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Aggressive behavior of the white-eye mutant crickets *Gryllus bimaculatus*.

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ABSTRACT

Aggressive behavior of white-eye mutant crickets was investigated and compared with that of wild-type crickets. In the dark, wild-type pairs performed long-lasting fights with significantly higher aggressive levels compared to those in the light. In contrast, fights between two white-eye mutants were not significantly different with those between two wild-type crickets both in duration and the aggressive levels. Ethograms of aggressive behavior showed that the mutants could show typical sequentially escalating fight with the same behavioral categories as the wild-type crickets. These results indicate that the white-eye mutants are able to express normal aggressive behavior.

INTRODUCTION

Aggression is one of the common principle behaviors in animals and has been extensively studied in many years. Male crickets *Gryllus bimaculatus* show intensive aggressive behavior when they come across another male and start to fight each other [1], and thus *G. bimaculatus* has been one of the most useful outstanding model system for the study of aggression [3, 8]. Although the neural mechanisms underlying aggressive behavior in the crickets are still largely unclear, there are many studies to show what kinds of sensory information they utilize during the fight to assess the opponent's strength and motivation [2, 7]. For instance, it has been reported that the blinded crickets show more intensive fights than intact crickets, suggesting that they utilize certain visual cues, probably size of the body and/or mandibles, to decide their own behavior [7].

In this study, we focused on the aggressive behavior of the white-eye mutants in *G. bimaculatus* (autosomal recessive; *gwhite*, [6]). So far, only little is known about behavioral properties of white-eye mutant crickets even though they are sometimes found in natural environment. The *gwhite* mutants do not have any screening pigments in the eyes and must have serious optical disadvantages. Here, we aimed to compare aggressive behavior of the *gwhite* mutants with that of the wild-type crickets to clarify their fighting ability and effects of visual information to aggressive behavior.

MATERIALS AND METHODS

Crickets *G. bimaculatus* were raised in a laboratory colony (25-30 °C, 14 hr: 10 hr light and dark cycle). The *gwhite* strain was given by Dr. Sumihare Noji (Tokushima University, Japan). Male crickets were used for the experiments 1-2 weeks after the imaginal molt. To reduce the effect of former fighting experiences, each cricket was individually separated in a 100 ml beaker (4.5 cm in diameter) at least 3 days before the behavioral experiments. To observe the fighting behavior, two males were put into a round glass arena (12 cm in diameter) just before the experiment. Before fighting, each cricket was separated with a partition, which was made

with acrylic plate, in the arena. Then, the partition was removed, and the behavior of two males was observed until the dominance hierarchy between them was established, at which the winner sings an aggressive song and chases up the loser while the loser flees from the winner. For the detailed behavioral analysis, the behavior of the crickets during the experiment was recorded by a digital video camera (NV-GS500, Panasonic, Osaka, Japan) located above the arena. For the experiments in the dark, an infrared LED lamp was used for illumination and behavior of the crickets was recorded by an infrared camera (WAT-902H, Watec, Yamagata, Japan).

To evaluate intensity of the fight, the aggressive level of the fight was scored depending on the maximum aggressive level of the loser (0-6; modified after [9]). Duration of the fight was also measured by counting the video flames in which both of the pair aggressively interacted. These data were statistically tested by Kruskal-Wallis test with Scheffe's post-hoc test.

RESULTS

Fighting behavior was observed in the following three conditions: wild-type pairs in the light, wild-type pairs in the dark and *gwhite* mutant pairs in the light. Fig. 1 shows ethograms of fighting behavior in three different conditions, in which behavior of each individual was analyzed every 100 ms. In the light condition, the wild-type pairs showed typical escalating fighting behavior consisted of 9 behavioral categories, approach, being approached, antennal contact, antennal fencing, mandible spreading, mandible engagement, tactile combat, chase and withdrawing (Fig. 1A). The *gwhite* mutants also show the same behavioral categories, and the sequence of behavior is quite similar to that of the wild-type pairs in the light (Fig. 1B), clearly indicating that the *gwhite* mutants are able to express normal aggressive behavior as wild-type individuals. On the other hand, the wild-type pairs in the dark showed more complex fighting behavior than those in the light. An additional behavioral category, kick, was observed in some cases, and the repetition of a part of the behavioral sequence was often observed (Fig. 1C). This fact suggests that certain visual cues from the opponent are crucial for sequential process of the fight.

Aggressive levels of the fights were significantly different among three conditions (Fig. 2A; $p < 0.001$, Kruskal-Wallis test). The highest levels were observed in the dark (Fig. 2A, dark), indicating that the visual information from the opponent suppressed aggressive behavior of the male individuals. Post-hoc tests showed that the aggressive levels of the wild-type pairs in the dark and the *gwhite* mutants in the light were significantly different (Fig. 2A; dark vs. *gwhite*, $p < 0.01$, Scheffe's test). Similar significant differences were also found in the durations of the fights: i.e. the pairs in the dark performed the longest fight (Fig. 2B; $p < 0.05$, Kruskal-Wallis test). This result also indicates that the crickets become more aggressive in the dark than in the light. By contrast, in the light, fighting behavior of the

gwhite mutant pairs was not significantly different from that of the wild-type pairs both in the aggressive levels and in the durations of the fight (Fig. 2AB; light vs. *gwhite*, $p > 0.05$, Scheffe's test).

DISCUSSION

It has been reported that, in fighting behavior of the cricket, visual cues from the opponent suppress aggression of the contestants [7]. Also in our study, the wild-type crickets in the dark fight longer with higher aggressive levels than those in the light, being coincident with previous studies. In the dark, the crickets often repeated the escalating behavioral sequence, i.e. antennal fencing, mandible spreading, mandible engagement and tactile combat, and in such cases, the dominance hierarchy was established only after several times of mandible engagement. By contrast, in the most cases in the light, the dominance hierarchy was established after they engaged mandible only once. This observation suggests that the crickets see the opponent's behavior and decide whether they continue fighting or withdraw. When they cannot get any visual information about the opponent's behavior, they tend to continue fighting longer. Unlike the wild-type pairs in the dark, the *gwhite* mutant pairs did not show any long-lasting fights. It is possible that the mutants could get some visual cues from the opponent, which is enough to suppress aggression. So far, optical functions of the *gwhite* mutants have not been investigated in detail. Further physiological and morphological studies are necessary to clarify their visual capability.

The *gwhite* mutant crickets could show typical escalating aggressive behavior similar to that of intact pairs indicates that the mutation does not affect aggressive behavior itself. In *Drosophila melanogaster*, it has been reported that the white-eye mutant, which is mutant for the *white* gene, showed serious impairment of aggression [4]. The *white* gene encodes the ATP binding cassette (ABC) transporter, by which eye pigment precursors are transported into the pigment granules [5]. The impairment of aggression in *white* mutants is caused not only by optical deterioration but also by missing certain neural functions in the CNS [4], in which the White ABC transporter would play a crucial role. Normal aggressive behavior in the cricket *gwhite* mutant implies that the gene responsible for white-eye does not affect to neural functions concerning aggression. Elucidation of molecular basis of the *gwhite* mutation in the cricket will give us better understanding to their behavioral properties in general.

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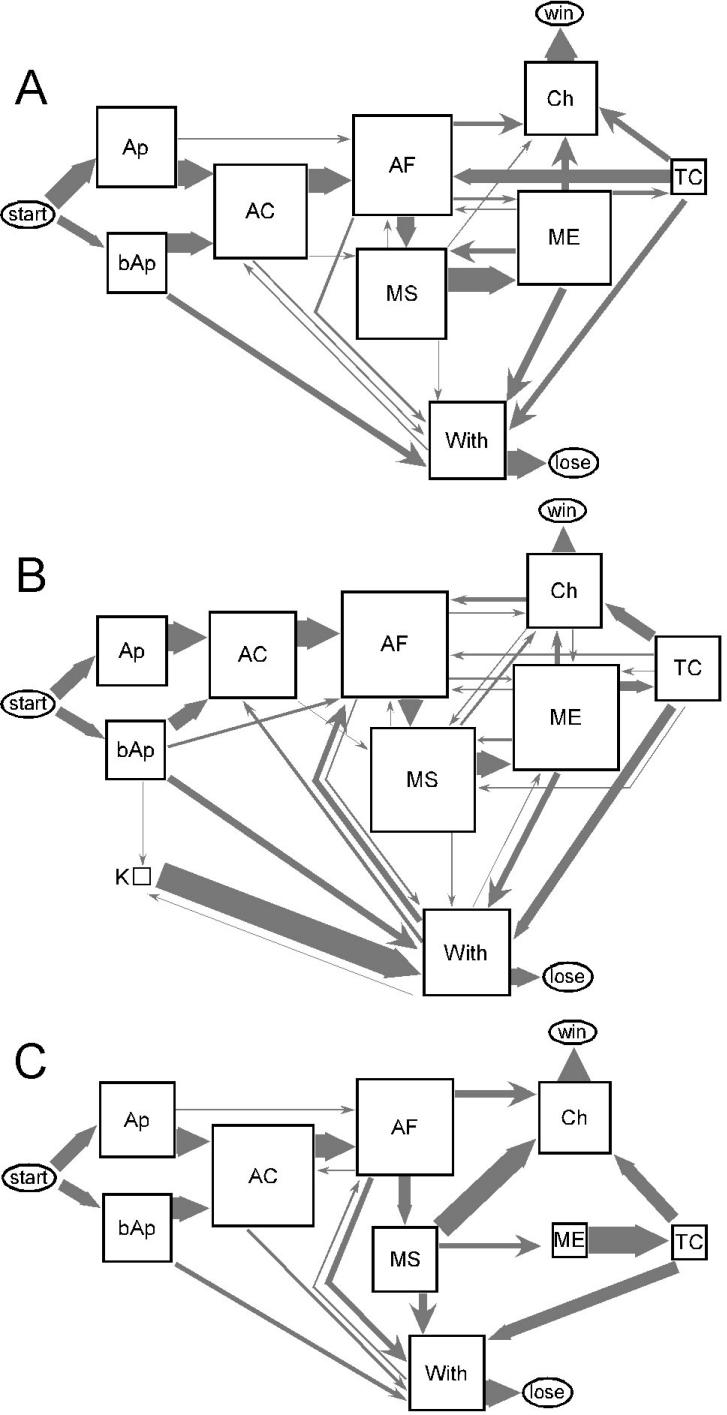
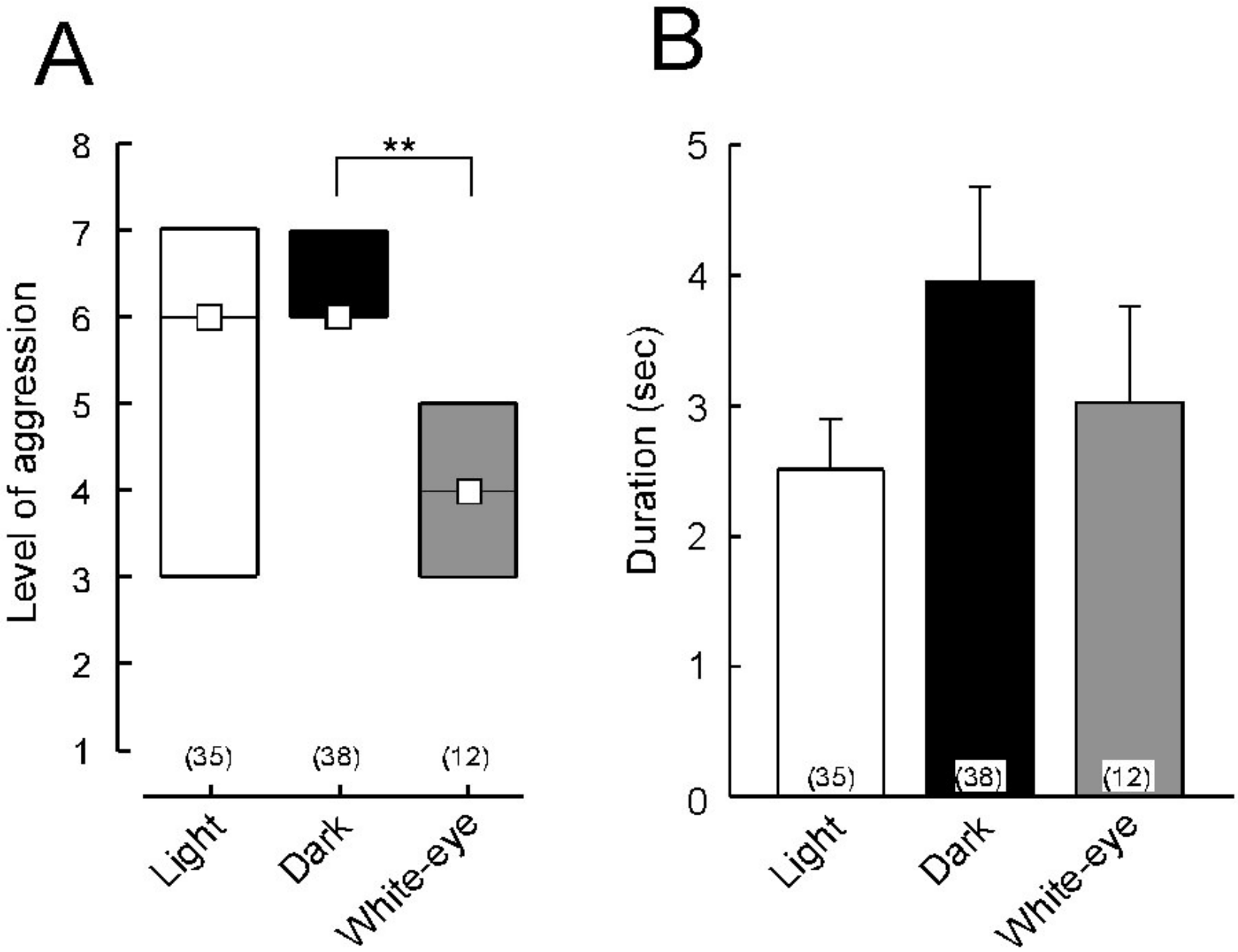
Figure legend

Fig. 1 Ethograms of the fighting behavior between intact crickets under the light condition (**A**)(n = 70), under the dark condition (**B**)(n = 76) and between *gwhite* mutant crickets under the light condition (**C**)(n = 24). Size of the square indicates the occurrence probability of each behavioral category, and thickness of the arrow indicates the transition probability between each behavioral category. **Ap**; approach, **bAp**; being approached, **K**; kick, **AC**; antennal contact, **AF**; antennal fencing, **MS**; mandible spreading, **ME**; mandible engagement, **TC**; tactile combat, **Ch**; chase, **With**; withdrawing.

Fig. 2 Level of aggression (median ± interquartile range) (**A**) and duration (median ± SEM) (**B**) of the fight between intact crickets under the light condition (white), under the dark condition (black) and between *gwhite* mutant crickets under the light condition (gray). **; $P < 0.01$, Scheffe's test. Parenthetical numbers indicate the number of experimented pairs.



Sakura et al. Fig. 1



Sakura et al. Fig. 2