



Bystanders to contests between conspecifics are primed for increased aggression in male fighting fish

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We performed two experiments in which we allowed a male fighting fish, *Betta splendens*, designated a bystander, to observe aggressive contests between pairs of male conspecifics. Another male (naïve male) observed an empty tank or two nonaggressive males, depending on the experiment. Immediately after these observation periods, we allowed the bystander and naïve male to interact in a neutral area. In both experiments, bystander males were dominant over naïve males in a significant number of the encounters. Bystander males performed significantly more aggressive behaviours (displays, chases and bites) than did naïve males. Differences in dominance were not due to chance differences in body size. These findings demonstrate that exposure to aggression between conspecifics increases aggressive motivation in bystander male fighting fish. We discuss briefly the implications of such social experience on the formation of dominance hierarchies.

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Aggressive contests between animals rarely occur in a social vacuum, but instead take place in the presence of conspecifics (McGregor 1993; McGregor & Peake 2000). A territorial dispute between rival males might be observed by other males as well as by potentially receptive females. Observers and participants can interact in several ways. If characteristics or behaviour of the observers affect the behaviour of the participants, this is called an audience effect (McGregor & Peake 2000). For example, the types of displays performed in territorial disputes between male Siamese fighting fish, *Betta splendens*, differ depending on whether the observer is male or female (Doutrelant et al. 2001). In other cases, observing a contest may induce changes in the behaviour of the observers. If aggressive motivation, and hence the probability of performing an aggressive act, increases as a result of this experience, this is called priming (Hogan & Bols 1980; Bronstein 1989). Oliveira et al. (2001) found that male cichlids, *Oreochromis mossambicus*, experienced significant increases in circulating androgen levels after observing fights between conspecifics. The extent to which priming increases success in subsequent or future contests remains largely unexplored (but see Hollis et al. 1995). Observers of aggressive behaviour may use the information they extract in future interactions, a phenomenon known as eavesdropping (Johnsson & Akerman 1998; Otter et al. 1999; Earley & Dugatkin 2002). For example,

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Oliveira et al. (1998) reported that, after observing a contest between conspecifics, male fighting fish responded differently towards the perceived 'loser' than towards the perceived 'winner'.

Interactions between bystanders and participants can have significant effects on dominance hierarchy formation (Chase 1982a, b; Dugatkin 2001). For example, fighting experience alters the outcome of subsequent aggressive interactions in a range of taxa (van de Poll et al. 1982; Drummond & Osorno 1992; Chase et al. 1994; Whitehouse 1997; Goessmann et al. 2000). Losing an encounter increases the chances that an animal will lose future encounters, even against otherwise inferior rivals (Beacham 1988; Beaugrand et al. 1991; Drummond & Osorno 1992). This decrease in fighting motivation, dubbed the loser effect, may last several hours to several days (Chase et al. 1994). Conversely, an animal that wins an aggressive encounter may experience a greater probability of winning future encounters, although the winner effect is usually less robust than the loser effect (Jackson 1991; Chase et al. 1994).

We used male Siamese fighting fish to test the hypothesis that exposure to conspecific aggression primes bystanders for increased aggression, thereby inducing a winner effect in them. Specifically, we predicted that a male fighting fish that observed a contest between conspecifics would defeat an otherwise similar rival that observed no such stimulus. Fighting fish are excellent subjects for this study because their aggressive behaviour is stereotyped and well documented (Simpson 1968;

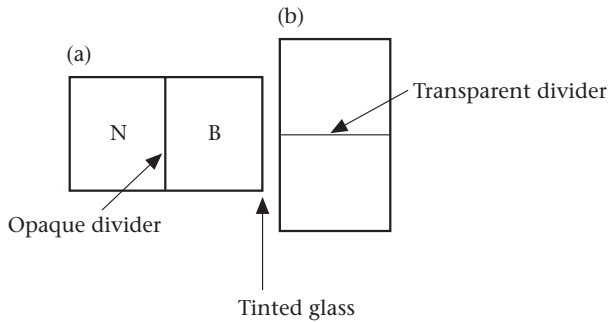


Figure 1. Schematic diagram of tanks from experiment 1, in which no control males were used, showing (a) the focal tank and (b) the stimulus tank. The focal tank housed the bystander male (B) and naïve male (N).

Bronstein 1983; Halperin et al. 1992), and previous research has examined aspects of audience, priming and eavesdropping effects in this species (Hogan & Bols 1980; Oliveira et al. 1998; Doutrelant & McGregor 2000; Doutrelant et al. 2001).

METHODS

We purchased male fighting fish from multiple commercial vendors to reduce the likelihood that individuals had had prior social contact. The fish were visually but not chemically isolated in divided 9.5-litre tanks for 2 weeks before any contests were initiated. We maintained tanks at 25°C on a 14:10 h light:dark cycle. We fed fish twice daily with freeze-dried bloodworms.

Experiment 1 used a population of 40 male fish. Contests were conducted between 22 February and 30 March 2001. Before each contest we randomly selected four fish from this population. Two fish, a bystander male and a naïve male, were assigned to isolated compartments in one 9.5-litre tank (focal tank; Fig. 1a). The other two fish were assigned to isolated compartments in another tank (stimulus tank; Fig. 1b) placed perpendicularly to the bystander's end of the focal tank. Tinted glass separated the bystander male from the stimulus tank. The males in the stimulus tank could see each other but not the bystander male. All four fish were allowed 5–6 h to acclimate to their respective tanks.

The divider separating the fish in the stimulus tank was removed and the two males were allowed to interact for 15 min. Bystander males oriented towards the interaction (through the tinted glass) in 100% of contests. Males in the stimulus tank never directed displays at the bystander males, confirming that the tinted glass functioned as one-way glass (we did not use one-way glass because the fish would react to their own reflection). Immediately following the contest in the stimulus tank, we removed the opaque divider between the bystander male and the naïve male and allowed them to interact for 15 min. We compared the number of agonistic displays and physical interactions to determine the winner of each contest (Simpson 1968; Bronstein 1983; Chase et al. 1994). Contests with no decisive winner were disregarded

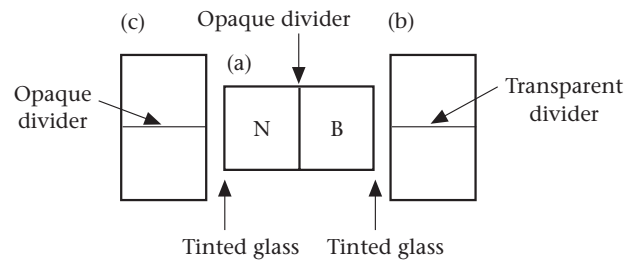


Figure 2. Schematic diagram of tanks from experiment 2, in which male controls were used. Tank arrangement was identical to that shown in Fig. 1 except that the control fish were housed in the tank labelled (c). The focal tank housed the bystander male (B) and naïve male (N).

from this analysis ($N=6$). We staged 26 contests using randomly selected opponents, of which 20 (76.9%) yielded a clear winner. Seventeen fish were used more than once (some as stimulus fish), but fish were never paired with the same opponent. To reduce the likelihood of confounding winner or loser effects, we allowed at least 72 h to elapse before a fish was used again (Chase et al. 1994).

We recorded three categories of aggressive behaviour: displays, chases and bites. The first category included both frontal (erecting the gill covers) and lateral (broad-side swimming, dorsal fin erection) displays. Chases included lunges that did not result in physical contact, as well as extended chases lasting several seconds or more. Lunges that resulted in physical contact were categorized as bites. These categories were modified from those used by other authors (Simpson 1968; Bronstein 1989; Halperin et al. 1998). To reduce interobserver differences, all behavioural data were collected by one of us (A.D.P.). Comparisons of aggressive behaviour between bystander and naïve males included the six contests that yielded no decisive winner ($N=26$ interactions). We included these contests so that our analyses would be as conservative as possible.

Experiment 2 was performed between 14 January and 25 March 2002. Instead of no control stimulus, the naïve males had the opportunity to observe two visually isolated male fighting fish (control tank; Fig. 2c). The naïve male was separated from these males by tinted glass identical to that used on the other side of the tank. This experiment used fish from a separate group of 40 males, although we performed fewer contests for experiment 2 due to constraints imposed by unrelated experiments. We staged 22 contests, of which 15 (68.2%) yielded a clear winner. Only six fish were used more than once, but none was matched against the same opponent. Other than the differences stated above, experiment 2 was in other respects identical to experiment 1. Comparisons of aggressive behaviours included data from all 22 contests.

At the end of each experiment, we weighed fish to the nearest 0.01 g with an electronic balance. Data were analysed using Statistix 7.0 (Analytical Software 2000). Tests are two-tailed and differences were considered significant at $P<0.05$. Means are presented \pm SE (error bars on Figures also represent SE).

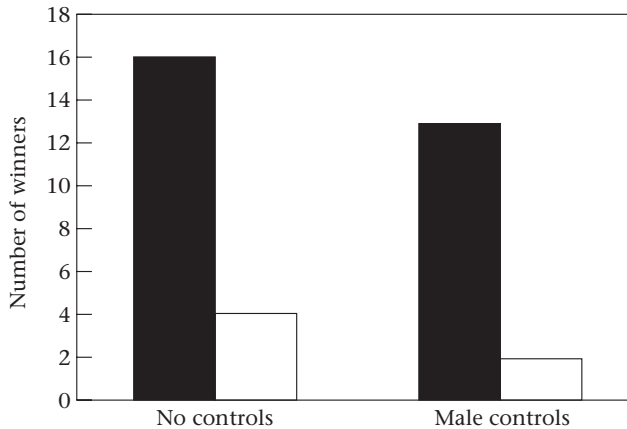


Figure 3. Males that were bystanders (■) to a contest between conspecifics were more likely to win a subsequent contest than were naïve fish (□).

Ethical Note

We restricted contests to the minimum duration necessary to determine a clear winner (15 min; Bronstein 1989; Oliveira et al. 1998; Doutrelant & McGregor 2000). We continuously monitored all contests and terminated them if serious injury to one of the combatants appeared imminent (Halperin et al. 1998). None of the fish died or suffered debilitating injury as a result of these encounters. Although several authors (Oliveira et al. 1998; Doutrelant et al. 2001) have performed similar studies in which fish were prevented from fighting, we opted to allow physical contact (see also Halperin et al. 1998; Oliveira et al. 2001). This decision was based on preliminary observations in our laboratory, which revealed that initial display intensity did not always predict the ultimate winner of an encounter (but see Simpson 1968).

RESULTS

In experiment 1, bystander fish won 16 of 20 (80%) encounters with their randomly selected naïve opponents (two-tailed proportion test: $Z=2.46$, $P=0.014$; Fig. 3). Winners of these encounters were usually quite clear; losers paled in coloration, retracted their fins and hid in the corners of the tank. Bystander males performed significantly more displays (paired t test: $t_{25}=2.21$, $P=0.037$), chases ($t_{25}=3.33$, $P=0.0027$) and bites ($t_{25}=2.72$, $P=0.012$) per 15 min than did naïve males (Fig. 4). This significant winner effect in bystanders was not due to chance differences in body mass between bystanders and naïve fish (bystanders: 1.57 ± 0.05 g; naïve: 1.55 ± 0.05 g; two-sample t test: $t_{34} = -0.28$, $P=0.78$).

In experiment 2, bystander fish won 13 of 15 (86.7%) encounters with naïve males (two-tailed proportion test: $Z=2.58$, $P=0.0098$; Fig. 3). Behavioural differences between bystander and naïve males were similar to the experiment described above. Bystander males performed more displays (paired t test: $t_{21}=2.45$, $P=0.023$), chases ($t_{21}=2.24$, $P=0.036$) and bites ($t_{21}=2.68$, $P=0.014$) per 15 min than did naïve males (Fig. 5). Bystander males and

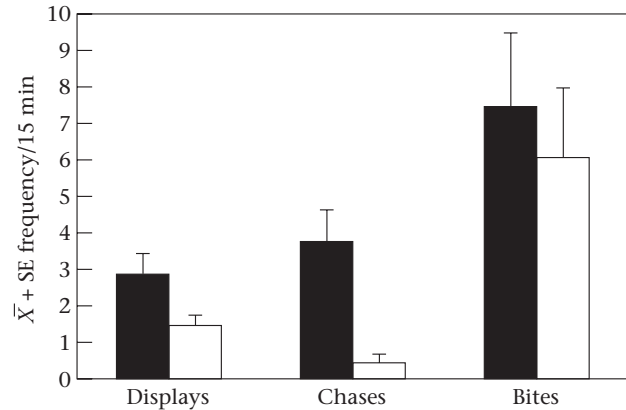


Figure 4. Males that were bystanders (■) to a contest between conspecifics performed significantly more aggressive behaviours than did naïve males (□) when naïve males were exposed to no control stimulus ($N=26$ pairs).

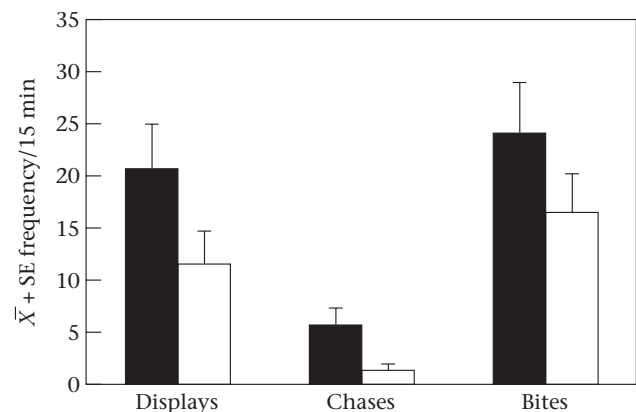


Figure 5. Males that were bystanders (■) to a contest between conspecifics performed significantly more aggressive behaviours than did naïve males (□) when naïve males were exposed to two nonaggressive males as a control stimulus ($N=22$ pairs).

naïve males were similar in body mass (bystanders: 2.07 ± 0.09 g; naïve: 1.94 ± 0.11 g; two-sample t test: $t_{34}=0.99$, $P=0.33$), suggesting that dominance of bystander males was not due to larger body size.

DISCUSSION

Bystander males defeated similarly sized naïve males in 80% or more of contests regardless of the type of control stimulus to which the naïve males were exposed. Comparisons of aggressive behaviours from all contests, including those with no clear winner, yielded a similar result: bystander males were more aggressive than naïve males. This result suggests that observing a contest between conspecifics not only increased the aggressive motivation of male fighting fish (Hogan & Bols 1980; Bronstein 1989) but also increased the likelihood of winning a subsequent encounter. In other words, priming induced a winner effect in the bystander fish. This result is comparable to that reported by Hollis et al. (1995), who showed that priming via Pavlovian

conditioning can significantly affect the outcome of aggressive interactions between male blue gourami fish, *Trichogaster trichopterus*.

Our study differed from others (Johnsson & Akerman 1998; Oliveira et al. 1998; Earley & Dugatkin 2002) in that bystanders were tested against naïve fish with which they had no previous experience. This distinction is important because it differs from what is typically called eavesdropping. Eavesdropping is said to occur when individuals extract information from an observed contest between others and then use this information in future encounters with one of those individuals. Priming, the phenomenon we observed, is more cognitively neutral. A change was induced in bystanders that altered their future behaviour, in this case their probability of dominating another male. The results of our study are also not consistent with those of a recent study on green swordtails, *Xiphophorus helleri*. R. L. Earley & L. A. Dugatkin (personal communication) found no significant priming effect using an experimental design similar to ours. Future studies will reveal how taxonomically widespread the priming phenomenon is.

Could pre-existing differences between bystander males and naïve males explain our results? This is sometimes referred to as the correlation model for dominance hierarchy formation (Chase 1986) and is often the null hypothesis against which other models are tested. To avoid the possibility of pre-existing differences confounding our results, we randomly selected fish for each contest. We performed post hoc comparisons of body mass and found no significant differences between bystander and naïve males. This is significant because body size is an important determinant of dominance in many fish, including fighting fish (Beacham 1988; Beauprand et al. 1991; Jaroensutasinee & Jaroensutasinee 2001). Although we cannot rule out unforeseen, pre-existing differences (e.g. circulating hormone levels) influencing the outcome of our experiments, we found no support for the correlation model.

The physiological mechanisms responsible for increased aggression in bystander fish are not known, but most likely involve changes in androgens such as testosterone and 11-ketotestosterone, or amines such as serotonin (Wingfield et al. 1990; Huber et al. 1997; Oliveira et al. 2001). We tested bystanders for a priming-induced winner effect immediately after exposing them to aggressive contests between conspecifics. Research in fish has shown that pure winner effects (those resulting from actual physical contests) are temporally variable, and may wane in as little as 1 h (Chase et al. 1994; but see Hsu & Wolf 1999). Hormone profiles in cichlids suggest that priming may be similarly short-lived (less than 2 h; Oliveira et al. 2001). Indirect evidence for the transient nature of priming also comes from the same study on green swordtails by R. L. Earley & L. A. Dugatkin (personal communication). In their study, bystanders observed conspecific interactions for 60 min, of which time contests were only 12.1 ± 2.9 min. Thus, any priming that might have occurred may have waned over the next 48 min. Future studies should examine the duration of priming-induced winner and loser effects to clarify their

role in shaping dominance interactions in nature, where animals do not come in contact with rivals every 15 min.

The traditional approach to the study of dominance in animals has been to construct hierarchies based on a series of dyadic interactions. There have been several attempts, however, to model the consequences of self-reinforcing (i.e. winner or loser) effects on the structure of dominance hierarchies (Theraulaz et al. 1995; Bonabeau et al. 1996; Dugatkin 1997; Hemelrijk 2000). In general, self-reinforcing processes are believed to increase hierarchy structure. They amplify initial asymmetries between individuals and, as the number of contests increases, increase the disparity between low- and high-ranking animals. Dugatkin (1997) found that significant winner effects alone produced a clear linear hierarchy, but significant loser effects alone produced a despotic hierarchy with only a clear alpha individual. Intermediate conditions produced populations with varying numbers of individuals with unambiguous ranks. Hemelrijk (2000) predicted that dominance hierarchies based on memory-based rank perception (i.e. prior social experience) would be less differentiated than those based on direct assessment of rank by fighting.

Priming effects, such as we observed in this study, and eavesdropping further complicate the assessment of dominance hierarchies. Not only is the behaviour of an individual influenced by its own recent fighting experience, it is potentially influenced by the dominance interactions of other animals. In his observations of domestic chickens, Chase (1982a, b) found that exposure to conspecific aggression often stabilized dominance relationships into transitive linear hierarchies. Similarly, Dugatkin (2001) expanded upon his own earlier model and predicted that audience effects, in concert with pure winner or loser effects, would produce stratified, linear dominance hierarchies. Thus, the addition of priming or eavesdropping effects to a system with either pure winner or loser effects can dramatically alter the structure of dominance relationships.

Despite the substantial literature that now exists on winner and loser effects, our understanding of priming and its effect on winner–loser effects is relatively incomplete. We now have a good theoretical framework from which to generate predictions (Dugatkin 1997, 2001). Empirical studies should examine the duration of priming, the concurrent existence of both winner and loser effects, and the fitness consequences of these effects in terms of resource acquisition or mating opportunities. Priming effects remind us to consider dyadic interactions in a larger social context, making it more difficult to predict the outcomes of interactions based on phenotypic characters such as age and body size. We must also be aware that dominance relationships between individuals are temporally variable and subject to changes in the immediate social environment.

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