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# TITLE: Two sides of the same coin? Consistency in aggression to conspecifics and predators in a female songbird

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Running title: Consistency in female aggression across contexts

## Abstract

Different forms of aggression have traditionally been treated separately according to function or context (e.g. aggression towards a conspecific versus a predator). However, recent work on individual consistency in behavior predicts that different forms of aggression may be correlated across contexts, suggesting a lack of independence. For nesting birds, aggression towards both conspecifics and nest predators can affect reproductive success, yet the relationship between these behaviors, especially in females, is not known. Here we examine free-living female dark-eyed juncos (*Junco hyemalis*) and compare their aggressive responses towards three types of simulated intruders near the nest: a same-sex conspecific, an opposite-sex conspecific, and a nest predator. We also examine differences in the strength of response that might relate to the immediacy of the perceived threat the intruder poses for the female or her offspring. We found greater aggression directed towards a predator than a same-sex intruder, and towards a same-sex than an opposite-sex intruder, consistent with a predator being a more immediate threat than a same-sex intruder, followed by an opposite-sex intruder. We also found positive relationships across individuals between responses to a same-sex intruder and a simulated predator, and between responses to a same-sex and an opposite-sex intruder, indicating that individual females are consistent in their relative level of aggression across contexts. If correlated behaviors are mediated by related mechanisms, then different forms of aggression may be expressions of the same behavioral tendency and constrained from evolving independently.

## Keywords:

Female aggression, nest defense, behavioral syndrome, personality, *Junco*, intra- and inter-specific aggression

## Introduction

Aggression in its broadest sense is any overt fighting behavior or signal of imminent behavior with the capacity to harm (Moyer 1968; Huntingford 1982; Nelson 2006). Such behavior has long been the focus of study because of its conspicuous nature and the risk of injury (Lorenz 1966; Moyer 1968; Nelson 2006). In seminal papers on aggression, Lorenz (1966), and later Huntingford (1976), argued for classifying aggression into three categories: predator towards prey, anti-predator aggression, and social aggression among conspecifics. Because these categories reflect very different functions, other authors developed more restrictive and specific definitions that limit use of the term aggression to contexts in which the behavior is directed only at conspecifics (Moyer 1968; Nelson 2006). Further, studies of the neural basis of aggression towards conspecifics in mammals reveal that even within the limited sphere of conspecific aggression, the mechanisms of aggression can depend on the category of conspecific confronted (Gammie and Lonstein 2006). For example, neurotransmitters (e.g. GABA) can inhibit female aggression towards male, but not female, intruders (Palanza et al. 1996), and male mice selected for short attack latencies (SAL) are aggressive to both sexes while males selected for long attack latencies (LAL) are less aggressive to females, a difference reflected in prefrontal cortex serotonin levels (Caramaschi et al. 2008). Consequently, current treatments of aggressive behavior have tended to consider aggression in separate contexts largely independently (but see Huntingford 1976; Johnson and Sih 2005; Duckworth 2006; Witsenberg et al. 2010).

In recent years it has become increasingly evident that there is considerable variation among individuals in behavioral response to a variety of stimuli, differences that are often consistent within individuals, across time and contexts (Sih et al. 2004; Groothuis and Carere 2005; Bell 2007). This phenomenon of within-individual consistency in behavioral response has been variously termed personality, behavioral syndrome or type, coping style, temperament, or behavioral profile (Sih et al. 2004; Groothuis and Carere 2005; Bell 2007). Research on these suites of behaviors, and the mechanisms underlying their regulation, is crucial to our understanding of basic questions related to the functions and causes of animal behavior (Sih et al. 2004; Groothuis and Carere 2005; Bell 2007). Furthermore, if behavioral traits are mechanistically linked (e.g., via gene expression or hormonal mediation), then that linkage may affect evolutionary trajectories, either by constraining optimal trait expression or facilitating rapid change (Dingemanse et al. 2004; Bell 2007; Ketterson et al. 2009).

While considerable work has been done on behavioral correlations in general, our understanding of the ways in which individuals covary in their aggressive responses across of a variety of contexts is still limited (Bell 2007b). The majority of studies examining relationships among behaviors in different contexts have focused on personality metrics such as shy/bold and fast/slow exploring (Gosling 2001;

Dingemanse et al. 2004; Sih et al. 2004; Groothuis & Carere 2005) or how aggression in one context is related to other behavioral traits (Carere et al. 2005; Duckworth 2006; Kortet & Hedrick 2007; Hollander et al. 2008). Other studies have examined aggression to predators or conspecifics, but have not examined individual consistency in their responses to those stimuli (Gottfried 1979; Slagsvold 1993; Parmigiani et al. 1998; Sandell 1998; Mays 2004; Parn et al. 2008; Hollander et al. 2008). Consequently, much less is known about how aggression in one context may relate to aggression in another despite the observation that both sexes often show marked aggression towards both conspecifics and heterospecifics in the contexts of mate acquisition, defense of monogamy or paternity, and nest defense (Yasukawa and Searcy 1982; Montgomerie and Weatherhead 1988; Slagsvold 1993; Sandell 1998; Jawor et al. 2004; Johnson and Sih 2005; Nelson 2006, O'Neal et al. 2008). There are however, a number of findings that suggest these behaviors are related; Huntingford (1976) described a positive relationship between conspecific and heterospecific aggression in sticklebacks (*Gasterosteus aculeatus*), the same pattern was reported in cooperative cichlids (*Neolamprologus pulcher*) (Witsenberg et al. 2010), and wild mice selected for increased nonbreeding aggression showed a correlated response during lactation (Ebert 1983).

For animals with eggs or dependent young, aggression towards both conspecifics and predators can have profound and immediate effects on the reproductive success of the individuals associated with the reproductive attempt (Gottfried 1979; Montgomerie and Weatherhead 1988; Martin 1995; Sandell 1998; Clotfelter et al. 2007; Rosvall 2008). Predation often plays a major role in life history evolution (Martin 1995) and failed predator defense can lead to the immediate and total loss of offspring (Montgomerie and Weatherhead 1988; Cawthorn et al. 1998). For species that experience heavy predation pressure, this can provide strong selective pressure for effective predator deterrence (Montgomerie and Weatherhead 1988; Martin 1995). Aggression towards conspecifics near the eggs or young may have similar immediate benefits if the intruder is likely to destroy the offspring (infanticide) and/or act as an intraspecific brood parasite. This form of aggression by females, termed maternal aggression, is well explored in mammals, where infanticide is common (Ebert 1983, Palanza & Parmigiani 1994; Gammie and Lonstein 2006; Nelson 2006). However, aggression by reproductive females towards conspecifics (of either sex) is also seen in instances in which there is no direct threat or consequence for offspring. For instance, female-female aggression may be favored if social monogamy is beneficial to the female and aggression restricts polygamy (Yasukawa and Searcy 1982; Slagsvold 1993; Sandell 1998). Alternatively, female aggression towards intruding males may be beneficial if the presence of foreign male reduces paternal certainty, favoring a reduction in paternal investment (Westneat and Stewart 2003).

By measuring aggression in the same individuals across multiple contexts we can address two key questions; are individuals consistent in their responses across context (i.e. how are different forms of aggression related), and does the population as a whole show consistent differences in the degree of response according to context (i.e. is the population predictably more aggressive towards certain stimuli)? The first question points to a mechanistic relationship between different types of aggression, while the second question explores the ultimate functions of aggression. To address these two objectives we determine whether female dark-eyed juncos (*Junco hyemalis*) show intra-individual consistency in aggression *across contexts*, indicating that different forms of aggression are not independent. We then test whether females show inter-individual consistency in aggressiveness *according to context*, reflecting the potential level of threat each type of intruder presents to female fitness. To accomplish this we assayed two groups of incubating females, one group in the contexts of a same-sex intruder and a nest predator, the second group in the contexts of a same-sex and an opposite-sex intruder.

## Methods

### ***Study species, site and field methods***

Subjects were free-living female dark-eyed juncos (*Junco hyemalis carolinensis*), a North American sparrow. The resident population was censused prior to onset of breeding activity and all adults were uniquely color banded. Social pairs were identified via observations of behavior around the nest (nest building, incubation, defense). Juncos are cryptic ground nesters; both sexes feed and defend young; females alone incubate, for 12 days on average, before eggs hatch (Nolan et al., 2002). Every effort was made to locate nests during building or laying so that incubation onset was known. However, some nests were located after incubation onset, consequently the age of the eggs in some nests was unknown. Details regarding the study site and field methods are described elsewhere (McGlothlin et al. 2005; Reed et al. 2006; McGlothlin et al. 2007). All breeding pairs were located on and around the grounds of the Mountain Lake

Biological Field Station, in Giles Co., Virginia (37°22’N, 80°32’W). The Institutional Animal Care and Use Committees at both Indiana University and the University of Virginia approved all aspects of this experiment.

Two groups of females were assayed for aggression and each individual within a group was assayed in two different contexts. Four females were assayed in all four contexts but in only two contexts per breeding season. All trials were conducted during the incubation period, with 24-48 hours elapsing between trials, between 01 May and 15 July 2008 and 2009. Behavior trials began two days after incubation onset (if eggs of known age), and at least 24 hours after the nest was located if age was not known. Juncos will occasionally abandon a nest if partially predated, especially early in incubation (Nolan et al. 2002); therefore we chose not to randomize the order of presentation of stimuli for this set of trials in order to minimize the probability of nest abandonment. Females in Group 1 (n= 18) were presented a same-sex intruder first, followed by the simulated predator 24-28 hrs later. Because the trials for Group 1 were not randomized there is a possibility that any differences in response between the two contexts is due to trial order rather than stimulus. To address this possibility we consulted a previous study done in this population that also examined female aggression towards a predator during incubation. Clotfelter et al. (2004) quantified the number of attacks directed toward a nest predator among females that had no previous testing. There was no detectable difference in the level of aggression in those females (n=18) versus females in this study (n= 18; Wilcoxon test, $X^2 = 0.08$, p= 0.77). This strongly suggests that any differences observed between response towards a predator and response towards a same-sex intruder is not due to conditioning or priming of aggression by an earlier trial. Females in Group 2 received the stimuli (male intruder, female intruder) in a random order (n= 27, both). The conspecific trials for Group 1 were 30min in length and the predator trials were 10min in length. To allow direct comparisons, we scored only the first 10min of the conspecific trials. For Group 2 all trials were 10min in length.

### *Conspecific aggression behavioral trials*

Conspecific aggression was measured in females by recording behavioral response to a caged conspecific (intruder), similar to McGlothlin et al. 2007. For both groups, five conspecifics of each sex were captured offsite and held in captivity through season. For each trial, the intruder (chosen at random from among the 5) was placed in a small wire cage (15 cm cube) with large openings so that the intruder could be clearly seen and the cage was positioned within 3m of the focal female's nest. A camouflage-patterned cloth with a line attached covered the cage until the trial began. If the female was incubating she was flushed using a long stick to preclude her from seeing the human observer approaching the nest during set-up. If the female was not present the stimulus was set-up and covered as above. The observer then retreated >15m, waited for the female to approach within 5m of the nest and in direct line of sight of the cage. The observer then uncovered the stimulus by pulling the line attached to the cloth. Once the stimulus was uncovered, behavioral observations began. A single observer conducted all trials (KEC for Group 1, MR for Group 2) using binoculars and noted all behaviors and locations to a second observer who transcribed data and operated a stopwatch. Behavioral data included amount of time spent within 0.25m, 0.25-1m, 1-5m, on the nest, and the number of dives (swoops at the stimulus without contact) and hits (contact with the intruder/cage). The day of incubation, number of eggs, and whether or not the male associated with the nest was present during the trial were also recorded.

### ***Anti-predator aggression trials***

Anti-predator aggression was assayed by recording response to a simulated nest predator, a taxidermic mount of the chief nest predator on this population, the eastern chipmunk (*Tamias striatus*), posed in a life-like position, as used in earlier investigations (Cawthorn et al. 1998; Clotfelter et al. 2004; O'Neal et al. 2008). The protocol and the behaviors recorded were identical to those for intra-sexual aggression trials except a different single observer observed all trials (KA).

### ***Statistical methods***

All statistics were run using JMP 8 for Mac. Because behaviors were intercorrelated and we were interested in the overall level of aggression, we used Principal Components Analysis (PCA) to extract a single aggression score for each trial. The variables included time spent within 0.25 m, time spent within 1-5m, and total numbers of attacks (hits and dives combined), loading details are listed in Table 1. In all four treatments the aggressive behaviors (time within 0.25m and attacks) loaded strongly on the first principal component, which explained greater than 55% of the variation in the suite of aggressive behaviors.  A backwards-stepwise regression revealed that day of incubation, number of eggs, and male presence did not show a significant relationship with aggression scores for any of the behavior trials (all $p > 0.20$, power< 20%). The identity of the intruder used for a trial did not have a significant effect on behavior (all $p > 0.60$, power<25%). Whether or not the female was flushed during set-up did not have a detectable effect on response (all $p>0.50$, power< 10%). Pearson's correlations (*r*) were used to relate aggression scores towards a female intruder and a predator for Group 1, and towards male and female intruders for Group 2. We also examined the relationship between single behaviors that contributed to the composite aggressive response (e.g. attacks, time spent within 0.25m, time within 1-5m) across contexts using Pearson's correlations, to determine if the specific behaviors were also correlated.

Songbirds (Passeriformes), including the junco, often use a variety of postures to indicate aggressive intent when in close range (Searcy et al. 2006). This is a subtle behavior that is not easily observed from a distance, particularly when executed on the ground and in vegetation. Distance to stimulus consistently predicts attack in other songbirds, indicating this measure is as an effective proxy for aggressiveness (Searcy et al. 2006). Consequently, to compare the overall strength of response to a given stimulus, we used time spent within 0.25m, which correlated highly with attacks (total number of hits and dives) in all trials (Pearson's correlations: Group 1, female $r= 0.82$, $p< 0.0001$, predator $r= 0.87$, $p< 0.0001$; Group 2, female $r= 0.65$, $p= 0.0002$, male, $r = 0.75$, $p< 0.0001$), was readily observable, and was directly comparable across trials. Because each female was tested twice, we used a restricted maximum likelihood linear mixed model (REML) to determine whether the strength of response (time within 0.25m) was significantly predicted by the type of stimulus experienced (simulated predator, same-sex or opposite-sex intruder), while controlling for the identity of the female (van de Pol and Wright, 2009). The model for Group 1 included the stimulus (predator or female) and female identity (random factor). The model for Group 2 included 3 fixed effect variables: whether it was the first or second trial, the sex of the intruder, and a sex by trial interaction term; identity of the focal female was a random effect. To determine whether females were more aggressive to one sex or the other in the first trial we employed a *t*-test to compare response to a same-sex and opposite-sex intruder.

## Results

### a. *Consistent response to same-sex conspecific and predator*

We found a positive correlation between predator and same-sex conspecific aggression scores (Fig. 1; female score & predator score, *r*= 0.56, p= 0.0127). Examining single behaviors that contributed to the composite aggressive response, we found significant or marginally significant positive relationships between each behavior used to calculate the composite aggression scores (Fig. 2; within 0.25m, *r*= 0.40, p= 0.081; time within 5m, *r*= 0.67, p= 0.001; number of attacks, *r*= 0.46, p= 0.046).

### b. *Consistent response to same-sex and opposite-sex conspecifics.*

There was a positive, significant relationship between same-sex and opposite sex conspecific aggression scores (Fig. 1; female score & male score, *r*= 0.45, p= 0.0170). Controlling for the order in which the sexes were presented in a multiple regression strengthened the relationship, though order was not significant in the model (Overall model: $R^2$= 0.36, p= 0.0046, Order; *t*= 1.05, p= 0.30). Examining the single behaviors that made up the PC score, we found significant positive correlations between all measures (time within 0.25m, $r$= 0.42, p= 0.026; time within 1-5m, $r$= 0.38, p= 0.049; number of attacks, $r$= 0.44, p= 0.018).

### c. *Differences in aggression intensity according to context*

In Group 1, females responded more aggressively to a predator than to a same-sex intruder (Fig. 2; n= 19, β= 53.3, SE= 24.5, p= 0.036). In Group 2, neither the sex of intruder (Fig. 2; n= 27, β= -1.8, SE= 11.95, p= 0.88) nor whether it was the first or second trial at the nest had an effect on the strength of response (β= -13.9, SE= 11.95, p= 0.26). However, the order in which the sexes were presented (sex by trial) had a robust and significant effect on response (β= -46.12, SE= 11.95, p= 0.024). Individuals presented with a same-sex intruder first (opposite-sex intruder second), were significantly more aggressive towards both the same and opposite-sex intruder; individuals presented with an opposite-sex intruder first (same-sex intruder second) showed markedly reduced aggression to both sexes. Comparing the strength of response using only behaviors from the first trial revealed that females as a group were more aggressive towards same-sex than opposite-sex intruders (time in 0.25m, $t_{1,27}$= 3.57, p= 0.002).

## Discussion

Here we report that females were consistent in their aggressive responses both across contexts and according to context. In one group we found a positive correlation between a female's response to a predator and a same-sex intruder; in a second group we found a positive correlation between a female's response to a same-sex and an opposite-sex intruder. Individual females were also consistent in all of the behaviors that contributed to the composite aggressive response across contexts. As a group, females differed in the strength of their response according to context; the strongest response was directed towards predators, followed by same-sex intruders, then opposite-sex intruders.

### *Implications of differences in the strength of response*

*Same-sex intruder vs. predator*— The robust difference in response to a predator versus a same-sex intruder suggests that a predator is perceived as a greater threat to the female or her nest. Juncos are open-cup ground nesters and highly vulnerable to predation, especially from rodents (e.g. *T. striatus* and *Peromyscus sp.*), **losing up to 85% of nesting attempts in some years** to predation (Nolan et al., 2002; Clotfelter et al. 2007). However, juncos have been observed to defend their nests effectively against small rodents (Clotfelter et al. 2007). Consequently, aggression that successfully deters a potential nest predator is likely to be very important to female reproductive success (Montgomerie and Weatherhead 1988; Martin 1995; Cawthorn et al. 1998; Clotfelter et al. 2007; O'Neal et al. 2008).

*Opposite sex intruders*— Our finding that females were more aggressive to female than to male intruders suggests that while both types of aggression may be important, a female intruder is a greater threat than a male intruder. Female aggression towards conspecifics in biparental species, especially those that do not engage in nest destruction or brood parasitism, is often explained as protection of paternal investment. If a male intruder signals the loss of paternity to the social mate, and thus favors reduced paternal investment, aggression towards male intruders would be advantageous (paternity-uncertainty hypothesis)(Westneat and Stewart 2003). Alternatively, aggression towards a female intruder may restrict polygyny, limiting competition from another female for paternal assistance or resources (maintenance of monogamy hypothesis)(Yasukawa and Searcy 1982; Slagsvold et al. 1992; Slagsvold 1993; Sandell 1998, Rosvall 2008). Previous studies have also reported that passerine females are more aggressive towards same-sex intruders than opposite-sex intruders in great tits (*Parus major*) (Slagsvold 1993), yellow-breasted chats (*Icteria virens*) (Mays 2004) and bluethroats (*Luscinia s. svecica*) (Parn et al. 2008). Together these findings suggest that heightened female aggression towards a same-sex intruder may be a general trend in passerines, i.e. that sharing a male's paternal effort with another female (polygyny) is more costly than increased male paternity uncertainty.

Our finding that females respond to the second conspecific intrusion according to the sex of the first intruder (the order by sex interaction) is intriguing but challenging to interpret. As far as we can determine, other studies examining female response to a male versus a female intruder either did not use a repeated measures design or did not test for an order or order by sex effect (Slagsvold 1993; Sandell 1998; Mays 2004; Parn et al. 2008). Consequently, it is difficult to say whether this is a common pattern or an unusual finding. However, previous work on banded wrens (*Thryothorus pleurostictus*) also found that subsequent behavioral responses were affected by earlier treatments in both sexes (Hall et al. 2005). Regardless, the stimulus on the first day had a clear effect on the female junco's response the second day, such that females that received the female intruder first were aggressive to both intruders, while females that received the male intruder first were not very aggressive to either. This suggests that there is some kind of carryover effect, possibly due to physiological factors. For instance, the female intruder might cause a change in sex steroids (or receptors, other signaling molecules, or gene expression) that has lingering effects 24 hours later during the second trial. Alternatively, the female may form a learned association between the sex of the intruder received in the first trial and the intrusion set-up itself (cage, cloth, etc.) and responded accordingly the next day regardless of the sex of the new intruder. If this were the case we would expect female's latency to respond would differ, relative to the first trial. However, we were unable to detect a difference in latency to respond when comparing the first trial to the second (matched pairs *t*-test, $P>0.15$).

### ***Implication of correlations between behaviors across contexts***

We report a strong relationship between aggressive responses across context. The relationship between functionally important behaviors is crucial to our understanding of the evolution of behavior (While et al., 2010; Sih et al. 2004; Groothuis and Carere 2005; Bell 2007). If the expression of these traits is tied tightly together, it may be that different types of aggression (e.g. towards conspecifics or predators) are not free to evolve independently, providing some explanation for seemingly maladaptive behavior, and suggesting the existence of common mechanisms for seemingly independent traits (Sih et al. 2004; Bell 2007). For example, in male bluebirds, strong aggressive responses to conspecifics are related to low mate provisioning levels, reducing nest success (Duckworth 2006). Conversely, recent findings in juncos indicate that, at least in some years, females that are more aggressive towards female intruders also are more likely to produce fledged young (own data). The correlations we see here hint at a potential mechanism behind this relationship. Greater same-sex aggression predicts greater predator aggression among females, suggesting that females with more aggressive phenotypes may be better able to deter predators, improving nest success, or be more capable of settling on higher quality (lower predator density) territories.

The strong relationship between responses across contexts suggests that there may be a common mechanism regulating these behaviors or that individuals evaluate stimuli and modulate responses in a consistent manner. There are currently few data regarding the mechanisms underlying correlated behaviors, but there is evidence in other species that they may have a genetic component (Ebert 1983, Edwards et al. 2006, Caramaschi et al. 2008), and/or may be hormonally regulated (Adkins-Regan 2005, Nelson 2006, Caramaschi et al. 2008, Ketterson et al. 2009), especially in males during the breeding season (Wingfield et al. 1987; Ketterson et al. 1991, but see Moore 1987). Less is known about the role of such hormones in female birds (Ketterson et al. 2005, but see Goymann et al. 2008). However, female juncos with experimentally elevated testosterone (T) show increased aggression towards same-sex conspecifics (Zysling et al. 2006), and recent findings show that individual variation in the ability to produce testosterone is positively related to aggression towards a female intruder (own data). In contrast, experimental elevation of T had no effect on female anti-predator aggression during the incubation period (Clotfelter et al. 2004), but it did when females were tending nestlings (O'Neal et al. 2008).

In conclusion, we found that a female's aggressive response in one context was strongly correlated with aggressive response in other contexts and that the level of response indicates that predators are perceived as the greater threat to individual fitness, followed by female intruders, and then male intruders. These findings suggest that aggression in different contexts is not independent and may be regulated by common mechanisms. If true, different forms of aggression may reflect the same behavioral tendency (Bell 2007a) and possibly be constrained from evolving independently, at least in the short term (Ketterson et al. 2009). Taken together these findings suggest that female aggression is an important component of the female behavioral phenotype. Further work is needed to determine the mechanistic basis, and costs and benefits of, variation in aggression in order to determine how and when selection acts on aggression in females.

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## Figure and Table Captions

Table 1: Loadings of individual behaviors on principal components with Varimax rotation. Note that aggressive behaviors (time in 0.25m, number of attacks) load heavily and positively on the first component in all trials.

Figure 1: Scatter plots illustrating the correlations between behavioral responses to a same-sex intruder and a simulated predator (upper panel), or a same-sex and an opposite-sex intruder (lower panel). Principal components were derived from variation among 3 behavioral variables exhibited upon presentation of a predator, a same-sex and an opposite-sex intruder. Points represent one individual's score in two contexts. Lines are regressions to illustrate relationships. Lines for Group 2 indicate relationships according to the order the sexes were presented, the dotted line: females receiving a female intruder first, dashed line: females that received male intruder first.

Figure 2: Groups 1 and 2 plotted according to time spent within 0.25 m (mean, +/- s.e.) of intruder and trial. Numbers in/over bars are samples sizes. For Group 1, response to predator was greater than to a same-sex intruder. For Group 2, there was no sex or order effect. However, note the pronounced effect of order of presentation and sex on the strength of response. If a same-sex intruder was presented first the response to same-sex and an opposite-sex intruder was strong; if an opposite-sex intruder was presented first the response to both intruders was minimal.

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