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The Use of Standard Aggression Testing Methods to Predict Combat Behaviour and Contest Outcome in *Rivulus marmoratus* Dyads (Teleostei: Cyprinodontidae)

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Abstract

Aggression plays an important role in animal contests, but the extent to which aggression correlates with dominance has been a topic of much debate. The relationship between aggression and dominance ability in the hermaphroditic fish, *Rivulus marmoratus*, was investigated using three standard protocols, the mirror test (Mi), model test (Mo), and standard opponent test (So). In each, display latency, attack latency, and biting frequency were quantified for a test individual towards its opponent. The general rank-order for eliciting strength of the three different stimuli was Mi > So > Mo. The relationships between the individual indices from the standard tests and three dyadic contest variables, initiator of display, initiator of attack, and winner, were analysed in contests between previously tested pairs to ascertain how well the standard protocols predicted dyadic contest behaviour/outcome. Display and attack latencies in the standard tests did not predict the level of analogous combat behaviour. Biting frequency differences between individuals in a pair in the So and Mo tests as well as display latency differences in the Mi test contributed to predictions of contest outcome. The individual that scored higher, relative to its opponent, won a significantly greater proportion of the bouts. These findings demonstrate the importance of relative differences in aggression in determining dominance. However, the predictive value of standard test behaviour is test-specific and, based on the available literature, depends on both the species used and the context in which they are employed.

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Introduction

Aggression is a quantifiable, overt behavioural response such as an animal's tendency to attack, and is an attribute of the individual (Hand 1986; Francis 1988). Dominance, on the other hand, is based on the outcome of a dyadic contest or relationship and depends more on the asymmetry of an interaction between individuals (e.g. Bernstein 1981; see Drews 1993 for a review). To examine how aggression relates to dominance, the two must be tested independently (Francis 1988). Standard aggression testing methods such as the mirror (e.g. Franck & Ribowski 1987; Holder et al. 1991), model (e.g. Barlow et al. 1986; Beeching 1992; Halperin et al. 1997), and standard opponent tests (e.g. Bakker & Sevenster 1983; Franck & Ribowski 1987) have been used to quantify aggressive responses of an individual towards its opponent (mirror image, dummy, and conspecific opponent, respectively) under controlled conditions. Usually, a single type of test is used, but the type varies among studies. An important question, then, is whether the three types of tests produce similar results.

Some consideration has been given to comparisons of these tests with respect to their effectiveness as stimuli for aggressive behaviour (e.g. Thompson 1963; Baenninger 1966; Dore et al. 1978). However, a simultaneous comparison of the eliciting strength of all three standard tests has yet to be made. The mirror image has been shown to evoke a greater response from a test fish than a conspecific opponent (e.g. Baenninger 1966; Dore et al. 1978; but see Rhoad et al. 1975) or a dummy (Thompson 1963; Thompson & Strum 1965). This often is attributed to the fact that the mirror image serves as a constant aggressive reinforcer (Thompson 1966) whose behaviour exactly mimics that of the test fish (Gallup 1968), while neither the conspecific opponent nor the dummy provides such reinforcement.

In none of the three testing procedures does the focal individual succeed in chasing the opponent away; that is, a dominance relationship is not explicitly established. In contrast, most dyadic contests result in a dominance relationship where the winner chases the loser. This difference between standard and dyadic contest conditions has spurred the debate of how accurately, and to what extent, behaviour exhibited within these tests can predict contest behaviour or outcome. Only a few studies have tested whether the aggressive behaviour exhibited in the standard tests is useful in predicting the behavioural responses of a focal individual in contest situations. In those that have, no significant predictive relationship has been found. For example, the display behaviour of *Betta splendens* toward its mirror image showed a low correlation with analogous display behaviour in a combat situation (Dore et al. 1978; Meliska et al. 1980).

Studies that have tested the association between aggression in the standard tests and dyadic dominance have yielded variable results that seem to depend both on the indices of aggression used and on the context in which aggression is studied. The indices of aggression range from threat displays (e.g. Baenninger 1968; Francis 1983, 1984) to potentially more costly behaviour such as biting frequencies (e.g. Bakker & Sevenster 1983; Franck & Ribowski 1987). However, no consensus has been reached with respect to which activities best predict the outcome of a dyadic encounter.

This lack of consensus among previous studies may stem from the fact that standard tests of behaviour are employed as potential indicators of contest outcome in a wide variety of dominance-related contexts, ranging from territoriality (e.g. FitzGerald & Kedney 1987) and dyadic dominance (e.g. Barlow et al. 1986) to group dominance (e.g. Baenninger 1968; Holtby et al. 1993). Furthermore, the goal of many of these studies was not to assess the utility of the standard tests but to examine some other factor involved in aggressive or dominance behaviour, such as the role of previous experience (Francis 1983), the effects of artificial selection for dominance ability (Francis 1984), or the effects of parasite load on aggressiveness (Hamilton & Poulin 1995). Studies that explicitly test the relationship between standard test behaviour and behaviour exhibited within, or the outcome of, a dyadic contest are necessary to understand the usefulness of the standard protocols as predictors of contest dynamics.

The first objective of this study was to investigate the eliciting strength of each standard test, with respect to the others, as a stimulus for aggressive behaviour. The second objective focuses on whether variation in the behaviour of individuals in the standard tests can accurately predict behaviour exhibited within a dyadic contest and its outcome.

Materials and Methods

Study Organism

The species used in this study was *Rivulus marmoratus,* a member of the killifish (Cyprinodontidae) family. *R. marmoratus* are functional hermaphrodites that produce homozygous clonal offspring (Harrington 1961; Harrington & Kallman 1968). This fish is quite aggressive both in the field (Huehner et al. 1985) and laboratory (Hsu & Wolf 1999). Three different clones were used: the DS and NA clones were derived from Florida and had been bred in captivity for over 30 generations; the B clone was the first generation from Belize. The three clones were used in order to facilitate comparisons between field (B) and laboratory-reared (DS, NA) fish. All clones were maintained and bred at Syracuse University for 3 yr (two generations).

Maintenance and Aggression Test Arrangements

Individual fish were maintained from hatching at a temperature of 26–27 °C with a 14 h light : 10 h dark photoperiod in separate, plastic isolation tanks (10 cm × 10 cm × 6 cm) filled with 300–400 ml of brackish water (160 g salt/10 l water, 12.3 ppt), and were fed brine shrimp (*Artemia*) nauplii each day. Test individuals were separated into pairs of similarly sized fish (< 1 mm difference in total body length) from the same clone. Each pair (DS = 18 pairs, NA = 12 pairs, B = 23 pairs) was arbitrarily assigned a sequence of three aggression tests; each individual in a pair received the same sequence. All six possible combinations of the three standard tests, the standard opponent (So), mirror (Mi), and model (Mo), were used to test for sequence effects.

The tests were conducted at intervals of 24 h between 09.00 h and 16.00 hours. The So and Mo tests were conducted in 11 cm × 6.5 cm × 19.5 cm tanks with the water at a height of 10 cm. The Mi test was conducted in the individual's original isolation tank, with the mirror attached to one side of the tank. A Plexiglas sheet placed over the top of each tank prevented the fish from jumping out.

The standard opponent was a fish of the same clone as the focal individuals and was at least 11% smaller than the mean length of all focal individuals from that particular clone to ensure that the test fish did not respond submissively to the opponent (standard opponent size: DS = 3.0 cm; NA = 2.8 cm; B = 3.0 cm). A transparent Plexiglas partition for the So test was positioned so that the focal individual had a swimming area of 7 cm × 6.5 cm × 10 cm and the standard opponent had an area of 4 cm × 6.5 cm × 10 cm. A gravel substrate was 1.5 cm deep in each of the test tanks.

The dummy for the Mo test was constructed of oven-baked clay with acetate fins. The size of the model depended on the size of the test individuals; for each clone, a model was used that had a size approximately equal to the mean size of all test individuals (mean size in cm: DS = 3.74 ± 0.05 SE; NA = 3.36 ± 0.02 SE; B = 3.64 ± 0.05 SE). Barlow et al. (1984) found that dummies approximating the size of the focal individuals elicited the most intense aggressive response. Colouration of the model was similar to that of *R. marmoratus*, with grayish-tan on the dorsal side and light tan on the ventral side. The model possessed no attributes relevant to contest behaviour such as threat display or attack postures. The dummy was suspended by a fishing line in the same tank as the test fish; it was positioned 3 cm below the water line, approximately at the centre of the tank and was held stationary throughout the experiment.

Before testing, the experimental tanks were cleaned and salt water (160 g/10 l, 12.3 ppt) was added. A Styrofoam partition blocked the standard opponent, mirror, or model from the view of the test fish until the test began. The test fish were put into their respective tanks 16 h prior to testing to provide an acclimatization period and were fed brine shrimp. The test began when the Styrofoam partition was lifted. The response of the test fish was videotaped for 30 min. Illumination was provided by a 100 W bulb approximately 15 cm above the centre of the experimental tank.

Dyadic Contests

Twenty-four hours after all three standard tests had been conducted, a dyadic contest was run between the two individuals in a previously designated pair, each of which experienced the same sequence of standard tests. The two fish of the dyad were distinguished by unique fin markings. The tank used for the contest had the same dimensions and substrate as the tanks used in the So and Mo tests. The dyad was separated for 16 h by a Styrofoam partition that divided the tank into two equal areas. After the partition was lifted, the fish were videotaped for 60 min.

The winner was the fish that bit or chased the other continually without return attacks or displays by its opponent (the loser). Continual retreat upon an approach from the other fish or emersion behaviour (jumping outside the test area and adhering to the surface of the tank) also indicated the loser.

Aggression Indices

The indices used to quantify the aggressive behaviour of the test fish towards the opponent in the standard tests were: (i) latency to first display(s); (ii) latency to first attack(s); and (iii) frequency of attack (bites/min from the start of the contest). To distinguish between a display and an attack, we confined the 'first display' to when the focal individual first oriented towards its opponent; this was often accompanied by erection of the dorsal and caudal fins and flaring of the operculum. Attack refers to physical contact between the focal individual and the opponent (the dummy in the Mo test or the conspecific in the dyadic contest), or the barrier between itself and the opponent (the mirror in the Mi test or the glass partition in the So test). In the dyadic contests we recorded: (i) the individual that first displayed; (ii) the individual that first attacked; and (iii) the winner and loser of the contest.

Statistical Analyses

The main effects of the experiment were quantified using a cross-over design multivariate ANOVA (MANOVA). A sequence effect tests the influence of the standard test order (e.g. Mi : Mo : So) on the aggressive behaviour of the test fish. The period effect tests whether the aggressive response of an individual changes over time, regardless of sequence. The statistical assumptions of the MANOVA were upheld and the most powerful test (Roy's Greatest Root) was used to draw conclusions about whether the independent variables differed significantly across the responses. Dunn–Sidak adjustments were conducted on the univariate analyses to minimize compounding of Type I errors ($\alpha_{adj} = 1-(1-\alpha)^{1/k}$; Sokal & Rohlf 1981).

A MANOVA also was used to test whether clone type or body size influenced aggressive responses and to determine whether the Mi, Mo, and So tests differed in their eliciting strength as stimuli for aggressive behaviour. Clone type and standard test type were entered as main effects with body size as a covariate; display latency, attack latency, and biting frequency were the response variables. Roy's Greatest Root was again used to determine significance and Dunn–Sidak adjustments were conducted on univariate analyses.

The predictive value of qualitative differences in aggression and its heterogeneity between clones was analyzed using a G-test. In this qualitative analysis, the explanatory variables were categorical (e.g. did one fish score higher than another in the standard tests?). Predictions from quantitative differences in aggression between paired contestants were determined using a logistic regression analysis with backward elimination, i.e. the explanatory variables were continuous. The quantitative approach allowed the importance of the magnitude of aggression differences between individuals to be assessed (e.g. how much higher did one fish score than another?). In the analysis, differences in display latency, attack latency, and biting frequency between paired individuals in each standard test (So, Mi, Mo) were regressed against outcome. The outcome refers to whether the fish which was arbitrarily designated Fish 1, won the contest. All independent variables that were eliminated in a stepwise fashion exceeded the 0.1 significance level; only variables that contributed significantly to the model are reported. The χ^2 statistic refers to the maximum likelihood chi-square.

Results

Effects of Sequence and Period in Standard Tests

Sequence effects were not significant for display latencies ($F = 1.11$, $p = 0.36$, $df = 5, 300$) or biting frequencies ($F = 0.61$, $p = 0.69$, $df = 5, 300$). The general MANOVA analysis suggested sequence effects for attack latencies ($F = 2.38$, $p = 0.04$, $df = 5, 300$) but the univariate analysis with Dunn–Sidak adjustments indicated no effects ($0.04 > [\alpha_{adj} = 0.017]$). Period had no significant effect on display latencies ($F = 0.42$, $p = 0.66$, $df = 2, 300$), attack latencies ($F = 1.94$, $p = 0.14$, $df = 2, 300$), or biting frequencies ($F = 0.4$, $p = 0.67$, $df = 2, 300$).

Clone Type, Body Size, and Test Effects on Aggressive Responses in Standard Tests

The effect of body size of the focal fish was not significantly different across responses ($F = 0.94$, $p = 0.42$, $df = 3, 310$); tests of parallelism ($F = 1.41$, $p = 0.25$, $df = 2, 311$) and levels ($F = 0.5$, $p = 0.48$, $df = 1, 312$) were also insignificant, indicating that variations in the responses with body size did not occur.

Aggressive responses differed significantly among clone types ($F = 16.1$, $p < 0.0001$, $df = 3, 311$) and test types ($F = 104.6$, $p < 0.0001$, $df = 3, 311$). Univariate analyses with Dunn–Sidak adjustments indicated clonal differences in attack latencies ($F = 3.66$, $p = 0.03$, $df = 2, 312$) and biting frequencies ($F = 15.5$, $p < 0.0001$, $df = 2, 312$), but not in display latencies ($F = 0.86$, $p = 0.42$, $df = 2, 312$). The DS clone exhibited significantly shorter attack latencies (Student Newman Keuls (SNK), $p < 0.05$) and higher biting frequencies (SNK, $p < 0.05$) than did the NA and B clones, but the latter two clones did not differ significantly with respect to either aggression index.

The mean latency to display was significantly different among tests ($F = 3.78$, $p = 0.02$, $df = 2, 312$). The Mi test elicited significantly shorter latencies to display than the So and Mo tests, which were not significantly different from each other (Fig. 1a). Mean latency to attack was also significantly different among tests ($F = 57.5$, $p < 0.0001$, $df = 2, 312$). Attack latency in the Mo test was significantly greater than attack latencies in both the So and Mi tests, which were not significantly different (Fig. 1b). Mean biting frequencies across the three standard tests were significantly different ($F = 92.9$, $p < 0.0001$, $df = 2, 312$). All paired comparisons, Mi–Mo, So–Mo, and So–Mi, were significantly different (Fig 1c). The data suggest a clear ordinal ranking for the tests' effectiveness as stimuli for overt aggressive behaviour: Mi > So > Mo.

Correlations Among Tests

The correlation coefficients for each comparison (Mi–Mo; So–Mo; So–Mi) were statistically homogeneous (χ^2 method described in Zar 1996) among the three clones for display latency and biting frequency (Table 1). Thus, the clone data were

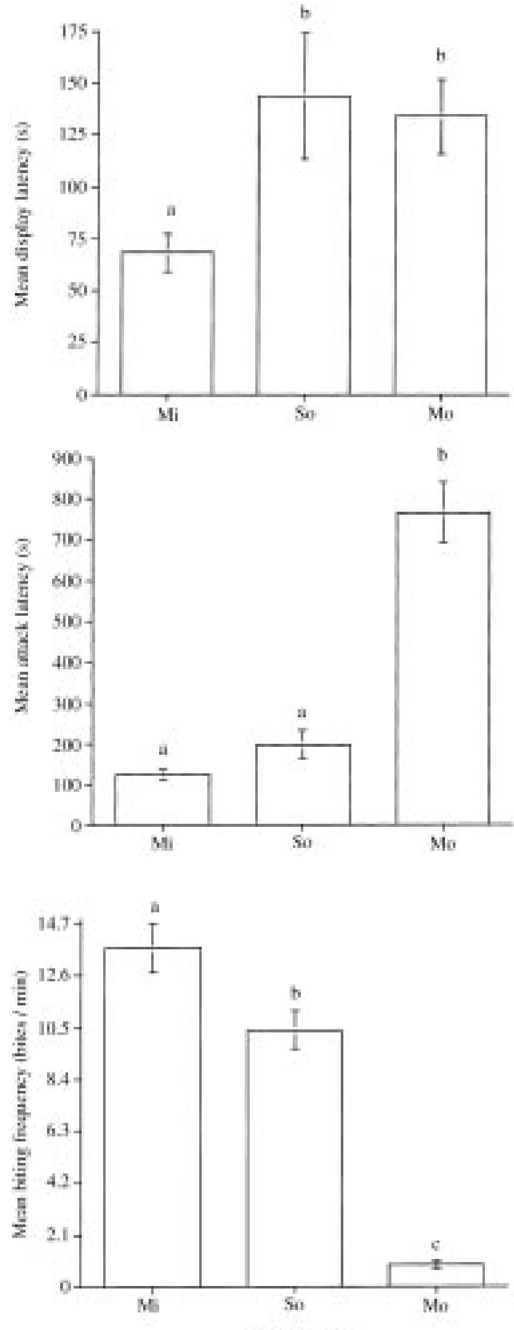

Fig. 1: The relationship between the mirror (Mi), standard opponent (So), and model (Mo) tests with respect to the mean: (a) display latency (SE, Mi = 11.5, So = 59.7, Mo = 151.6); (b) attack latency (SE, Mi = 0.98, So = 0.77, Mo = 0.15); and (c) biting frequency (SE, Mi = 9.03, So = 50.7, Mo = 18.2). Error bars represent ± 1 SE. Mean responses with different letter labels are significantly different from one another ($p < 0.05$; Student Newman Keuls multiple comparisons)

Table 1: Correlations among the standard tests (So, Mi, Mo) for display latencies, attack latencies, and biting frequencies. The corrected heterogeneity chi-square (χ_p^2) appears in bold with df = 2 and the correlation coefficient appears in plain type with df = 104

	display latency			attack latency			biting frequency		
	So	Mi	Mo	So	Mi	Mo	So	Mi	Mo
So	–	**3.98**	**0.10**	–	**5.85**	**0.49**	–	**1.70**	**0.52**
		0.042	0.203*		0.080	0.130		0.375‡	0.110
Mi			**3.41**			**6.20***			**0.12**
	–	–	0.168	–	–	DS: 0.380*	–	–	0.097
					–	NA: −0.050			
						B: −0.150			

* $p < 0.05$; ‡ $p < 0.0001$.

pooled for the correlation analysis among tests for these variables. For attack latency, the clones were homogeneous for both the So–Mo and So–Mi combinations but not for Mi–Mo (Table 1). The attack latency data were pooled for So–Mo and So–Mi but each clone was analysed separately for Mi–Mo. Significant correlations were found only for the pooled So–Mo display latency comparison, the pooled So–Mi biting frequency comparison, and the Mi–Mo attack latency comparison for the DS clone (Table 1). Hence, the aggression indices were not reliably correlated among the three standard tests.

Predictive Power of Standard Test Behaviour: Qualitative Analyses

The predictive relationship between behaviour in the aggression tests and the indices of the dyadic contest was first tested for homogeneity among the three clones (DS, NA, and B). The clones were homogeneous with respect to the predictive nature of their responses within each aggression test (heterogeneity, G-tests, $0.05 \leqslant G \leqslant 5.86$, $0.053 \leqslant p \leqslant 0.975$, df = 2; the G and p-values for each of the 27 situations described in Tables 2, 3 and 4 fell between the intervals shown). Therefore, the behavioural data for all three clones were pooled for each of the three tests.

The individual to initiate display faster in the standard tests was not significantly more likely to display first in the dyadic contest, attack first in the contest, or win the contest (Table 2). The individual of a pair with a shorter latency to attack in the standard tests was not significantly more likely to initiate display in the dyadic contest or to win the contest. However, individuals that attacked faster in the Mo test tended to initiate attack in the contest a significant proportion of the time while attack behaviours in the So and Mi tests did not predict analogous contest behaviour (Table 3). Individuals with higher biting frequencies in the stan-

Table 2: The probability that the individual of a pair to display sooner in the standard aggression tests will: (i) initiate display in the contest; (ii) initiate attack in the contest; or (iii) win the contest. N/A refers to situations where neither individual of the pair displayed towards the opponent, or where the two fish had the same display latency

	Yes	No	N/A	G-test	
Initiator of display in contest					
Mirror	24	26	3	0.08	ns
Model	23	27	3	0.3	ns
Standard opponent	20	31	2	2.4	ns
Initiator of attack in contest					
Mirror	22	26	5	0.3	ns
Model	29	20	4	1.7	ns
Standard opponent	24	25	4	0.02	ns
Winner of the contest					
Mirror	20	31	2	2.4	ns
Model	32	19	2	3.4	ns
Standard opponent	27	25	1	0.08	ns

Table 3: The probability that the individual of a pair to attack sooner in the standard aggression tests will: (i) initiate display in the contest; (ii) initiate attack in the contest; or (iii) win the contest. N/A refers to situations where neither individual of the pair attacked the opponent, or where the two fish had the same attack latency

	Yes	No	N/A	G-test	
Initiator of display in contest					
Mirror	33	19	1	3.8	ns
Model	23	21	9	0.1	ns
Standard opponent	27	25	1	0.08	ns
Initiator of attack in contest					
Mirror	29	21	3	1.3	ns
Model	29	15	9	4.5	*
Standard opponent	28	22	3	0.7	ns
Winner of the contest					
Mirror	26	27	0	0.02	ns
Model	27	18	8	1.8	ns
Standard opponent	27	26	0	0.02	ns

* $P < 0.05$.

dard tests were not likely to display first in the contest or win the contest. Biting frequency in the Mo test predicted the initiator of attack in the contest, while biting frequency in the So and Mi tests did not (Table 4).

Table 4: The probability that the individual of a pair with the higher biting frequency in the standard aggression tests will: (i) initiate display in the contest; (ii) initiate attack in the contest; or (iii) win the contest. N/A refers to situations where neither individual of the pair bit the opponent or where the two fish had the same biting frequency

	Yes	No	N/A	G-test	
Initiator of display in contest					
Mirror	30	22	1	1.2	ns
Model	26	18	9	1.5	ns
Standard opponent	29	23	1	0.7	ns
Initiator of attack in contest					
Mirror	25	25	3	0.0	ns
Model	30	13	10	6.9	*
Standard opponent	29	21	3	1.3	ns
Winner of the contest					
Mirror	33	20	0	3.2	ns
Model	28	17	8	2.7	ns
Standard opponent	31	22	0	1.5	ns

* $P < 0.05$.

Predictive Power of Standard Test Behaviour: Quantitative Analyses

Quantitative differences in aggressive behaviour between individuals may also aid in predicting contest outcome. As differences in display latency between paired fish increased in the Mi test, the individual with the shorter display latency was more likely to win a subsequent contest than was the individual that took longer to display ($\chi^2 = 6.3$, $p = 0.012$, $df = 1$, $\beta = 0.015$). As biting frequency differences between the paired individuals increased in the So ($\chi^2 = 8.9$, $p = 0.0029$, $df = 1$, $\beta = 0.194$) and Mo ($\chi^2 = 9.04$, $p = 0.0026$, $df = 1$, $\beta = 1.888$) tests, so too did the probability that the individual of a pair with the higher frequency would win. Attack latency differences in the standard tests were not predictive of contest outcome.

Discussion

General Discussion

The aggression indices were not strongly correlated among the standard tests (So, Mi, Mo) but the tests differed substantially, if not always significantly, in eliciting strength. The weak correlations demonstrate that responses by the test fish were quite variable and highly test specific. Individual aggression levels among tests suggested a general rank-order for eliciting strength, Mi > So > Mo, which coincides with results from many studies that have employed these aggression tests (e.g. Thompson 1963; Thompson & Strum 1965; Dore et al. 1978; but see Rhoad et al. 1975).

This study also illustrates that few indices of aggression from the standard tests successfully, or reliably, forecast the winner of a dyadic encounter and that the predictive power of the standard tests with respect to contest behaviour and outcome is inconsistent. Initiation behaviour (latency to display, latency to attack) in the standard tests did not generally predict analogous combat behaviour. The quantitative analyses, which assessed the magnitude of aggressive differences between paired fish, demonstrated that as the magnitude increased, the individual that scored higher in the standard tests had a better chance of winning the dyadic contest. In the So and Mo tests, an increased disparity in biting frequency between individuals led to a greater probability that the fish with the higher frequency would win. A similar result was obtained in the Mi test, with display latency differences.

The correlation analyses and the comparisons among standard tests with respect to their eliciting strength as stimuli for aggressive behaviour reveal that the aggressive responses of the focal fish may be highly dependent on the actions of the opponent. Game theoretical models (e.g. Maynard Smith & Price 1973; Maynard Smith & Parker 1976) and applications of sequential assessment models in animal contests (Enquist & Leimar 1983; Enquist et al. 1990; Koops & Grant 1993) emphasize the influences that one individual's behaviour, or assessment tactics, can have on the behaviour exhibited by another. Specifically, dyadic dominance is established through a series of increasingly escalated interactions, where the decision of one individual to continue to fight or to submit depends on the behavioural strategies utilized by its opponent. In our investigation of *R. marmoratus* behaviour, the rank-order for eliciting strength (Mi > So > Mo) corresponded well with the intensity of aggression often exhibited by the opponent types, indicating a direct correlation between opponent and test fish behaviour.

The effect of opponent type on the aggressive response of the test fish has been clearly demonstrated in studies that utilize dummy opponents (e.g. Barlow & Siri 1994). Dummies are useful in the study of aggressive behaviour because they serve as constant visual stimuli and effectively eliminate the confounding effects of opponent behaviour variation on the response of the test fish. However, dummy attributes such as colour, size, posture, and movement may vary substantially. These characteristics, though not necessarily behavioural, also affect the aggressive response of the test fish (ter Pelkwijk & Tinbergen 1937; Seitz 1940; Thompson 1963; Rowland 1975; Barlow et al. 1984; Barlow & Siri 1994; Beeching 1995), and ultimately may affect the aggression scores. In the present study, opponent behaviour variability was likely to have been high within the So tests. The same standard opponent was tested against all fish of the same clone, but its behaviour was unlikely to be constant against every focal individual and it may have elicited varying amounts of aggression from the test fish.

The influence of opponent type and behaviour between (and even within) tests poses potential problems for analysing aggression via standard testing methods. Standard test methodologies (i.e. the opponents used) vary substantially among studies, thus making it difficult to assess the general utility of the tests across species. Additionally, opponent variability in behaviour or morphology, and the consequent variations in the response of the test fish, may confound interpretations about the meaning of aggression scores with respect to dominance ability.

For most studies, the standard tests are used to measure how individuals would perform in contests. Initiation behaviour (display and attack latencies) in the standard tests failed to predict analogous combat behaviour in *R. marmoratus*. This result corresponds well with those of Dore et al. (1978) and Meliska et al. (1980) who found minimal correlations between mirror display and combat-elicited display in *Betta splendens*. The propensity of an individual to initiate display or attack in a contest may be tightly linked to the contest situation (i.e. the opponent). Initiation behaviour may not be expected to predict analogous contest behaviour if, as is likely, the standard tests provide different stimulus situations than do the dyadic contests (Gallup 1968; Franck & Ribowski 1987; Ruzzante 1992).

The comparisons of individual aggressive differences in the standard tests, to dominance ability in the dyadic contests, illustrate that the manner in which each individual behaved relative to its counterpart in the standard tests predicted dominance outcome better than did absolute aggression. Games of sequential assessment predict that as the degree of asymmetry (e.g. size differences) between contestants increases, dominance outcome in favour of the individual on the positive end of the asymmetry becomes easier to predict (Maynard Smith 1982; Enquist et al. 1990). Symmetry among individuals leads to more escalated contests of longer duration and the dominant individual is often the contestant who is willing to exhibit more costly behaviour, i.e. differences are manifest behaviourally.

R. marmoratus dyads were matched for size and experienced identical sets of standard test sequences prior to the ensuing contest. Without asymmetrical cues such as size and experience-related differences in behaviour, each individual of the pair is expected to have an equal probability of winning the subsequent contest. Thus, one might expect little predictability of contest outcome from standard test behaviour unless substantial differences in intrinsic aggression levels exist between individuals. The quantitative analysis suggests that as differences in intrinsic aggression levels between paired opponents increases, more accurate predictions of contest outcome can be made. This result corresponds well with game theoretical predictions of symmetrical contest dynamics and it is thus not surprising that the standard test behaviour fails to accurately forecast contest outcome without significant variation in individual aggression levels. The role of individual differences in aggression has also been shown in the courtship of Midas cichlids (*Cichlasoma citrinellum*, Barlow 1998), where pairing success was determined not by the absolute aggression scores of the males and females, but rather by the aggression levels of the females relative to the males.

The most highly escalated behaviour (biting frequency) was that which, in a majority of the tests (So and Mo), best predicted contest outcome. Although direct measures of contest costs (i.e. energetics), as they relate to interaction intensity and outcome, have provided the most substantial support for game theoretical models of animal combat (Smith & Taylor 1993; Hack 1997), behaviour that incurs substantial risk of injury is also often considered costly to perform (e.g. biting frequency and mouth wrestling; Enquist et al. 1990). Biting frequency in the standard tests may indicate an individual's willingness to persist or escalate in a contest and is potentially the most costly of the three indices used in the present study. Variations among individuals in their tendency to escalate to more costly behavioural tactics in the standard tests, as opposed to differences in initiation behaviour, may be the most useful in forecasting dyadic dominance in otherwise symmetrical contests.

Synthesis

Substantial evidence for the use of standard tests as general tools to assess dominance is lacking and, so too, is a consensus on the behaviour that best predicts contest outcome (Table 5). Of the 15 studies reported, seven revealed no predictive relationship between behaviour measures in the standard tests and contest outcome, four indicated that aspects of display behaviour such as frequency, intensity, or duration were good predictors of outcome while the remaining four forecast outcome through escalated responses such as attack latency or SAM (swim against mirror), a highly escalated behaviour accompanied by violent head shaking and jaw snapping (See Table 5 for details). In the present study, only three of nine indices predicted outcome. Differences in biting frequency in the standard tests with relatively passive opponents (So, Mo) predicted outcome, while display latency in the Mi test which, of the three standard tests, provided the most aggressive opponent, predicted outcome.

This failure to reach definitive conclusions about the utility of standard tests in studies on aggression or dominance may stem from one of many potential reasons. Interpretation of standard test responses is made more complex by the presence of opponents whose behaviour may be highly variable or unnatural. Furthermore, the test-specificity of the individual responses may render conclusions about the relationship between aggression and dominance valid only under certain, limited circumstances.

An underlying factor that often is not taken into account, but which can influence an individual's response to an opponent, is the context in which the fight takes place (e.g. a territorial dispute, a dyadic contest, or a fight for position in a hierarchy). Standard testing methods have been employed to analyse each of these three different types of contests. Bakker & Sevenster (1983), Hamilton & Poulin (1995), and FitzGerald & Kedney (1987) concluded that the frequency with which an individual attacks an opponent does not forecast the winner of the bout. In each of these studies, territorial aggression was of primary interest. Most often, a territory owner need not engage in highly escalated bouts in order to chase off an intruder; the owner may only need to attack the intruder for it to flee. Indeed, FitzGerald & Kedney (1987) found that the rapidity of attack served as a better indicator of dominance (or territory ownership) than frequency of attack.

Baenninger (1968) and Holtby et al. (1993) used individual aggressive responses to mirror-image stimulation to predict group dominance status in *Betta splendens* and *Oncorhynchus kisutch*, respectively. Frequency of display predicted the alpha-individual in *B. splendens* groups while SAM predicted group dominance in *O. kisutch*. Despite these predictions, dominance relationships between pairs of

Table 5: A sample of studies addressing the utility of using standard aggression tests to predict subsequent dominance and the results they obtained while employing various aggression indices

Author, year	Species	Standard tests	Aggression indices	Predict dominance?
Baenninger 1968[2]	*B. splendens*	Mi	gill cover extension frequency	YES
Evans 1985[2]	*B. splendens*	So	gill cover erection (GCE)	YES
Meliska et al. 1975[2]	*B. splendens*	Mi	threat display intensity	YES
			approach duration	YES
			display frequency	YES
Meliska et al. 1980[1]	*B. splendens*	Mi	GCE duration (GCEDUR)	NO
Barlow et al. 1986[3]	*C. citrinellum*	Mo	aggression score = sum of subjects' aggressive responses to the dummy	NO (long-term) YES (short-term)
Bakker & Sevenster 1983[1]	*G. aculeatus L.*	So*	% of time spent biting or bumping at the intruder	NO
Bakker 1986[1]	*G. aculeatus L.*	So*	% of time spent biting or bumping at opponent	NO
FitzGerald & Kedney 1987[3]	*G. aculeatus L.*	So*	latency of attack	YES
			duration of attack & orientation	NO
			frequency of attack & orientation	NO
			% time spent in attack & orienting (orientation toward intruder)	NO
Hamilton & Poulin 1995[1]	*G. breviceps*	Mi	number of attacks	NO
			time in aggression zone	NO
Francis 1983[1]	*M. opercularis*	Mi, So	threat display duration in Mi	NO
			frontal display duration in So	NO

Table 5: cont.

Author, year	Species	Standard tests	Aggression indices	Predict dominance?
Francis 1984[1]	*M. opercularis*	Mi, So	threat display duration in Mi	NO
			frontal display duration in So	NO
Holtby et al. 1993[3]	*O. kisutch*	Mi	lateral display 1 (LAT1)	NO
			lateral display 2 (LAT2)	NO
			swim against mirror (SAM)	YES (overall best)
			swim along mirror (SLM)	NO
			submissive display (SUB)	NO
			SAM + SLM = OVAGGR	YES (stream – tank)
			LAT + OVAGGR = AGGR	YES (paired contests)
			AGGR + SUB = TOTBEHAV	YES (paired contests)
Rosenau & McPhail 1987[2]	*O. kisutch*	Mi	threat display duration	YES
Berejikian et al. 1996[3]	*O. mykiss*	Mi	swim against mirror (SAM)	YES
			display duration	NO
Earley et al. present study[2,3]	*R. marmoratus*	Mi, So, Mo	relative biting frequency	YES (So, Mo)
			relative display latency	YES (Mi)
			relative attack latency	NO
Franck & Ribowski 1987[1]	*X. helleri*	Mi	biting frequency	NO

The abbreviations for standard tests are as in the text.
* refers to a type of standard opponent test called the 'model-bottle' test which is used in studies concerned with territorial aggression.
The table is arranged in alphabetical order according to the test species used.
[1] indicates those citations in which no predictions were found.
[2] indicates that display behaviours predicted outcome.
[3] indicates that escalated behaviours predicted outcome.

individuals, as assessed through pairwise competitions, show low correlation with the rank of those same individuals in a hierarchy (Nelissen 1985). Social rank may be determined by additional aggression-related factors, or 'group factors', such as the intervention of high-ranking individuals in fights between lower ranking group members (Nelissen 1985; Ruzzante 1992). These factors, which are often manifest in group hierarchies, may not be evident in dyadic encounters, thus standard aggression tests do not appear to be applicable in studies of this sort.

Another potential source of variability among the studies employing standard tests is the presence of confounding factors, including prior contest experience which is known to influence the outcome of later contests (Chase et al. 1994; Hsu & Wolf 1999). For example, *Macropodus opercularis* individuals experiencing a win or a loss prior to aggression testing (Mi, So) did not differ in their scores for aggression displays but differed substantially in their success in future dyadic encounters (Francis 1983). Similar results were obtained after artificial selection for social dominance (Francis 1984). Since aggression testing towards the mirror or conspecific opponents always followed dominance or subordination experience, no comparison could be made between pre-experience aggression and dominance. Prior experience may also have influenced the aggression scores or dominance outcomes of contests in sticklebacks (*Gasterosteus aculeatus* L.; Bakker & Sevenster 1983; FitzGerald & Kedney 1987) and *B. splendens* (Baenninger 1968).

Conclusions

The results of standard testing seem to be test-specific and context-specific. The current use of these tests (So, Mi, Mo) may not be sufficient to reveal the complex relationship between aggression and dominance in dyadic contests. Nevertheless, the standard tests do seem to have considerable utility in describing individual differences in aggressive tendencies. If individual responses towards a particular opponent were shown to be repeatable, the value of standard tests for comparing intrinsic aggression levels among individuals could be realized. Franck & Ribowski (1987) showed high repeatability in individual responses to a mirror image between two successive exposures at intervals that ranged from 2 h to 2 months; responses toward the same conspecific opponent were also reproducible on the order of 2 h. Many more studies of this sort are needed to confirm the degree to which behaviour within each test is repeatable.

If the standard tests are to be employed to predict dominance outcome, however, conspecific opponents seem the most practical to use for they respond to the test fish in ways that simulate an actual contest, in contrast to the unnatural 'behaviour' of the mirror-image and model. Video playback has emerged as another method to analyze aggression in fishes (Rowland et al. 1995; McKinnon & McPhail 1996) but some of the same limitations, as those described above, also apply in this technique (Fleishman et al. 1998).

Indices of aggression that have the highest potential of relating to actual contest outcome should be chosen. Enquist & Leimar (1983) proposed that, in a symmetrical contest, outcome should be most evident later in the bout when more costly behaviour is exhibited. Additionally, individuals who attack first or more frequently, and those who are more willing to escalate most often go on to win dyadic contests (e.g. Figler et al. 1975; Figler & Einhorn 1983; Barlow et al. 1986; Jackson 1991). Forms of escalated behaviour seem to be the best candidates for use as aggression indices in the standard tests. Furthermore, since the behaviour exhibited by the test fish depends on opponent responses, emphasis should be given to recording the behaviour of both the aggressor and the receiver in the standard tests as well as analyses of the relative differences in aggression scores between paired individuals.

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