factors also affect the degree of aggressiveness of individuals. For example, the aggressiveness of some animals can be enhanced or reduced by artificial selection experiments (Bakker, 1986; Doyle and Talbot, 1986).

The Siamese fighting fish *Betta splendens* is one of the model species to study competition in animals because the males of this species exhibit typical aggressive behavior (Simpson, 1968; Robertson and Sale, 1974). *B. splendens* females also exhibit aggressive behavior against other females (Braddock and Braddock, 1955). The innate aggressiveness of the *B. splendens* males is known to be a predictor of the outcome of male-male competition (Bronstein, 1985). *B. splendens* males monitor the aggressive interactions of other males, and this information affects their aggressiveness in the future fights (Oliveira et al., 1998; McGregor et al., 2001; Clotfelter and Paolino, 2003). It is known that previous social status also influences the aggressiveness of the males in the subsequent fights; the dominants behave more aggressively, whereas the subordinates exhibit a lesser degree of aggressive behavior (Wallen and Wojciechowski-Metzlar, 1985). However, to date, the influence of genetic factors and sex on aggressiveness have not been thoroughly examined.

In the present study, we examined the influence of sex, genetic factors, and previous fight experiences on the aggressiveness of male and female *B. splendens*. We predicted the following results: (1) In the natural environment, *B. splendens* males compete with other males to acquire a territory that is an essential resource for their

reproduction (Jaroensutasinee and Jaroensutasinee, 2001, 2003). Thus, it is possible that males exhibit a greater aggressive behavior than females. (2) Individuals possessing genetic tendencies to behave more aggressively may win fights with their opponents having less aggressive tendencies. (3) The influence of a previous fight experience on subsequent aggressiveness may be determined by the degree of rewards or stresses of the previous fight, i.e., winners who expect to obtain rewards with a relatively high value may exhibit a high frequency of aggressive behavior, whereas losers with greater stresses may exhibit less aggressive behavior.

2. Materials and methods

2.1. Study animals

We obtained parental males and females exhibiting different body colorations from several local wholesalers in Tokyo, Japan. It is known that Japanese wholesalers imported *B. splendens* from breeders in southeastern Asia and these breeders usually breed *B. splendens* individuals having different body colorations separately in order to maintain their coloration (Abe and Yamazaki, 2000). Therefore, it can be considered that fish exhibiting different body colorations belonged to different sub-populations, i.e., lines (Falconer and Mackay, 1996).

In the present study, test fish (F1 generation) of three original lines were obtained by pairing parental males and females with different body colorations, i.e., line 1 was obtained by pairing a red male and a yellow female; line 2 by pairing a green male and an azure female; and line 3 by pairing a blue male and a purple female. Each parental pair was maintained within an aquarium and bred once. The offspring of each parental pair were reared within an aquarium (45 × 30 × 30 cm) with circulated water for 6 months. After 6 months, each individual offspring was maintained singly within a tank (13 × 13 × 10 cm) for one month prior to the experiment. In order to visually isolate subjects from other fish, the tanks were covered by opaque films. We obtained 16 males and 14 females of line 1, 16 males and 8 females of line 2, and 16 males and 14 females of line 3. The fish were maintained on a 12 h:12 h light: dark cycle at 25-30°C, and they were fed newly hatched brine shrimp nauplii.

2.2. *Experimental procedure*

We used small aquariums ($15.6 \times 11.6 \times 11.3$ cm) for the experiment. Each aquarium was filled with 1 l water. A 20-watt daylight lamp was placed 20 cm above the aquarium, and the water temperature was maintained at 27°C. Three sides of the aquarium were covered by opaque boards to exclude any disturbances.

We conducted three trials for each test fish: (1) the measurement of the aggressive behavior of the fish against its mirror image in order to quantify the innate aggressiveness, (2) a direct fight with another individual of a different line, and (3) the measurement of the aggressive behavior of the fish against its mirror image to quantify the influence of the direct fight on their subsequent aggressiveness. In each trial, we recorded fish behavior by using a digital video camera (DCR-TRV30, Sony, Japan) and measured the number of gill cover erection (GCE) as a parameter for the aggressiveness of each fish. GCE is known to be one of the typical aggressive behaviors in *B. splendens*, and it is a reliable indicator of their fighting ability (Clayton and Hinde, 1968; Evans, 1985; Abrahams et al., 2005).

On the day prior to the direct fight trial, we measured the innate aggressiveness of each test fish against its mirror image. It is known that *B. splendens* individuals frequently exhibit aggressive behavior against their mirror images, and the frequency of this behavior was used as a parameter for the aggressiveness of the fish (Baenninger, 1984; Wallen and Wojciechowski-Metzlar, 1985; Abrahams et al., 2005). A mirror that was covered with an opaque board was placed at one side of the aquarium. A test fish was placed in the aquarium. After a 10-min acclimation period, the opaque board was removed, and the aggressive behavior exhibited by the test fish against its mirror image was recorded for 20 min. The test fish exhibited frequent aggressive behavior against their mirror images through the trial. The test fish was then transferred to its original tank immediately after the trial.

In the direct fight trial, we used a pair of fish having the same sex from different lines (Table 1). The difference in the body size between the fighting pair was 1.9 ± 0.3 mm (mean \pm S.E.) in males ($N = 24$ pairs) and 1.1 ± 0.2 mm in females ($N = 18$ pairs). The experimental aquarium was divided into two compartments of identical

sizes by an opaque partition. Artificial weeds were placed at a corner of each compartment as a refuge. One individual of the fighting pair was placed in one of the compartments, and the other individual was placed in the other compartment. After a 10-min acclimation period, the opaque partition was removed, and the fish behavior was recorded for 90 min. When one individual ceased to exhibit aggressive behavior and hid itself in the weeds, we judged that the outcome of the fight was determined. The retreated individual was referred to as a loser, whereas its opponent was a winner. In the preliminary experiment, the fight outcomes of the intrasexual competition in *B. splendens* were usually determined during 90 min. In addition to the number of GCE by each individual, we recorded time (min) spent by the fish for the fight (fight duration). After the end of the fight, the winner often performed GCE against the loser, while the loser did not exhibit GCE. We also recorded the number of receiving GCE by the losers from the winners from the end of the fight to the end of the trial.

In order to quantify the influence of the direct fight experience on subsequent aggressiveness of the test fish, we measured the number of GCE of each fish against its mirror image by using a method identical to that used for the measurement of innate aggressiveness. We repeated this measurement on 1, 5, 10, 15, 20, and 30 days after the direct fight trial for each test fish. During this period, the test fish were visually isolated from other fish.

2.3. *Statistical analysis*

We conducted two-way ANOVA to examine the influence of sex and line on the innate aggressiveness of the test fish. In the direct fight trial, we performed three-way ANOVA to measure the effect of sex, line, and the outcome of the fight on the aggressiveness of the test fish during the fight. In addition, the aggressiveness within the fighting pair was compared by paired *t*-test. To clarify the effect of body size of the fish on aggressiveness in the direct fight, we also conducted ANCOVA with the number of GCE or fight duration as the dependent variable, the line type as a factor, and body size as a covariate. In order to quantify the influence of the direct fight on the subsequent aggressiveness of the test fish, we calculated the differences (%) in the number of GCE on each day (1, 5, 10, 15, 20, and 30 days) after the direct fight and that

in the trial prior to the direct fight, i.e., innate aggressiveness. The difference was analyzed by three-way repeated-measures ANOVA. All data satisfied the assumptions of parametric tests, such as normality and equality of variances. Data were analyzed using StatView 5.0 (SAS institute, NC, USA) and JMP 5.1.2 (SAS institute).

3. Results

No sexual difference was observed in the innate aggressiveness that was measured on the day prior to the direct fight trial in terms of the number of GCE (ANOVA: $F_{1,78} = 0.1$, $P = 0.71$; Tables 2). The aggressiveness of the test fish was significantly influenced by its line type ($F_{2,78} = 21.9$, $P < 0.001$). Fish of line 3 exhibited more GCE than those of lines 1 and 2 (Fisher's PLSD, $P < 0.01$), and fish of line 2 invested more effort in GCE than those of line 1 ($P < 0.01$; Table 2). The interaction between the sex and line type did not affect the innate aggressiveness of the test fish (ANOVA: $F_{2,78} = 0.01$, $P = 0.99$). The innate aggressiveness did not differ between the males who were winners and losers in the direct fight on the next day (number of GCE: paired t -test, $t_{23} = 1.5$, $P = 0.15$; Table 2). In the females, the innate aggressiveness did not differ between the winners and losers in the direct fight trial (number of GCE: $t_{17} = -0.2$, $P = 0.83$; Table 2). Therefore, the innate aggressiveness of the test fish was primarily determined by the line type.

In the direct fight trial, fewer males (25%) of line 1 were winners, whereas a greater number of males (62.5%) of both lines 2 and 3 were winners ($G^2 = 6.2$, $P = 0.04$; Table 1). In the females, the frequencies of the winners and losers in the direct fight trial did not differ among the lines ($G^2 = 0.6$, $P = 0.75$; Table 1). The body sizes (standard length) of the males did not differ between the winners (mean \pm S.E.: 39.1 ± 0.4 mm) and losers (39.3 ± 0.4 mm, ANOVA: $F_{1,42} = 0.9$, $P = 0.34$). The body sizes of males also did not differ among the line type (line 1: 38.5 ± 0.4 mm; line 2: 39.7 ± 0.6 mm; line 3: 39.4 ± 0.4 mm; $F_{2,42} = 1.9$, $P = 0.16$), and the interaction between the fight outcomes and line type was not influenced by their body sizes ($F_{2,42} = 0.5$, $P = 0.60$). In the females, no difference was observed between the body sizes of winners (36.2 ± 0.3 mm) and losers (35.8 ± 0.4 mm, ANOVA: $F_{1,30} = 1.6$, $P = 0.21$). The body sizes of females were not significantly different among the line type (line 1: 35.5 ± 0.3 mm; line

2: 36.6 ± 0.7 mm; line 3: 36.2 ± 0.3 mm; $F_{2,30} = 1.9$, $P = 0.16$), and the interaction between the fight outcomes and line type was not affected by their body sizes ($F_{2,30} = 3.0$, $P = 0.06$). These results indicate that body size of the test fish did not influence the fight outcome although the line type affected the fight outcome in the males.

In the direct fight trial, the male fighting pairs exhibited a longer fight duration than females (Tables 2, 3). The fight duration was also influenced by the line type (Table 3). Individuals of line 1 spent a shorter time for the fight than those of lines 2 and 3 (Fisher's PLSD, $P < 0.05$), while the fight duration did not differ between lines 2 and 3 ($P = 0.34$; Table 2). As a result of ANCOVA, the fight duration of the males was not significantly influenced by both their body sizes (ANCOVA: $F_{1,44} = 0.01$, $P = 0.94$) and line type ($F_{2,44} = 1.7$, $P = 0.19$). In the females, neither body size (ANCOVA: $F_{1,32} = 0.4$, $P = 0.54$) nor line type ($F_{2,32} = 1.4$, $P = 0.25$) influenced the fight duration.

During the direct fight, the males invested considerably more effort than the females in the number of GCE (Tables 2, 3). No other factors or interactions among the factors significantly influenced the number of GCE during the direct fight (Table 3). In the males, effects of both body size and line type on the number of GCE were not significant (ANCOVA: body size: $F_{1,44} = 1.4$, $P = 0.24$; line type: $F_{2,44} = 0.3$, $P = 0.76$). The number of GCE of the females was not influenced by both body size (ANCOVA: $F_{1,32} = 3.1$, $P = 0.09$) and line type ($F_{2,32} = 0.2$, $P = 0.83$). In the male fighting pairs, no significant difference was observed in the number of GCE between the winners and losers (paired t -test, $t_{23} = 0.9$, $P = 0.38$; Table 2). In the female fighting pairs, the number of GCE did not differ between the winners and losers ($t_{17} = -0.5$, $P = 0.38$; Table 2). The number of GCE exhibited by the winners showed a high correlation with that exhibited by the losers (males: $r = 0.89$, $N = 24$, $P < 0.001$; females: $r = 0.89$, $N = 18$, $P < 0.001$). These results indicate that the test fish exhibited aggressive behavior with a similar frequency to their fight opponents.

In the direct fight trial, the number of GCE received by the losers from the winner after the fight was influenced by the line type (ANOVA: $F_{2,36} = 20.6$, $P < 0.001$). The losers of line 1 received more GCE than those of lines 2 and 3 (Fisher's PLSD, $P < 0.01$; Fig. 1). The losers of line 2 also received more GCE than those of line 3 ($P < 0.01$). The number of GCE received by the losers was not influenced by sex ($F_{1,36} = 0.4$, $P = 0.54$) or the interaction between sex and line ($F_{2,36} = 0.7$, $P = 0.49$; Fig. 1).

The outcome of the direct fight trial showed a remarkable influence on the subsequent aggressiveness of the test fish (Table 4). The winners exhibited more GCE against their mirror images as compared to those prior to the direct fight trial, whereas the losers exhibited fewer GCE (Figs. 2, 3). In the repeated-measures ANOVA, the number of days after the direct fight trial and the interaction of this factor with the fight outcome significantly influenced the number of GCE (Table 4); the influence decreased following several days after the direct fight, but the influence persisted for a longer duration in the losers than in the winners (Figs. 2, 3). The interaction among the number of days after the direct fight, line type, and fight outcome also influenced the number of GCE (Table 4). Immediately after the direct fight, the individuals of line 1 changed their aggressiveness more drastically than those of the other lines, i.e., the winners and losers of line 1 exhibited more and fewer GCE, respectively, on the day after the direct fight when compared with their innate aggressiveness (Figs. 2, 3). The sex, line, and other interactions among the factors did not significantly affect the aggressiveness of the test fish after the direct fight trial (Table 4). The subsequent aggressiveness of the male losers on the day after the direct fight negatively correlated with the number of GCE received by them from winners after the fight in the direct fight trial ($r = -0.60$, $P = 0.002$, $N = 24$). In females, the correlation between the subsequent aggressiveness by the losers on the day after the direct fight and the number of GCE received by them from winners after the fight in the direct fight trial was not significant ($r = -0.43$, $P = 0.07$, $N = 18$). Therefore, the negative effect of losing experience on the subsequent aggressiveness in males increased when the losers received more GCE from their opponents after the fight.

4. Discussion

The results of this study indicate that the innate aggressiveness of *B. splendens* is influenced by the line type. Although Noble (1939) has commented on the possibility of a genetic effect on the social dominancy in *B. splendens*, detailed information has not been reported. In the present study, no differences in the body size and age of the test fish of the same sex were observed among the lines. Therefore, the influence of the line type on the innate aggressiveness appears to be due to a genetic

effect. Since no sexual difference was observed in the innate aggressiveness of the test fish, the genetic effect may function with a similar manner in both sexes. The line type also affected the fight duration in the direct fight trial. The individuals of line 1 that exhibited lesser innate aggressiveness spent a shorter time for the direct fight than those of other lines. In the direct fight trial, the males of line 1 were often losers when compared with those of other lines. These results suggest that to a certain extent, the genetic factors may determine the investment of effort in intrasexual competition and may influence the fight outcome in the competition in *B. splendens*.

In the direct fight trial, the frequency of the aggressive behavior of the test fish increased to a level identical to that of their fight opponents regardless of their innate aggressiveness. This result indicates that individuals have to invest their effort in the competition at a similar level to their competitors during the fight. If the frequency of the aggressive behavior of some individuals is lower than that of their opponents during the fight, it may reduce their chance of winning the competition (Bronstein, 1985; Evans, 1985). The result of this study suggests that the effect of social environment on the aggressiveness during the fight may be greater than that of genetic tendencies in animals.

Although no sexual difference was observed in innate aggressiveness, the fight duration of females in the direct fight trial was shorter than that of males. Moreover, in the direct fight, the females invested less effort in GCE than the males. In the natural environment, *B. splendens* males fight with other males to acquire territories (Jaroensutasinee and Jaroensutasinee, 2001, 2003). The winners of this male-male competition can acquire a high-quality territory and mate with females within the territory. Therefore, it is essential for the males to win in the intrasexual competition in order to obtain reproductive success. Hence, males invest considerably more effort in the direct competition than females.

Following the experience of a direct fight with other individuals, the outcome of the fight remarkably affects the aggressiveness in both sexes. The winners of the previous fight exhibited an increase in their aggressiveness, whereas the losers exhibited a decrease in their aggressiveness. The result of this study is consistent with previous knowledge on male *B. splendens* (Baenninger, 1984; Wallen and Wojciechowski-Metzlar, 1985). Similar tendencies have also been reported in other fishes (Braddock, 1945; Frey and Miller, 1972). Recently, Frost et al. (2007) demonstrated that prior

experience influence the degree of boldness in rainbow trout *Onchorhynchus mykiss*. In *B. splendens*, since the winners of the intrasexual competition may obtain resources, i.e., territories in the case of males and high social rank in the case of females (Noble, 1939; Badura and Friedman, 1988; Jaroensutasinee and Jaroensutasinee, 2001; Sneksler et al., 2006), they should behave more aggressively against other rivals to defend their resources. However, the influence of the previous fight diminished following several days after the fight. In the present study, because the winners of the direct fight trial did not obtain any resources such as territory, they might revitalize their motivation to fight.

The number of GCE exhibited by the test fish after the direct fight trial was also influenced by the interaction among the number of days after the fight, line type, and fight outcome. Individuals of line 1 drastically changed their aggressiveness on the day after the fight. They exhibited GCE at a similar level to their fight opponents during the direct fight, although their innate aggressiveness was lower than that of the other lines. In addition, in the direct fight trial, the losers of line 1 received more GCE from their opponents after the fight than those of the other lines. This experience may have a severe negative effect on the losers of line 1, resulting in a decrease in their aggressiveness on the day after the direct fight. Indeed, the male loser exhibited aggressive behavior with lesser degree when they had received more GCE from their opponents in the direct fight trial in the previous day. The individuals of line 1 exhibited a low degree of innate aggressiveness, and the males of this line tended to be losers in the direct fight trial. Hence, it is possible that the winners of line 1 exhibit a greater aggressive behavior after the direct fight in order to defend their resources because they have fewer opportunities to access the resources.

In conclusion, in *B. splendens*, the sex influenced the investment of effort in a direct fight with other individuals, although this factor did not affect their innate aggressiveness. The innate aggressiveness and the outcome of the direct fight were influenced by genetic factors. However, the frequency of aggressive behavior during the direct fight was determined by that of the fight opponents and was not influenced by the line type. The outcome of the previous fight apparently influenced the subsequent aggressiveness in future fights. These factors as well as other factors such as physiological conditions and the information on aggressiveness of other individuals (Oliveira et al., 1998; McGregor et al., 2001) may combine and determine the

aggressiveness and fight outcome of *B. splendens* in nature.

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Table 1. The numbers of fighting pairs and winners in the direct fight trial. The number of winners indicates that of the line in the row.

	Fighting pair			Winners		
	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3
Males						
Line 1		8	8		1	3
Line 2			8	7		3
Line 3				5	5	
Females						
Line 1		4	10		3	3
Line 2			4	1		3
Line 3				7	1	

Table 2. Mean \pm SE of the number of GCE (gill cover erection) by the test fish on the day prior to the direct fight, and those of fight duration and the number of GCE by the test fish during the direct fight with other fish.

Categories (n)	Previous day of	During the direct fight trial	
	<u>the direct fight</u>	Fight	Number of
	Number of	duration (min)	GCE
	GCE		
Sex			
Males (48)	63.3 \pm 2.8	68.9 \pm 2.1	134.6 \pm 6.1
Females (36)	64.6 \pm 3.2	48.1 \pm 2.8	76.9 \pm 4.0
Line			
Line 1 (30)	49.9 \pm 2.8	53.1 \pm 3.0	104.5 \pm 7.5
Males (16)	48.9 \pm 3.9	63.1 \pm 3.5	125.2 \pm 10.4
Females (14)	51.1 \pm 4.2	41.7 \pm 2.7	80.9 \pm 6.8
Line 2 (24)	64.5 \pm 3.2	66.0 \pm 3.5	115.6 \pm 10.1
Males (16)	64.3 \pm 4.4	71.2 \pm 3.1	138.8 \pm 10.7
Females (8)	65.1 \pm 4.0	55.8 \pm 7.5	69.4 \pm 8.0
Line 3 (30)	77.3 \pm 2.9	62.0 \pm 3.8	110.7 \pm 8.6
Males (16)	76.8 \pm 3.9	72.4 \pm 4.1	139.9 \pm 10.7
Females (14)	77.9 \pm 4.6	50.1 \pm 5.0	77.4 \pm 6.3
Winners within fighting pair			
Males (24)	67.9 \pm 3.7		136.4 \pm 8.9
Females (18)	63.8 \pm 4.3		76.3 \pm 5.6
Losers within fighting pair			
Males (24)	58.8 \pm 4.1		132.8 \pm 8.4
Females (18)	65.4 \pm 4.8		77.6 \pm 5.8

Table 3. Results of ANOVA for fight duration and the number of gill cover erections (GCE) of test fish in the direct fight trial.

Factors	Fight duration			Number of GCE		
	df	F	P	df	F	P
Sex	1	32.3	<0.001	1	48.2	<0.001
Line	2	3.9	0.02	2	0.1	0.91
Fight outcome				1	0.03	0.87
Sex × line	2	0.3	0.71	2	0.8	0.43
Sex × fight outcome				1	0.001	0.98
Line × fight outcome				2	0.8	0.45
Sex × line × fight outcome				2	0.4	0.70
Residual	78			72		

Table 4. Results of repeated-measured ANOVA for the number of gill cover erections by the test fish on the particular days (1, 5, 10, 15, 20 and 30 day) after the direct fight trial.

Factors	df	F	P
Subjects			
Sex	1	0.2	0.64
Line	2	1.5	0.23
Fight outcome	1	19.5	<0.001
Sex × line	2	0.8	0.46
Sex × fight outcome	1	0.001	0.99
Line × fight outcome	2	0.7	0.52
Sex × line × fight outcome	2	0.04	0.96
Residual	72		
Within-subjects			
Day	5	2.5	0.03
Day × sex	5	1.2	0.29
Day × line	10	0.8	0.66
Day × fight outcome	5	20.3	<0.001
Day × sex × line	10	0.6	0.82
Day × sex × fight outcome	5	0.4	0.85
Day × line × fight outcome	10	2.3	0.01
Day × sex × line × fight outcome	10	1.0	0.48
Residual within-subjects	360		

Figure legends

Fig. 1. Mean (\pm S.E.) of the number of receiving gill cover erection (GCE) by the losers from the winners after the fight during the direct fight trials.

Fig. 2. Mean (\pm S.E.) of the differences in the number of gill cover erection (GCE) by males against their mirror images on the day prior to the fight and on each day after the direct fight trial.

Fig. 3. Mean (\pm S.E.) of the differences in the number of gill cover erection (GCE) by females against their mirror images on the day prior to the fight and on each day after the direct fight trial.

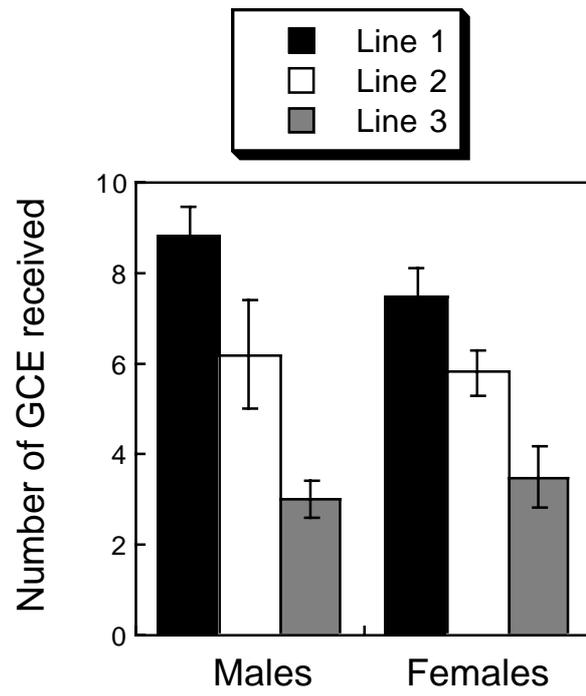


Fig. 1

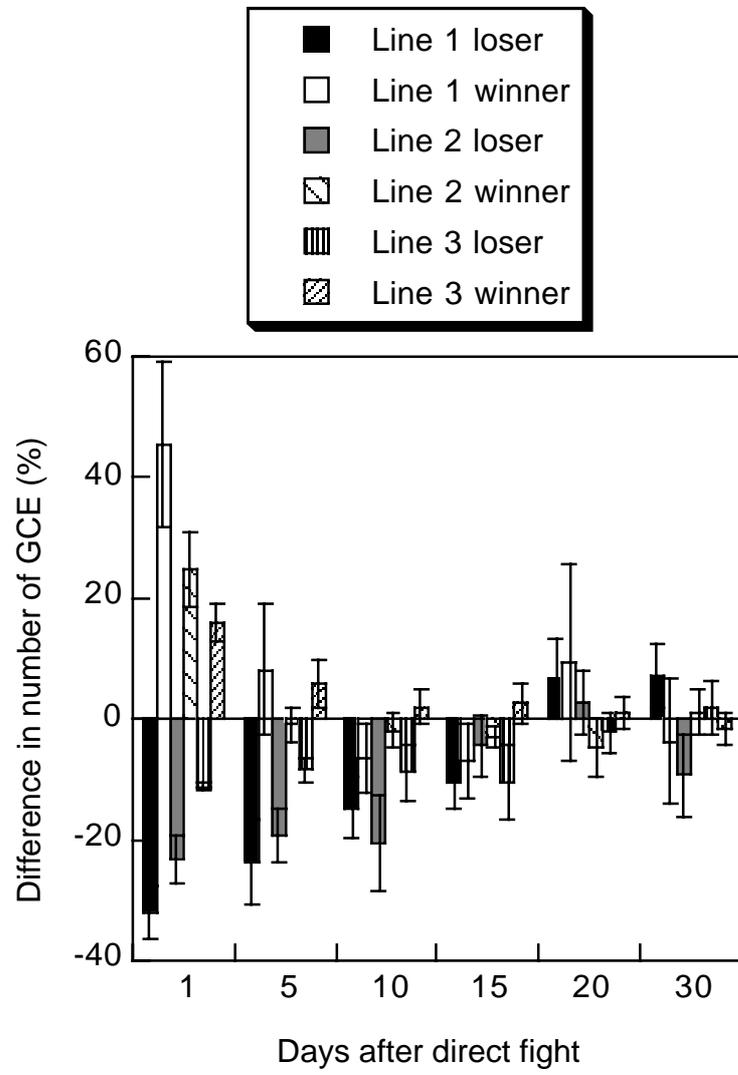


Fig. 2

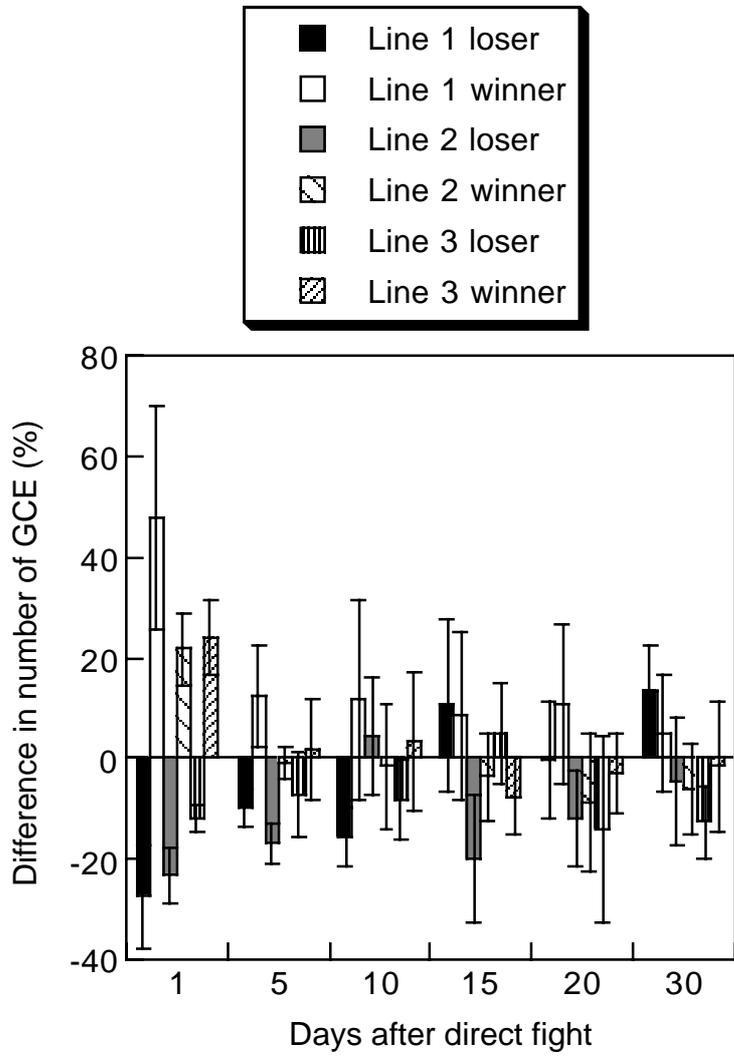


Fig. 3