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LEARNING EXPERIENCE AND AGONISTIC PLASTICITY IN *BETTA SPLENDENS*

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Abstract

Learning behaviour in a population evolves when it improves fitness of the individual. Interactions within the members of population may require the cognitive abilities which gives advantages in their lifetime reproductive fitness. Individuals may gather information and use the experience later in their life. In this study the learning behaviour of *Betta splendens* and its advantages in the social environment was investigated. It was hypothetized that organism will take advantages by learning from others, especially from the conspecific, to promote their lifetime reproductive fitness, in changing environment. Aggressive behaviour of two males *Betta splendens* were observed by the learners from separate tank (experiment 1). The social interaction between the winner from the first experiment and the learners were also observed (experiment 2). The learners received information through visual learning took advantages from prior experience. When learners encounters with the winners, they rarely compete to fight with them. In contrast, when they met with the losers, they always try to compete with them. The non-experienced individuals usually try to compete with each other unless they were injured during combat between them. These results clearly show that learners took advantages from prior social experience by showing behavioural plasticity in the changing environment. It was concluded that learning behaviour was simply evolved during the individual lifetime since adoption of learning gives some benefits to their life time reproductive fitness.

Keywords: learning, aggressiveness, evolutionary fitness, experience

Introduction

Learning simply defined as the acquisition of a new behavior or change in behaviour through interaction with the stimulus. Learning behavior is a common phenomenon among vertebrates especially found in birds, fish and mammals. It provides individual fitness, effective foraging, access to mates and predator avoidance. Knowledge on learning behavior is essential to the understanding of individual adaptation through which an organism responds to its changing environment.

The interaction among members of the same species reflects the sociality of the species. Different sensory cues such as vision and olfaction might be utilized by different species for communication. Members from a population might use different sensory cues to recognize each other in some animals. Thus, learning behaviour plays an important role in the evolution of animal behavior.

Baldwin (1896) proposed that a trait, which is evolutionary beneficial, is selected in one generation and passed it on to later generations successfully, then the trait will be genetically specified and appear in subsequent generations. Maynard-Smith (1972) developed an evolutionary stable strategy (ESS) which is a strategy once adopted by a majority of the members of population, it cannot be replaced with any alternative strategy through natural selection.

Learning ability depends on memory of the individuals and the memory reflects the individual's neuroplasticity. In recent decades, many researchers had been attempted to clarify the complexity between the learning behavior and the brain function in many different species.

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However, the understanding on the mechanism of learning behaviour is still a challenging problem for neuroscientists.

Many researchers have been used game theory to analyze animal behaviour such as predator-prey interactions, learning, communication, habitat selection etc. Game theory also provides researchers to assess the potential benefits of novel strategies upon introduction into a population. In this study, learning behaviour of *B. splendens* was investigated in order to understand their cognitive ability and the payoff towards the fitness of the individual through learning experience.

B. splendens is well known for its aggressive behaviour, showing sequences of threats (display) and attacks to opponents, and it is one of the most suitable fish for observing agonistic plasticity (Baenninger, 1984; Bronstein, 1985; Evans, 1985; Galizio et al., 1985; Halperin et al., 1992; Robertson, 1979; Wallen and Wojciechowski Metzlar, 1985). The differences in individual aggressiveness of *B. splendens* might be the result of prior social experience and the individual physiological condition. In their natural habitat, they may receive information from various sources such as encountering with conspecific and visual learning (Cain, 1980). We hypothesized that individuals experienced with visual learning may avoid physical damage from intensive aggressiveness and allow fostering pay-offs to invest in their lifetime reproductive fitness. In this study, we will investigate the learning ability of *B. splendens* and their agonistic plasticity with the two continuous experiments.

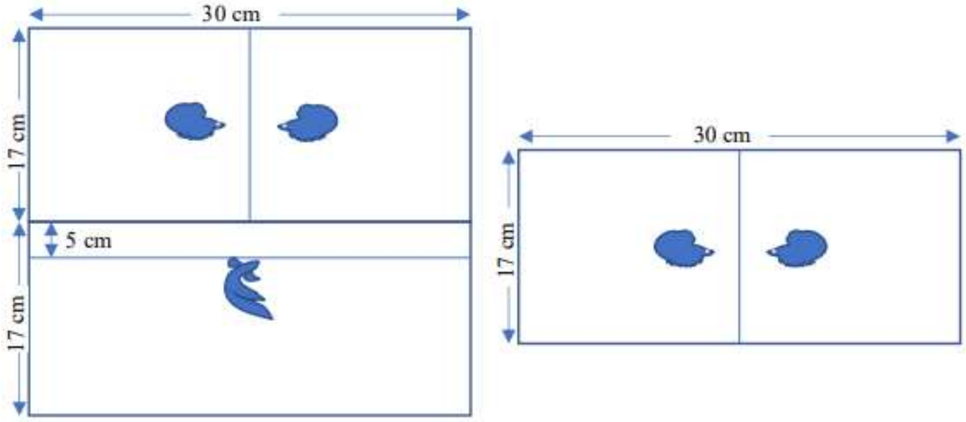
Materials and Methods

Learning ability of *B. splendens* and their plasticity in aggressive behaviour were investigated from May 2019 to July 2020. A total of ninety adult males Siamese fighting fish were obtained from a local supplier and kept separately in 1 liter glass bowls. They were visually

isolated from one another before starting of the experiment. All fish were fed food pallet twice a day and kept under a 12:12 light dark cycle. Water in the plastic bottles was refreshed once a week in order to maintain the health of the fish and if there was infection of disease, fish were treated with medicine. Individual fish were housed in the experimental tank 48 hr prior to the experiment to acclimatize with the new environment. The following experiments were conducted to understand the learning behaviour of *B. splendens* and their behaviour plasticity. Experiment 1 (Observing the learning behaviour of *B. splendens*)

Two matched size males *B. splendens* were randomly assigned into the two compartment tank (30x22x17cm), which is separated with a transparent plastic sheet in the middle of the tank (Fig.1), 48 hr prior to the experiment. An opaque glass was placed between the two compartments to prevent fish seeing from each other before the experiment and it was removed once experiment start. Another one compartment tank (30x22x17cm), which was designed to see from one side using the sun visor thus the fish from outside of the tank could not see inside of the tank, was placed next to the experimental tank (Fig. 1). A similar sized male was placed into one compartment tank in order to allow the fish to observe the behaviour of fish from the two compartment tank. Hence, learner fish could see both of the two males from two compartment tank but the encounters could not see learner fish. The experiment was lasted for fifteen minutes and all fish behaviour were recorded with a digital video camera (Canon EOS 7D Mark II. Experiment 2 (observing the agonistic plasticity)

To observe the learning experience, an experimental tank was equally divided into two compartments with a transparent plastic plate. Both learner and winner fish were placed into each compartment, and were observed the frequency of mutual attack between them for 15 minutes (N=15). If one individual defeats the other within the observation period, the experiment was finished and recorded the time. The observed fish from the two experiments were not repeated. The same experimental design was conducted using with observer and loser fish from the first experiment (N=10). The following behavior were recorded during the experiment: (a) frontal display: duration of fish showing frontal display within 5cm of the wall, (b) attack: number of biting and tail beats (c) escape: duration of fish outside of 5cm of the wall, (d) threat: duration of gill erection, and (e) learning: duration of fish learning. Experiment was lasted 15 minutes and testing was terminated when one fish fled at the approach of the other.



(experiment 1) (experiment 2)

Figure 1 Top view of the experimental tank designed to observe the learning behaviour of male *B. splendens* (experiment 1) and agonistic plasticity (experiment 2)

Statistical analysis

All data were checked for normality test using the Kolmogorov-Smirnov test. Some of the distributions were significantly deviating from the normal distribution then we used non-parametric test. When the data were normally distributed, we used parametric test. All statistics were analyzed using the SPSS 24.0 package.

Results

In experiment 1, the learning behaviour of B. splendens was observed (N=30). The competition between paired males was also recorded (N=30). Twenty five out of 30 pairs of male *B. splendens* established clear dominant/subordinate relationship during the experiments. Two pairs of males never attacked and avoided each other and 3 pairs of males were convinced to get draw in the competition during the trial. The duration of frontal display of paired males were significantly different (t-test: t= 3.281, p = 0.002; dominant = 364.0 $\pm$ 59.84 seconds; subordinate = 306. 0 $\pm$ 65.06 seconds; Fig. 2). Dominant males performed more biting and tail beats (t = 4.725, p = 0.001; dominant = 7.80 $\pm$ 2.79; subordinate = 4.56 $\pm$ 1.98; Fig. 3) and more gill cover erection (t= 8.421, p = 0.001; dominant = 42.72 $\pm$ 8.08; subordinate = 25.56 $\pm$ 6.19; Fig. 4). Escape behavior was found in subordinate male 183.6 $\pm$ 58 but this behavior was not found in dominant males. All the learners participated in learning and the duration of time spent on this behaviour was ranged from 300 to 800 seconds. The percentage of time spent by the learners during the experiment was highest in learning (58%) followed by display (24%), gill cover erection (17%) and biting and tail beats (1%), respectively (Fig. 5).

In experiment 2, the agonistic plasticity of learner males was observed. When learner males encountered with dominant male from the experiment 1, a significant difference in frontal display was found (U=11, p<0.001; dominant = 324.67 $\pm$52.63; learner = 172.0$\pm$ 70.02). Number of attack between paired males (t = 7.491, $p<0.001$; dominant = 9.47 ± 2.59; learner = 2.87 ± 2.36), and number of gill erection (t = 14.823, $p<0.001$; dominant = 37.93 ± 3.43; learner =12.20 ± 9.85) were significantly different (Fig. 6 and Fig, 7). Highly significant difference was found in frontal display (Mann-Whitney U Test: U=6, $p<0.001$; learners = 260.0 ±39.44 ; subordinate = 334.0 ± 34.38), escape behaviour (t = -7.791, $p<0.001$, learners = 335.0 ±91.56; subordinate = 96.0 ± 32.04), number of biting and tail beat (t =7.793, $p<0.001$; learners = 2.1 ± 0.99 ; subordinate = 10.50 ± 2.46), number of gill erection (t = 10.770, $p<0.001$; learners = 23.80 ± 2.52 ; subordinate = 41.10 ± 2.56) respectively (Fig. 8 and Fig, 9). Different display patterns between learner males who combat with dominant and subordinate males was found in the experiment 2. Number of biting and tail beating (U= 35.0, $p<0.05$; D_learner = 2.87 ± 2.36; S_learner = 2.1 ± 0.99) and number of gill erection (U= 12.0, $p<0.001$; D_learner = 12.20 ± 9.85; S_learner =23.80 ± 2.52)

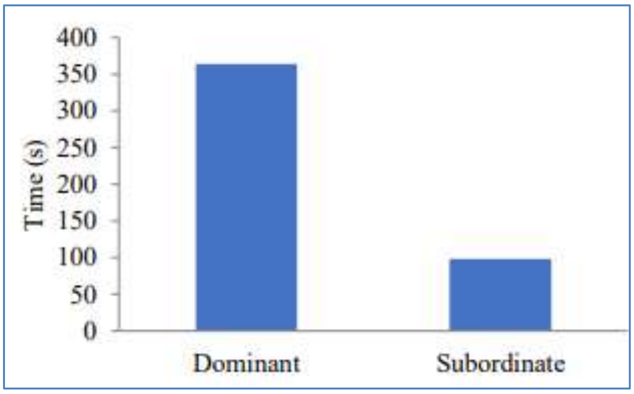


Figure 2 Interaction (display) between dominant and subordinate males

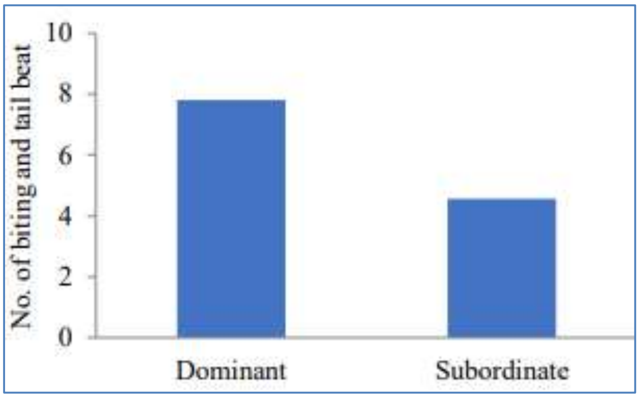


Figure 3 Number of biting and tail beat between dominant and subordinate males

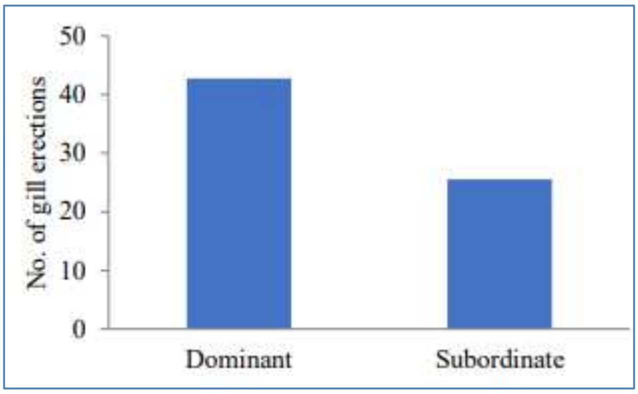


Figure 4 Number of gill erections between dominant and subordinate males

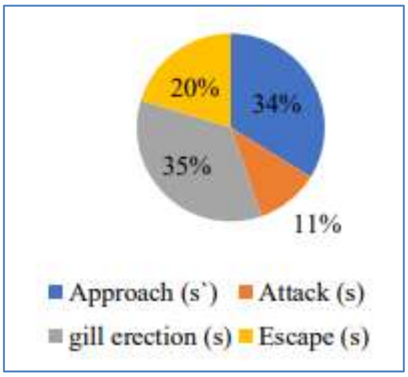


Figure 5 Percentage of display pattern of learner fish

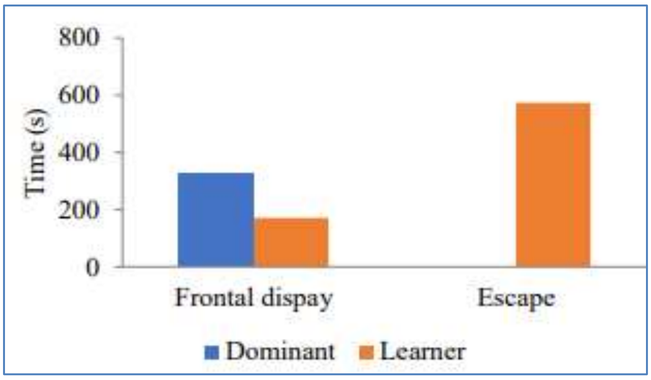


Figure 6 Interaction (display and escape) between dominant and learner males

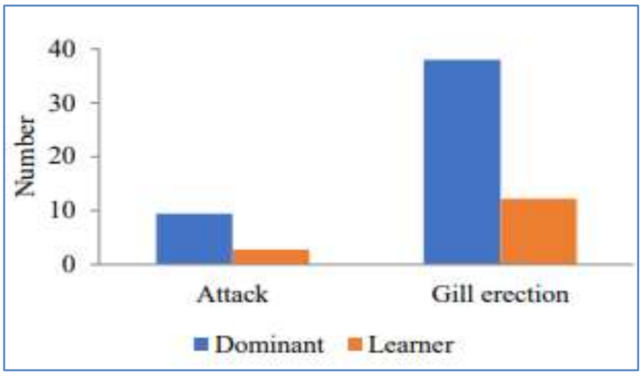


Figure 7 Interaction (attack and gill erection) between dominant and learner males

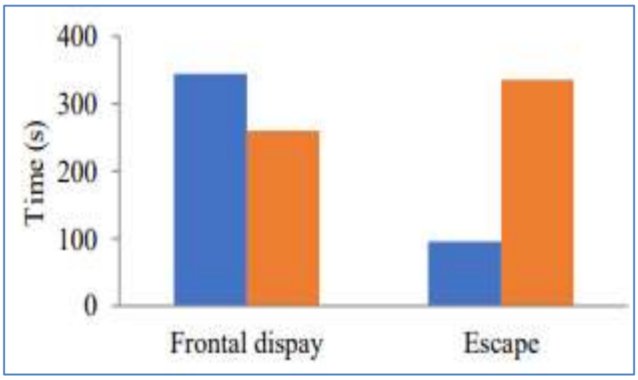


Figure 8 Interaction (display and escape) between subordinate and learner males

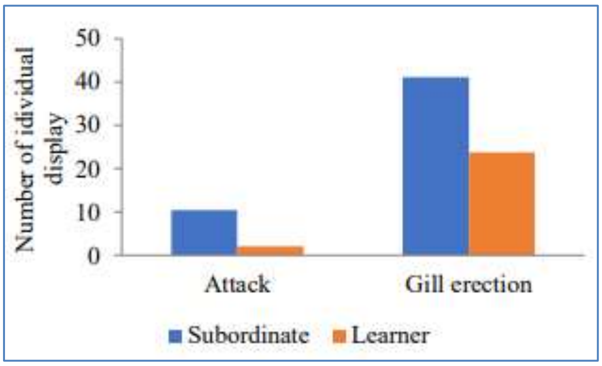


Figure 9 Interaction (attack and gill erection) between subordinate and learner males

Discussion

Learning behaviour of *B. splendens* was investigated with two continuous experiments, observing the learning behaviour of male *B. splendesn* and observing its agonistic plasticity. The results from these two studies clarify the effect of learning or prior visual experience on *Betta* agnoistic plasticity. When two males encounters, both males showed agonistic behaviour to the opponents until they reach to clear social hierarchy. We found that non-experience individuals (paired males) showed more aggressiveness and this finding is consistent with the results of others (Bronstein, 1984; Cain, 1980; Haller, 1994). This result explains that non-social- experience males spend high energetic cost in social combat which may potentially result injury or death. Dominant males showed more gill cover erections than the losers since gill cover erection is correlated with ability of males and its dominancy (Even, 1985). Number of bites and tail beats were also significantly different between two paired-males. Halperin et al., (1998) reported that biting and tail beating have been shown to be an honest indicator of energetic and male quality. Animals who do not directly involve in an interaction have been called audiences and they might receive various information from visual learning (Evans and Marler, 1984). In this experiment, most of the learners pay attention to social combat between two paired males indicating that learning behaviour is very common in *B. splendens* and/or in their natural habitats.

When we investigated the plasticity of agonistic behaviour of male *B. splendens* (experiment 2), we found that learners usually avoid dominant or winner males which indicated that learner males may take advantages from the prior experience to mitigate high energetic cost in fighting. In contrast, learners tried to compete with subordinate or loser males since submissive displays of loser in the prior experiment might be an honest indicator of loser condition and/or motivation (Evans, 1985; Halperin *et a*l., 1998; Simpson, 1968). Prior social experience such as dominant and subordinate roles resulting from paired encounters (Baenninger, 1970; Lobb & McCain, 1976; Meliska et al., 1975), prior visual experience with a conspecific (Meliska and Meliska, 1976), and visual and combat experience with responsive or passive conspecifics and non-conspecifics (Johnson and Johnson, 1973) might operate on agonistic plasticity of male *B. splendens*. The results from this study clearly explain that there was a positive correlation between learning experience/prior social experience and agonistic plasticity in male *B. splendens.*

Conclusion

The effect of learning experience on agonistic plasticity was investigated. The non-experienced males (paired males) actively involved in fighting display and males with prior visual experience took advantages from previous learning to mitigate high energetic cost from fighting. From this research it was concluded that learning behaviour is common in *B. splendens* and learning experience might affect on the agonistic plasticity of *B. splendens*.

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