Eavesdropping and the audience effect in fighting fruit flies reveal feedback between social information use and production

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Keywords: social information, Drosophila melanogaster, aggression, audience effect, eavesdropping

Abstract: Social information use and production occur simultaneously during social learning events; however, these two processes are often studied independently. We propose that social information use and production represent a feedback loop: eavesdroppers influence the behaviors of those being observed, which then alters the eavesdroppers' social decisions. Here, we used Drosophila melanogaster to investigate the relationship between social information use and production in an aggressive context. We quantified aggressive behavior between two males being observed by either a male, female, or no one. We then measured variation in social information use by allowing the eavesdroppers to interact directly with the observed males and compared their behavior to individuals who did not observe the aggressive encounter. Male eavesdroppers caused the observed males to decrease their aggressive behaviors, thus altering their social information production. Male eavesdroppers were then less likely to attack observed contest winners than males without social information. Female eavesdroppers did not elicit any change in the aggression of the two males, and female eavesdroppers did not use social information to inform their mating decisions. Overall, we found support for the feedback loop in males, but not females: information was exchanged bi-directionally during eavesdropping events, resulting in the modification of behavior by all parties.

Keywords: aggression, audience effect, Drosophila melanogaster, eavesdropping, social information

Introduction

Individuals are able to acquire information by observing the behavioral interactions of others (McGregor, 2005; McGregor & Peake, 2000; Wiley, 1983). We refer to these third-party conspecifics as 'social eavesdroppers': observers who acquire social information from other individuals' signaling behavior, cues, and/or direct interactions (Bonnie & Earley, 2007; Dall et al., 2005; É. Danchin et al., 2004; McGregor, 2005). For example, female black-capped chickadees, *Poecile atricapilla*, eavesdrop on male song contests and then use the information they acquired to adjust their reproductive decisions (Mennill et al., 2002). Social eavesdropping could provide a low-risk alternative to direct interactions with others, as eavesdroppers can learn about the quality or ability of another individual without engaging in interactions that could be risky or costly (McGregor & Peake, 2000; Mery et al., 2009a). Females of many species have been shown to eavesdrop on the mate choice decisions of other females and then copy those preferences, an example of eavesdropping as a cost-avoidance strategy (Vakirtzis, 2011). Furthermore, acquiring and applying information learned from watching other individuals is key to the formation and maintenance of behavioral traditions (Whiten, 2021).

In many cases, an interacting pair can detect the presence of a social eavesdropper, which may lead to a shift in behavior known as the 'audience effect' (Fichtel & Manser, 2010; McGregor, 2005). Individuals that adjust their behavior to maximize their benefit in response to observers would be evolutionarily favored (Zuberbühler, 2008). The audience effect is widespread and well-documented, with studies demonstrating the effect in fish (Doutrelant et al., 2001; Plath et al., 2008), birds (White et al., 2002), primates (Townsend & Zuberbuhler, 2009), and humans (Bond & Titus, 1983). For instance, substantial work has demonstrated that the intensity and/or frequency of alarm calls depends on the audience's

characteristics in primates (Chapman et al., 1990; Pollick et al., 2005; Wheeler, 2008). Additionally, ravens have also been found to modulate their defensive call rates based on the identity of their audience (Szipl et al., 2018).

While social eavesdropping and the audience effect are two well-described phenomena, they have historically been studied as two distinct processes (McGregor, 2005). Yet, social eavesdropping and the audience effect occur in tandem during social learning events (Bonnie & Earley, 2007). During these events, the expression of social behaviors – and thus, the "production" of social information – may be altered depending on the identity of the eavesdropping individual (Desjardins et al., 2012; Fitzsimmons & Bertram, 2013; Plath et al., 2008). At the same time, how that eavesdropper makes decisions - i.e., the "use" of social information - can change depending on the information that they observe from the interacting pair (Peake et al., 2001). Taken together, we can hypothesize the possibility of a feedback loop between social information production and use, in which the presence of a social eavesdropper alters the social behavior of the interacting pair, which in turn influences the behavior of the interactants and the eavesdropper.

The existence of a feedback loop between the production and use of social information has the potential to alter our current understanding of how social behaviors are expressed and have evolved, especially in group contexts. If there is no feedback between these processes, then research dedicated to how individuals use and produce social information can continue to be informative when each one is studied independently. However, if a feedback loop does exist, then studying only one-half of the feedback loop at a time - i.e., studying interacting pairs without eavesdroppers or vice versa - is ignoring critical participants during these interactions, which has serious implications for how we understand social learning, animal communication, and social behaviors.

For example, many studies of social eavesdropping are conducted by allowing an individual to observe an interaction between others, while the eavesdropper is obscured from view (and other sensory modalities; Magnhagen, 2006; Oliveria et al., 1998, Peake 2006). To create an environment in which the eavesdropper can observe, but not be seen, by the interacting pair, researchers often employ one-way mirrors, special lighting, or other visual effects. Making the eavesdropper undetectable to the interacting pair is done purposefully in order to avoid inciting changes in social information production elicited by the presence of the eavesdropper, i.e., eliminating any possible audience effects. However, in real social networks, the interacting individuals often can detect and respond to the presence of the eavesdropper, and *vice versa* (Zuberbuhler 2008). Thus, forming predictions about the costs, benefits, and/or evolution of eavesdropping based on studies where the audience effect or eavesdropping is purposefully excluded may be misleading. Despite these important implications, we know of no other studies that have investigated the feedback between social information use and production.

Here, we utilized the social information feedback framework proposed above to investigate social decision-making specifically in the context of aggressive interactions. In animal societies, aggression is an important social signaling behavior that is used to secure and defend resources such as mates or territory (Kin, 1973; Trainor & Marler, 2019). Aggression often takes place in an environment where social eavesdroppers are present and thus may be able to observe contests between others and extract information about the interactants' fighting ability (Oliveira et al., 1998; Peake et al., 2001). Previous work has shown that social eavesdroppers can obtain information from the aggressive behaviors performed, such as threat displays or overt physical interactions, or the contest outcome and dynamics, such as the identity of the winner and loser (Johnstone, 2001; Magnhagen, 2006; Oliveira et al., 1998; Tibbetts et al., 2020; Van Breukelen & Draud, 2005).

Decades of research, primarily in fish, have been dedicated to investigating how individuals change their aggressive behavior as the result of the presence of an audience, or how the outcome of a contest affects the future behavior of eavesdropping individuals (see Peake & Mcgregor, 2004 for review). Despite this, social eavesdroppers in these experiments rarely have the opportunity to make decisions about who to interact with (but see studies on mate choice in an aggressive context, Van Breukelen & Draud, 2005). Rather, experimental designs frequently designate the eavesdropping individuals to be randomly paired with one of the contest participants (Earley & Alan Dugatkin, 2002; Johnsson & Åkerman, 1998; Oliveira et al., 1998; Tibbetts et al., 2020). These designs prevent us from gaining insight into how individuals can process and utilize social information to make decisions about how they will behave toward other group members. Recent calls for the need to explain and understand social decision-making, especially in conflict settings, can be solved by utilizing an experimental design that gives the eavesdropper the choice of interacting with both contest participants (Hobson, 2020).

To investigate the proposed feedback loop and social decision-making, we combined standard methods used for testing how individuals produce and how individuals use social information in a single experiment. In the first half of each trial, we allowed individuals to eavesdrop on two males engaged in an aggressive contest; and in the second half of the trial, the eavesdroppers could then interact with both aggressors directly. We standardized the genotypes and prior experience of the aggressor males to ensure that we could directly link eavesdropper sex and presence to the interacting pair's social information production. We used 3 different genotypes of social eavesdroppers, which allowed us to achieve sufficient replication while also testing for the robustness of the effect of social information use across different genotypes.

Thus, the first phase of each trial –the observation phase-- allowed us to test for variation in how the interacting pair of males produced social information, and whether this variation depended on the sex

or presence of the observer. The second phase – the interaction phase-- allowed us to test how the produced social information influenced the eavesdropper's behavior. For male social eavesdroppers, we recorded their aggressive interactions with the interacting pair. For female social eavesdroppers, we recorded which of the paired males she mated with. By allowing the social eavesdropper to interact directly with both males involved in the aggressive contest, we were also able to measure social decision-making. To accomplish this, we quantified the social interactions that the eavesdropper engaged in with the winner and loser of the contest. Measuring both social information use and production in our experimental trials allows us to directly examine how an individual's ability to influence the production of social information results in differences in social information use.

We predicted that the presence and sex of an eavesdropper will drive changes in the behavior of the interacting aggressive pair. We further hypothesize that the social information observed by the social eavesdroppers would then alter their behaviors (i.e., aggression in males or mating in females), specifically their social decision-making. Observing both of these results in our data will support the existence of a feedback loop between these processes.

Methods

Study system

We used *Drosophila melanogaster* to investigate how social eavesdroppers differ in their access to and use of social information. *D.melanogaster*, the fruit fly, is an excellent study system for investigating the relationship between social information use and production because of their well-characterized suite of behavioral interactions. *D.melanogaster* forms non-random social groups on their food sources both in nature and in the lab, and social interactions, such as aggression, take place in an environment with ample conspecific social eavesdroppers (Saltz, 2013; Stamps et al., 2005; Wertheim et al., 2006). Fruit

flies have a well-studied repertoire of aggressive behaviors (Chen et al., 2002; Saltz, 2013). Fruit flies also have the necessary cognitive capacity for social information use and cultural transmission (E. Danchin et al., 2018; Mery et al., 2009b; Nöbel et al., 2018).

Genotypes and Rearing

To conduct the experiment, we used inbred fly lines that were originally derived from a wild population in Raleigh, NC (MacKay et al., 2012). The genotypes used as eavesdroppers were 136, 229, and 429. For the standardized interacting pair, we used one male from genotype 365 and one male from genotype 765. All genotype numbers are arbitrarily used to identify the fly lines and have no further significance.

Experimental flies for this study were reared in vials under standard conditions that minimized variation in larval density: 10 females and 10 males were placed into a vial containing standard fly food medium. Virgin male and female progeny from these vials were then collected the morning that they eclosed, and subsequently housed in individual vials to control for any prior social experiences. Flies aged up for 3 or 4 days before experimental trials and were maintained on a 12:12 hour light:dark cycle.

Behavioral Arena

We utilized a novel arena design to test for variation in social information use and production (Figure 1). The main part of the arena was composed of two Petri dishes (3 cm x 1 cm) taped together, with a small food patch (1.5 cm x 1 cm) placed on the bottom of the arena, which contained standard fly food medium and a dot of active yeast. There are two small holes in the arena: one on the side of the arena, for the insertion of flies via aspiration, and another hole in the center of the top of the arena. A pipette tip with a mesh base was placed over this hole, creating an observation tower for the eavesdropper fly to watch aggressive contests. This arena design mimics how flies in nature can perch above a social

group and observe the interactions of others before deciding to join the group (Stamps et al., 2005). The thin mesh barrier that separated the eavesdropper fly from the paired males allowed for the collection of visual as well as olfactory signals from the aggressive contest (Wang & Anderson, 2010). We observed occasional interactions at this mesh between the eavesdropper fly and the interacting pair, however, we did not observe any aggressive or courtship behaviors between the eavesdroppers and the interacting pair.

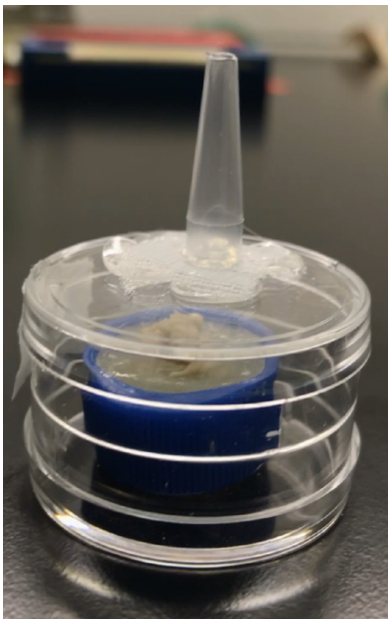

Figure 1. The behavioral arena used to conduct experimental trials. The pipette tip on top housed an eavesdropper fly; the opening was then covered to prevent them from escaping. The paired males were inserted into the petri dish arena with the blue cap that was filled with standard food and topped with yeast paste.

Overview of trials

Each trial consisted of two phases: the observation phase and the interaction phase. In the observation phase, an eavesdropper fly was placed into the observation tower while the paired males in the main part of the arena engaged in an aggressive contest. The first phase of the experimental trials allowed us to test for variation in how individuals produced social information, and whether this depended on the sex and/or presence of the observer. In the interaction phase, the eavesdropper fly was added to the arena with the paired males, and their social interactions with the males were recorded. The second phase of the experimental trials allowed us to determine whether access to social information influenced the eavesdropper's later social interactions.

Experimental Protocol

We randomly dusted the paired males with colored powder (orange or yellow) to create two distinct male phenotypes. This allowed human observers to easily differentiate between the two flies for scoring aggressive behavior, and this distinctive coloring also may have aided the eavesdropper flies in distinguishing between the two males (E. Danchin et al., 2018; Mery et al., 2009b; Monier et al., 2018). The males were then inserted into the main petri dish arena, and the eavesdropper fly was placed into the observation tower on top of the arena. Flies were allowed to acclimate for five minutes before experimental trials began. We used two morning time blocks for trials, with one set of trials beginning 15 minutes after subjective dawn, and the second time block beginning 1 hour after subjective dawn. This time period reflects peak activity time for fruit flies (Allada & Chung, 2010).

We measured aggressive behaviors performed by the paired males for 30 minutes. We recorded five measures of aggressive behavior: lunging, fencing, wing threat, chasing, and boxing (Chen 2002). Every two minutes, an observer recorded all aggressive behaviors performed by the two male flies in the arena for 20 seconds. During this period, the eavesdropper fly was also able to observe the aggressive contest without interacting directly with the two males. Immediately after the 30-minute aggressive contest, we gently inserted the eavesdropper fly into the arena with the two demonstrator males that they just observed. We utilized AKASO cameras (EK700) to video record the second half of the experiment, and then used BORIS (Friard and Gamba 2016), an event logging software, to code the social interactions that occurred between the eavesdropper and the paired males for another 30 minutes.

We conducted three different experimental treatments, which are explained further below.

Female Eavesdropper

In this treatment, we used a female fly as the eavesdropper. For the first phase of the experiment, a female fly observed an aggressive encounter between the paired males (n=41). For the second phase of

the experiment, we recorded whether the female mated and, if so, which male the female chose to mate with (n=38).

Male Eavesdropper

In this treatment, we used a male fly as the eavesdropper. For the first phase of the experiment, a male fly observed an aggressive encounter between the paired males (n=43). For the second phase of the experiment, we recorded all lunging behavior that the male eavesdropper displayed while interacting with the two males (n=32). We measured only lunging behavior for the second phase of the experiment because we found it to be highly correlated with other aggressive measures (Figure S1).

No Eavesdropper/No Social Information

In this treatment, there was no eavesdropper present for the first phase of the experiment, providing us with a baseline measure of how the paired males would behave without an audience present (n=82). In the second phase of the experiment, a male (n=32) or female fly (n=38) who did not observe the aggressive encounter was inserted into the arena. The same behaviors were recorded as described in the male and female eavesdropper treatments, depending on the sex of the fly that was added.

Female Eavesdropper - Single Choice

After completing our female eavesdropper trials, we observed that there was a strong bias in female mating preferences: in the 59 mating events that were recorded, females mated with the genotype 365 male in 51 of those trials. This bias for males from genotype 365 obscured any effects of social information on mate choice preferences. As a result, we did a follow-up experiment, where we conducted the female eavesdropper (n=47) and no eavesdropper trials (n=38) using the same protocol as described above, except we used two males from genotype 365 as the paired males engaged in an

aggressive encounter. Using this approach, the only difference between the males was their behavior during the observation phase, clarifying the effects of social information on female mate choice.

Analysis

The goal of our analysis was to investigate how individuals used and influenced the production of social information. To accomplish this, we created generalized linear mixed models using the glmmTMB package in R (v4.1.0, R Core Team, 2021, Bolker et al., 2009). Generalized linear mixed models allow us to fit a variety of distributions to our data, include random effects in our models, and account for data that is zero-inflated (Brooks et al., 2017). We created models that tested how the paired males produced social information, how males used social information, and how females used social information. To investigate how social information specifically altered social decision-making, we assigned a winner and loser to each of the aggressive contests in the first phase of the experimental trials, which was defined as the male who performed the most and least aggressive behaviors, respectively. If the paired males displayed the same number of aggressive behaviors, we recorded it as a tie and neither male was designated as a winner or loser. We then created models that looked at male and female responses to contest winners and losers. For all models, we checked model fit by utilizing the DHARMa diagnostic package in R, which conducts simulation-based dispersion tests (Hartig, 2022). In order to investigate our hypotheses around how access to social information can influence both social information production and use, we tested multiple interaction terms between our fixed effects in our models, explained in detail further below. For the final model selection, we only retained interaction terms that improved the fit of the model according to AIC weights.

Social Information Production Models

Our first model tested how the presence and sex of the eavesdropper influenced the aggressive behavior, i.e., the social information production, of the paired males (SI Production Model, Table 1). The response variable for this model was the total aggression displayed by the paired males in the first phase of the experiment. We calculated the total aggression by summing the five measures of aggressive behavior that each fly performed. We had two fixed effects in this model: our first was treatment, which described the presence or absence of an eavesdropper as well as the sex of the eavesdropper. An effect of treatment in this model would indicate that the presence and/or sex of the eavesdropper altered the social information that the eavesdropper observed. Our second fixed effect was age, as we used both 3- and 4-day post-eclosion flies for all experiments. Date was included as a random effect to indicate which flies were tested on the same day. A negative binomial error distribution with a logit link function was used for this model, and a term was included to account for zero inflation, as some pairs had no recorded aggressive interactions during the experimental trials. We also conducted a model to investigate how the eavesdropper's genotype influenced social information production, this model and its results are reported in the Supplemental Information (Table S1).

Male Social Information Use Models

Our next set of models tested how access to social information influenced the aggressive behavior and social decision-making of the males that were added into the arena. We modeled the number of lunges that the added male directed towards the contest winner (SI Use Male Response to Winner Model, Table 2) or the loser (SI Use Male Response to Loser Model, Table 3) during the interaction phase of the experiment.

Fixed effects in the SI Use Male Response to Winner model included treatment, which described if the male was an eavesdropper or was not allowed to observe social information. We also included a fixed effect for the genotype of the added male to account for genotypic variation in male lunging behavior.

Our model selection process led us to retain the interaction we tested between genotype and treatment, as this interaction term improved the fit of the model (delta AIC = 1.59). An effect of this term would indicate that genotypes differed in their use of social information. The total aggression displayed by the paired males in the first phase of the experiment was also used as a fixed effect, to determine if our sample of their aggressive behaviors predicted the behavior of the added male. Our final fixed effect was the age of the flies, and date was included as a random effect. We tested an interaction between the aggression of the paired males and treatment, however, that interaction did not improve the AIC of our model (delta AIC = 1.86). A negative binomial error distribution with a logit link function was used for this model, and a term was included to account for zero inflation, as some added males had no recorded aggressive interactions during the experimental trials.

For the SI Use Male Response to Loser model, we used the same fixed effects of treatment, genotype, displayed aggression by the paired males, and age, and a random effect of date. We also tested for the same interactions as the winner model, however, our model selection process did not support the retention of either the interaction between displayed aggression and treatment (delta AIC = 1.87) or the interaction between genotype and treatment (delta AIC = 3.48).

Female Social Information Use Models

Our last models tested how access to social information influenced the mating choices of the female eavesdroppers when they were added into the arena in the second phase of the experiment. We only analyzed data from our female follow-up experiment (see above), where females observed and were allowed to interact with two males of their preferred male genotype, 365.

To investigate how females used social information, we first modeled whether females mated, or not (SI Use Female Model, Table 4), with a binary response variable. Females that mated were assigned a value of 1 (n =53 females), and females who did not mate were assigned a 0 (n = 32 females). Treatment was

tested as a fixed effect, to investigate how access to social information modified the likelihood of female mating. The total aggression displayed by the paired males in the first phase of the experiment was also used as a fixed effect, to determine if socially produced information influenced the mating behavior of the added female. We included the interaction between treatment and total paired male aggression to investigate how observed social information influenced mating decisions, as it improved model fit according to AIC (delta AIC = 0.51). We also tested the genotype of the added female as a fixed effect, to account for genetic variation in mating preferences. We did not retain the interaction between genotype and treatment, as it did not improve the model's AIC (delta AIC = 1.46) We utilized a binomial error distribution for all models.

To further investigate how social information specifically altered female social decision-making, we modeled whether each mated female chose the winner (SI Use Female Response to Winner Model) or the loser (SI Use Female Response to Loser Model) of the aggressive contest. We had smaller sample sizes for these models: of the 85 females tested, only 21 mated with the winner, and only 9 mated with the loser. These lower samples were a result of many trials resulting in a "tie" between the paired males, and females often choosing not to mate with either male (n=55). The full description of these models and their results can be found in supplemental tables 2 and 3.

Correction for Multiple Testing

We created multiple models to investigate our hypotheses regarding social information production, use, and social decision-making, which required a correction for multiple testing. We applied Bonferroni corrections for multiple testing on all the P values for our models that are reported below and in tables 1-4.

Results

Social Information Production

The paired males altered their behavior based on the presence and sex of the eavesdropper (Figure 2, SI Production Model: X2 = 10.1, df = 2, p-value = 0.012, Table 1). The paired males were the least aggressive when a male eavesdropper was present (SI Production Model: least-squares means for signaling male aggression in the male eavesdropper treatment = 1.84). When a female eavesdropper was present, the paired males did not significantly alter their behavior, compared to when there was no eavesdropper present (SI Production Model: least-squares means for signaling male aggression in the female eavesdropper treatment = 2.27, no eavesdropper treatment = 2.45). We did not see an effect of eavesdropper genotype on paired male aggression (Supplemental Table 1).

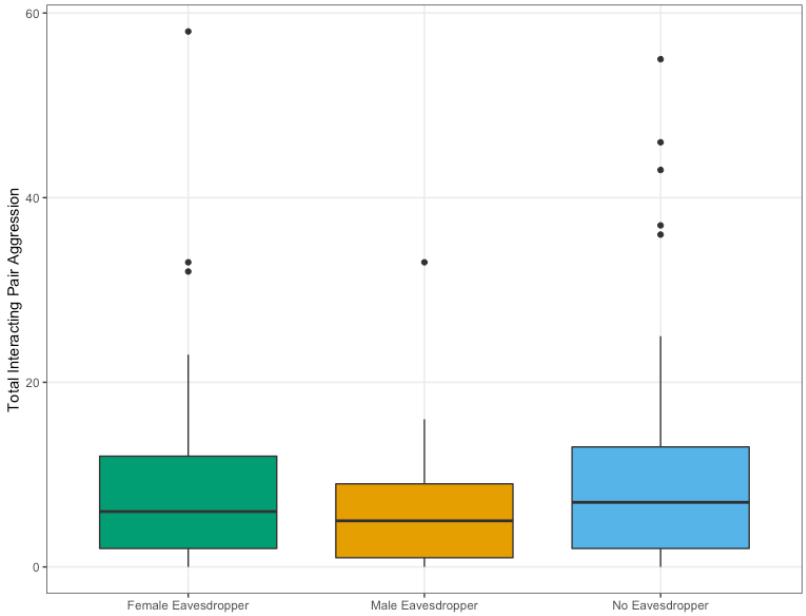

Figure 2. Variation in social information production based on audience identity. The total aggression of the paired males in relation to the sex and presence of an eavesdropper observing them. We calculated total aggression by summarizing five measures of aggressive behavior we recorded for each of the paired males. Analysis via linear mixed models revealed a significant effect of sex and presence of the eavesdropper on paired male aggressive behavior (see text).

Male Social Information Use

Male flies altered their behavior based on their access to social information. Males with social information were less aggressive towards the contest winner, compared to males without social information (SI Use Male Response to Winner Model: X2 = 8.4, df = 1, p-value = 0.008, Table 2). We observed genetic variation in how males responded towards the contest loser (SI Use Male Response to Loser Model: X2 = 8.38, df = 2, p-value = 0.04, Table 3), but not the contest winner (SI Use Male Response to Winner Model: X2 = 4.5, df = 2, p-value = 0.22, Table 2). We did not see a significant effect of the interaction term between genotype and treatment on male aggressive behavior towards the contest winner (SI Use Male Response to Winner Model: X2 = 6.3, df = 2, p-value = 0.08, Table 2). The total aggression of the paired males in the first phase of the experiment did not significantly predict how aggressive the male flies would behave towards the contest winner or loser (SI Use Male Response to Winner Model: X2 = 0, df =

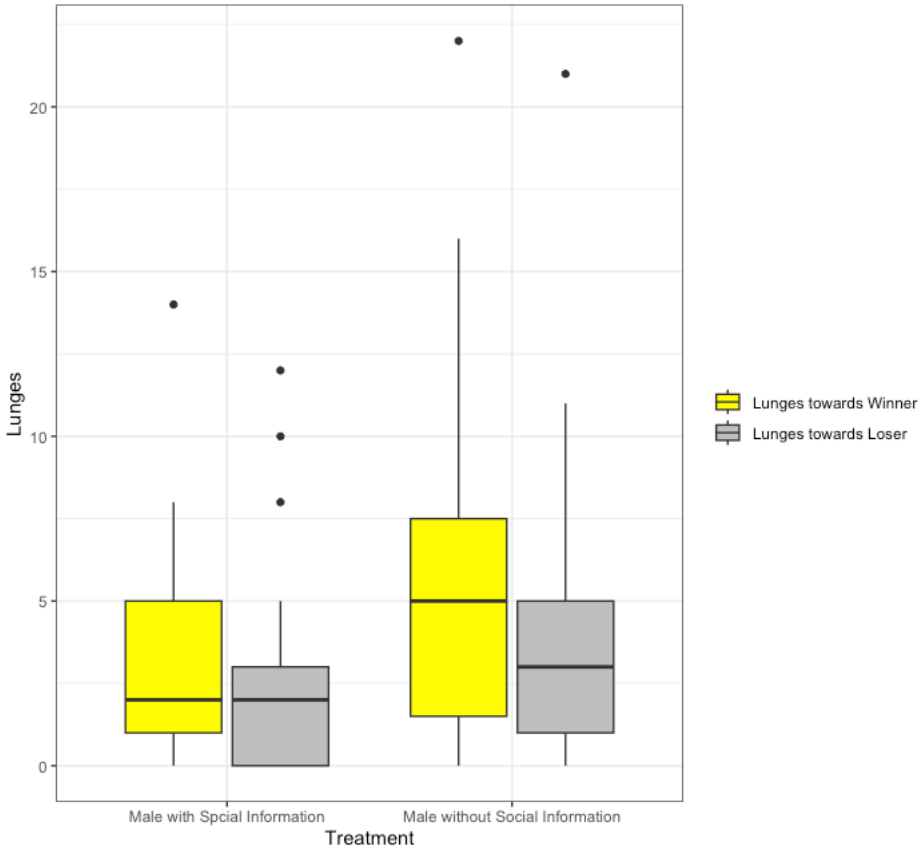

Figure 3. Social decision making in male social information use. The number of times that the male lunged at the contest winner or loser in the second half of experimental trials is represented on the y-axis, and the x-axis shows the two treatments: male eavesdroppers and males who did not have access to social information. Males with social information lunged significantly less often at observed contest winners than males without social information.

1, p-value = 2; SI Use Male Response to Loser Model: X2 = 2.32, df = 1, p-value = 0.26, Table 2 & 3). Age also did not significantly predict male aggressive behavior towards the contest winner or loser (SI Use Male Response to Winner Model: X2 = 1, df = 1, p-value = 0.64, SI Use Male Response to Loser Model: X2 = 2.74, df = 1, p = 0.2, Table 2 & 3).

Female Social Information Use

Female flies displayed strong genotypic differences in likelihood to mate (SI Use Female Model 1: X2 = 14.01, df = 2, p-value = 0.003, Table 4). Specifically, females from genotype 229 almost always mated (females mated in 27/29 trials), and females from genotype 136 rarely mated (females mated in 8/27 trials). Neither access to social information nor the displayed aggression of the interacting pair significantly predicted the likelihood of mating (SI Use Female Model: treatment X2 = 3.4, df = 1, p-value = 0.2; displayed aggression X2 = 4, df = 1, p-value = 0.14, Table 4). The interaction between total aggression and treatment was also not significant in predicting female mating likelihood (SI Use Female Model: X2 = 2.4, df = 1, p-value = 0.38).

We did not find support for any of our fixed or random effects significantly predicting the identity of who the female mated with (the winner or the loser), these full results are presented in supplementary tables 2 and 3.

Discussion

In this study, we experimentally demonstrate the existence of a feedback loop between social information production and use. To our knowledge, this work represents the first time that this feedback has been demonstrated experimentally. Traditionally, social information use and production have been studied separately, which limits our understanding of how these two processes interact as

they happen simultaneously in nature (McGregor 2005, Bonnie and Earley 2007). By combining these two processes into a single experiment, we found that male eavesdroppers substantially decreased the aggression of our standardized interacting pair, compared to pairs of males from the same genotypes, but who were not observed by an eavesdropper (Figure 2, Table 1). Furthermore, the ability to collect social information caused eavesdropping males to change their aggressive behaviors: male eavesdroppers lunged at the contest winner less than males who did not have the opportunity to observe social information (Table 2). These results were consistent across different genotypes of male eavesdroppers.

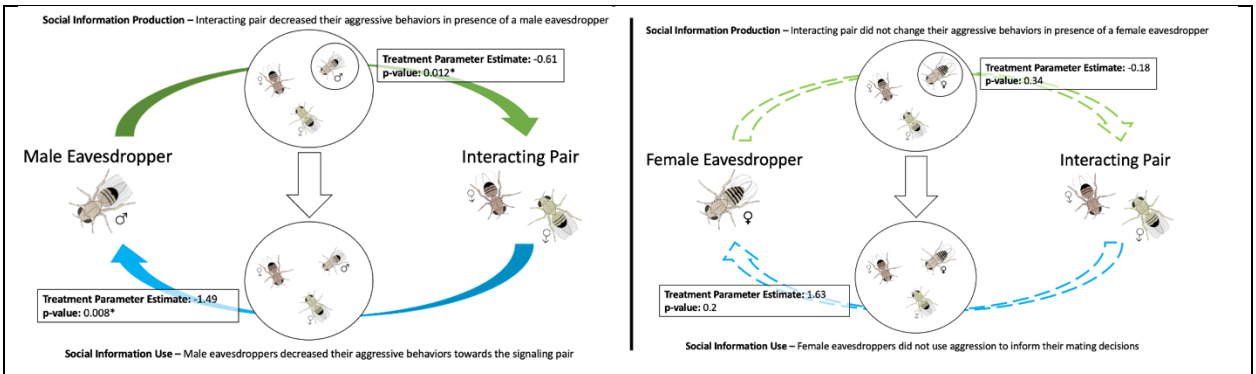

Figure 4. Diagrams depicting how our study's findings support the existence of a sex-specific feedback loop between social information use and production. Males simultaneously influence the production of and use social information resulting from an aggressive contest. Females do not influence social information production and do not use social information resulting from an aggressive contest.

We cannot be certain about the exact social information that the male eavesdroppers were using to inform their decision-making. We measured the aggressive behavior of the interacting males, and while we did not see a direct effect of this measure on added male fly behavior (tables 2 and 3), documented winner-loser effects in flies provide evidence that flies can learn from aggressive contests (Penn et al., 2010; Yurkovic et al., 2006). Eavesdroppers could have extracted information from a variety of sources

in the arena, such as observations of how the paired males interacted with their environment or chemical signaling behaviors (Kent et al., 2008). Another mechanism that could cause male eavesdroppers to change their behavior in response to the paired males is priming. Priming occurs when observing an aggressive encounter alters an individual's motivation to fight, making it more likely that they will also win their subsequent aggressive encounter (Earley et al., 2005; Hsu et al., 2006; Tibbetts et al., 2020). We did not see increased aggression in our male eavesdroppers: instead, males who observed an aggressive encounter were *less* aggressive than males who did not (Figure 3). Additionally, under priming ,the effect of observing a contest on eavesdropper behavior is expected to generalize across all opponents (Clotfelter & Paolino, 2003). Social eavesdropping, on the other hand, is expected to be individual-specific – the eavesdropper remembers information and learns from the behaviors of who they observed. Therefore, while priming may have occurred in our trials, our results are more consistent with social eavesdropping, as we saw an effect of social information (i.e., treatment) on male behavior towards winners, but not losers (Table 2). Further research will be necessary to identify causal relationships between eavesdropping, paired male behavior, and the eavesdropper's later aggression.

Despite this outstanding gap in knowledge, we can still be confident that our results show that social information use and production are linked processes: we documented that paired males only altered their social information production in specific social contexts, and eavesdropper males only altered their behavior after observing the paired males. Because we could tightly control the genotype and prior experience of each fly in our experiment, we know that each interacting pair was composed of males of identical genotypes, who were reared in identical conditions and had no prior experience. Similarly, the flies that were used as eavesdroppers and those without social information were likewise reared and controlled. Being able to manipulate and control the flies until experimental trials means that the only difference between our male eavesdroppers and males without social information treatments was the

ability to collect social information. Overall, these findings indicate that there is a bidirectional transfer of information between the participants and the eavesdroppers in an aggressive context.

Social eavesdropping can benefit individuals by allowing them to assess their opponents' potential before engaging in aggressive behavior, thus reducing the costs of fighting (Earley 2010, Johnstone 2001). Our results are consistent with previous work that shows obtaining social information about the fighting ability of an opponent may allow individuals to avoid prolonged or escalated encounters (Magnhagen, 2006; Valone 2005); notably, these results were obtained while preventing any influence of the eavesdropper presence on the social information that was produced. Our qualitatively similar findings suggest that, while studying this feedback better informs our predictions, they do not qualitatively alter the patterns that others have previously observed, at least in this case. Importantly in our study, we see that observed males reduced their aggressive behavior, and as a result, male eavesdroppers then reduced their aggression toward the contest winner based on what they observed. In this context, the feedback produced substantially lower overall aggression than would be predicted by simply studying pairs of males without eavesdroppers (e.g., in our no eavesdropper treatment), or investigating male eavesdropping without also considering its effect on social information production (e.g., in our no social information treatment). Thus, further investigations into this feedback will continue to better inform our predictions on how social information is used and produced within social groups.

Our experiments with female eavesdroppers also support the feedback between social information use and production, although it does so in a pattern that is opposite from what we observed with the male flies. The feedback loop between social information use and production proposes that changes in eavesdropper behavior are predicated on changes in the behavior that they observe from the social information producers. In other words, the presence of a social eavesdropper should cause an

adjustment in social information production, which then would lead to changes in eavesdropper behavior. However, in our study, we did not observe a change in the behavior of the interacting males in the presence of a female social eavesdropper (Figure 2). Rather, the interacting pair displayed similar aggression levels whether a female was observing them or if there was no eavesdropper present. Accordingly, we did not observe any differences in the mating decisions between female eavesdroppers and females without social information (Table 4). Together, these findings lend additional support for the feedback loop: changes in eavesdropper behavior are dependent upon modifications in the social information that is produced in their presence.

We found evidence for sex differences in our proposed feedback loop between social information use and production. Paired males only significantly changed their aggressive behaviors only when another male was watching; they did not make any adjustments when a female observed instead. Correspondingly, we did not find any evidence that females used aggressive behaviors as social information, as they did not alter their mating behaviors or mate choice after watching an aggressive encounter. This is in contrast to our male results, who we do have evidence for using social information as they changed their behaviors after observing a contest. Thus, our evidence suggests that in our system, any influence of male-male aggression on female mate choice may be indirect, context-dependent, or absent (reviewed in Wong & Candolin 2005). Rather, males strategically suppress their aggression when in the presence of other males, likely in order to avoid elevated aggressive encounters.

Our experiments are well suited for studying the feedback between social information production and use; however, we note some important limitations. We tested only three, inbred genotypes for how they either used or influenced the production of social information, which is not representative of genetic variation in the wild. Experiments including more genotypes with more genetic diversity will be needed to assess how genetic variation interacts with the feedback, if at all (Araya-Ajoy et al., 2020;

Cantor et al., 2021). Additionally, all of our flies were naïve, with no prior social experience. While this provided a standardized methodology to test all of our flies, different results may be obtained for individuals that have had prior social interactions with aggression and mating behaviors (Taborsky & Oliveira, 2012). Our female data also presented several challenges: any effect of social information was obscured in our initial set of experiments due to a strong preference for one of the tested male genotypes. In our follow-up trials, we often were not able to assign a winner and loser during the experimental trials due to a high number of ties, and surprisingly, our females were not always willing to mate, although they were unmated for their entire adult lives. While we were still able to obtain enough data via our follow-up trials to investigate the feedback loop, continued work is necessary to better understand how females use social information from male-male aggressive contests.

This study has revealed several areas of further research necessary to continue to better elucidate the relationship between social information use and production. The sex differences that we observed emphasize the need for continued testing and modeling of these processes in diverse populations, as accounting for individual differences is vital for accurate predictions of social information spread in populations (Duboscq et al., 2016). Additionally, we need a better understanding of the selection pressures in social networks that are introduced via this feedback loop. In this system for example, in order for increased aggression to be adaptive, the benefits must outweigh the costs, but these will likely change depending on the social environment that the individual is experiencing (Duckworth, 2006). Further research can tackle this by quantifying the costs and benefits of plasticity in social information production and use.

Overall, our study lends the first empirical support for a feedback loop between how social information is produced and used in different social contexts. Utilizing the social information feedback framework to

investigate animal behaviors can contribute to a better understanding of how behaviors related to conflict, sociality, and group living are expressed and evolve.

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Table 1. Parameters, test statistics, and their interpretation in the model for social information production. P-values are corrected for multiple testing.

Parameter	**p-value**	**Chi**	**Interpretation**
Treatment	0.012	10.1	Paired males altered their aggressive behavior based on the presence and sex of an eavesdropper
Age	0.07	4.48	No effect of age on paired male aggression
Date	0.25	2.32	No effect of experimental day on paired male aggression

Table 2. Parameters, test statistics, and their interpretation in the model for male social information use towards contest winners. P-values are corrected for multiple testing.

Parameter	**p-value**	**Chi**	**Interpretation**
Treatment	0.008	8.4	Access to social information altered how aggressive males were towards previous contest winners
Genotype	0.22	4.5	No detected genotypic variation in how males directed their aggression toward the winner
Treatment x Genotype	0.08	6.3	No significant genotype variation in how males used social information to direct their aggressive behaviors toward winners
Age	0.64	1	No effect of age on male aggression
Paired Male Aggression	2	0	Measured aggression in the previous contest did not affect how males directed their behaviors toward winners
Date	2	0	No effect of experimental day on aggression

Table 3. Parameters, test statistics, and their interpretation in the model for male social information use towards contest losers. P-values are corrected for multiple testing.

Parameter	**p-value**	**Chi**	**Interpretation**
Treatment	1.76	0.02	Access to social information did not alter how aggressive males were towards previous contest losers
Genotype	0.04	8.38	Genotypic variation in how males directed their aggression toward the loser
Age	0.2	2.74	No effect of age on male aggression
Paired Male Aggression	0.26	2.32	Measured aggression in the previous contest did not predict how males directed their behaviors toward losers
Date	2	0	No effect of experimental day on aggression

Table 4. Parameters, test statistics, and their interpretation in the model for female likelihood to mate. P-values are corrected for multiple testing.

Parameter	**p-value**	**Chi**	**Interpretation**
Genotype	0.0027	14	Genotypic variation in likelihood to mate
Paired Male Aggression	0.14	4	Measured aggression displayed in the previous contest did not alter mating behaviors
Treatment	0.2	3.4	Access to social information did not significantly predict likelihood to mate
Treatment x Paired Male Aggression	0.38	2.4	Observing the aggression of the paired males did not significantly change their likelihood to mate
Date	0.81	1.2	No effect of experimental day on aggression

Allada, R., & Chung, B. Y. (2010). Circadian organization of behavior and physiology in Drosophila. *Annual Review of Physiology*, *72*, 605–624. https://doi.org/10.1146/ANNUREV-PHYSIOL-021909-135815

Araya-Ajoy, Y. G., Westneat, D. F., & Wright, J. (2020). Pathways to social evolution and their evolutionary feedbacks. *Evolution*, *74*(9), 1894–1907. https://doi.org/10.1111/EVO.14054

Bond, C. F., & Titus, L. J. (1983). Social facilitation: A meta-analysis of 241 studies. *Psychological Bulletin*, *94*(2), 265–292. https://doi.org/10.1037/0033-2909.94.2.265

Bonnie, K. E., & Earley, R. L. (2007). Expanding the scope for social information use. *Animal Behaviour*, *74*(2), 171–181. https://doi.org/10.1016/J.ANBEHAV.2006.12.009

Cantor, M., Maldonado-Chaparro, A. A., Beck, K. B., Brandl, H. B., Carter, G. G., He, P., Hillemann, F., Klarevas-Irby, J. A., Ogino, M., Papageorgiou, D., Prox, L., & Farine, D. R. (2021). The importance of individual-to-society feedbacks in animal ecology and evolution. *Journal of Animal Ecology*, *90*(1), 27–44. https://doi.org/10.1111/1365-2656.13336

Chapman, C. A., Chapman, L. J., & Lefebvre, L. (1990). Spider monkey alarm calls: honest advertisement or warning kin? *Animal Behaviour*, *39*(1), 197–198. https://doi.org/10.1016/S0003-3472(05)80742-0

Chen, S., Lee, A. Y., Bowens, N. M., Huber, R., & Kravitz, E. A. (2002). Fighting fruit flies: A model system for the study of aggression. *PNAS*, *99*(8), 5664–5668. www.pnas.orgcgidoi10.1073pnas.082102599

Clotfelter, E. D., & Paolino, A. D. (2003). Bystanders to contests between conspecifics are primed for increased aggression in male fighting fish. *ANIMAL BEHAVIOUR*, *66*, 343–347. https://doi.org/10.1006/anbe.2003.2227

Dall, S. R. X., Giraldeau, L. A., Olsson, O., McNamara, J. M., & Stephens, D. W. (2005). Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution*, *20*(4), 187–193. https://doi.org/10.1016/J.TREE.2005.01.010

Danchin, É., Giraldeau, L.-A., Valone, T. J., & Wagner, R. H. (2004). Public Information: From Nosy Neighbors to Cultural Evolution. *Science*, *305*(5683), 487–491. https://doi.org/10.1126/SCIENCE.1098254

Danchin, E., Nöbel, S., Pocheville, A., Dagaeff, A. C., Demay, L., Alphand, M., Ranty-Roby, S., Van Renssen, L., Monier, M., Gazagne, E., Allain, M., & Isabel, G. (2018). Cultural flies: Conformist social learning in fruitflies predicts long-lasting mate-choice traditions. *Science*, *362*(6418), 1025–1030. https://doi.org/10.1126/SCIENCE.AAT1590

Desjardins, J. K., Hofmann, H. A., & Fernald, R. D. (2012). Social Context Influences Aggressive and Courtship Behavior in a Cichlid Fish. *PLOS ONE*, *7*(7), e32781. https://doi.org/10.1371/JOURNAL.PONE.0032781

Doutrelant, C., McGregor, P. K., & Oliveira, R. F. (2001). The effect of an audience on intrasexual communication in male Siamese fighting fish, Betta splendens. *Behavioral Ecology*, *12*(3), 283–286. https://doi.org/10.1093/BEHECO/12.3.283

Duboscq, J., Romano, V., MacIntosh, A., & Sueur, C. (2016). Social information transmission in animals: Lessons from studies of diffusion. *Frontiers in Psychology*, *7*(AUG), 1147. https://doi.org/10.3389/FPSYG.2016.01147/BIBTEX

Duckworth, R. A. (2006). Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. *Behavioral Ecology*, *17*(6), 1011–1019. https://doi.org/10.1093/BEHECO/ARL035

Earley, R. L., & Alan Dugatkin, L. (2002). Eavesdropping on visual cues in green swordtail (Xiphophorus helleri) ghts: a case for networking. *Proc R Soc Lond*, 943–952. https://royalsocietypublishing.org/

Earley, R. L., Druen, M., & Dugatkin, L. A. (2005). Watching fights does not alter a bystander's response towards naïve conspecifics in male green swordtail fish, Xiphophorus helleri. *Animal Behaviour*, *69*(5), 1139–1145. https://doi.org/10.1016/J.ANBEHAV.2004.07.015

Fichtel, C., & Manser, M. (2010). Vocal communication in social groups. *Animal Behaviour: Evolution and Mechanisms*, 29–54. https://doi.org/10.1007/978-3-642-02624-9_2/COVER

Fitzsimmons, L. P., & Bertram, S. M. (2013). Playing to an audience: the social environment influences aggression and victory displays. *Biology Letters*, *9*(4). https://doi.org/10.1098/RSBL.2013.0449

Hobson, E. A. (2020). Differences in social information are critical to understanding aggressive behavior in animal dominance hierarchies. *Current Opinion in Psychology*, *33*, 209–215. https://doi.org/10.1016/J.COPSYC.2019.09.010

Hsu, Y., Earley, R. L., & Wolf, L. L. (2006). Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. *Biological Reviews of the Cambridge Philosophical Society*, *81*(1), 33–74. https://doi.org/10.1017/S146479310500686X

Johnsson, J. I., & Åkerman, A. (1998). Watch and learn: preview of the fighting ability of opponents alters contest behaviour in rainbow trout. *Animal Behaviour*, *56*(3), 771–776. https://doi.org/10.1006/ANBE.1998.0824

Johnstone, R. A. (2001). Eavesdropping and animal conflict. *Proceedings of the National Academy of Sciences*, *98*(16), 9177–9180. https://doi.org/10.1073/PNAS.161058798

Kent, C., Azanchi, R., Smith, B., Formosa, A., & Levine, J. D. (2008). Social Context Influences Chemical Communication in D. melanogaster Males. *Current Biology*, *18*(18), 1384–1389. https://doi.org/10.1016/J.CUB.2008.07.088

Kin, J. A. (1973). *THE ECOLOGY OF AGGRESSIVE BEHAVIOR*.

MacKay, T. F. C., Richards, S., Stone, E. A., Barbadilla, A., Ayroles, J. F., Zhu, D., Casillas, S., Han, Y., Magwire, M. M., Cridland, J. M., Richardson, M. F., Anholt, R. R. H., Barrón, M., Bess, C., Blankenburg, K. P., Carbone, M. A., Castellano, D., Chaboub, L., Duncan, L., … Gibbs, R. A. (2012). The Drosophila melanogaster Genetic Reference Panel. *Nature 2012 482:7384*, *482*(7384), 173–178. https://doi.org/10.1038/nature10811

Magnhagen, C. (2006). Information about an opponent can decrease aggression in male common gobies competing for nest sites. *Animal Behaviour*, *71*(4), 879–884. https://doi.org/10.1016/J.ANBEHAV.2005.07.017

McGregor, P. K. (2005). *Animal communication networks*. 657. https://books.google.com/books/about/Animal_Communication_Networks.html?id=B58DSFCaQpwC

McGregor, P. K., & Peake, T. M. (2000). Communication networks: social environments for receiving and signalling behaviour. *Acta Ethologica 2000 2:2*, *2*(2), 71–81. https://doi.org/10.1007/S102110000015

Mennill, D. J., Ratcliffe, L. M., & Boag, P. T. (2002). Female eavesdropping on male song contests in songbirds. *Science*, *296*(5569), 873. https://doi.org/10.1126/SCIENCE.296.5569.873/ASSET/9738A624-9342-466E-B23E-A8E978823815/ASSETS/SCIENCE.296.5569.873.FP.PNG

Mery, F., Varela, S. A. M., Danchin, É., Blanchet, S., Parejo, D., Coolen, I., & Wagner, R. H. (2009a). Public Versus Personal Information for Mate Copying in an Invertebrate. *Current Biology*, *19*(9), 730–734. https://doi.org/10.1016/j.cub.2009.02.064

Mery, F., Varela, S. A. M., Danchin, É., Blanchet, S., Parejo, D., Coolen, I., & Wagner, R. H. (2009b). Public Versus Personal Information for Mate Copying in an Invertebrate. *Current Biology*, *19*(9), 730–734. https://doi.org/10.1016/J.CUB.2009.02.064

Monier, M., Nöbel, S., Isabel, G., & Danchin, E. (2018). Effects of a sex ratio gradient on female mate-copying and choosiness in Drosophila melanogaster. *Current Zoology*, *64*(2), 251–258. https://doi.org/10.1093/CZ/ZOY014

Nöbel, S., Danchin, E., & Isabel, G. (2018). Mate-copying for a costly variant in Drosophila melanogaster females. *Behavioral Ecology*, *29*(5), 1150–1156. https://doi.org/10.1093/BEHECO/ARY095

Oliveira, R. F., Mcgregor, P. K., & Latruffe{, C. (1998). Know thine enemy: fighting fish gather information from observing conspecific interactions. *Proc Biol Sci*, *265*(1401), 1045–1049.

Peake, T. M., & Mcgregor, P. K. (2004). Information and aggression in fishes. *Animal Learning & Behavior 2004 32:1*, *32*(1), 114–121. https://doi.org/10.3758/BF03196012

Peake, T. M., Terry, A. M. R., McGregor, P. K., & Dabelsteen, T. (2001). Male great tits eavesdrop on simulated maletomale vocal interactions. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*(1472), 1183–1187. https://doi.org/10.1098/RSPB.2001.1648

Penn, J. K. M., Zito, M. F., & Kravitz, E. A. (2010). A single social defeat reduces aggression in a highly aggressive strain of Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(28), 12682–12686. https://doi.org/10.1073/PNAS.1007016107

Plath, M., Blum, D., Schlupp, I., & Tiedemann, R. (2008). Audience effect alters mating preferences in a livebearing fish, the Atlantic molly, Poecilia mexicana. *Animal Behaviour*, *75*(1), 21–29. https://doi.org/10.1016/J.ANBEHAV.2007.05.013

Pollick, A. S., Gouzoules, H., & De Waal, F. B. M. (2005). Audience effects on food calls in captive brown capuchin monkeys, Cebus apella. *Animal Behaviour*, *70*(6), 1273–1281. https://doi.org/10.1016/J.ANBEHAV.2005.03.007

Saltz, J. B. (2013). Genetic composition of social groups influences male aggressive behaviour and fitness in natural genotypes of Drosophila melanogaster. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1771). https://doi.org/10.1098/RSPB.2013.1926

Stamps, J., Buechner, M., Alexander, K., Davis, J., & Zuniga, N. (2005). Genotypic differences in space use and movement patterns in Drosophila melanogaster. *Animal Behaviour*, *70*(3), 609–618. https://doi.org/10.1016/J.ANBEHAV.2004.11.018

Szipl, G., Ringler, E., & Bugnyar, T. (2018). Attacked ravens flexibly adjust signalling behaviour according to audience composition. *Proceedings of the Royal Society B*, *285*(1880). https://doi.org/10.1098/RSPB.2018.0375

Taborsky, B., & Oliveira, R. F. (2012). Social competence: an evolutionary approach. *Trends in Ecology & Evolution*, *27*(12), 679–688. https://doi.org/10.1016/J.TREE.2012.09.003

Tibbetts, E. A., Wong, E., & Bonello, S. (2020). Wasps Use Social Eavesdropping to Learn about Individual Rivals. *Current Biology*, *30*(15), 3007-3010.e2. https://doi.org/10.1016/J.CUB.2020.05.053

Townsend, S., & Zuberbuhler, K. (2009). Audience effects in chimpanzee copulation calls. *Http://Www.Tandfonline.Com/Action/AuthorSubmission?JournalCode=kcib20&page=instructions*, *2*(3), 282–284. https://doi.org/10.4161/CIB.2.3.6796

Trainor, B. C., & Marler, C. A. (2019). *Aggression and Territoriality*. https://doi.org/10.1016/B978-0-12-809633-8.90064-5

Vakirtzis, A. (2011). Mate Choice Copying and Nonindependent Mate Choice: A Critical Review. *Https://Doi.Org/10.5735/086.048.0202*, *48*(2), 91–107. https://doi.org/10.5735/086.048.0202

Van Breukelen, N. A., & Draud, M. (2005). The roles of male size and female eavesdropping in divorce in the monogamous convict cichlid (Archocentrus nigrofasciatus, Cichlidae). *Behaviour*, *142*(8), 1023–1035. https://doi.org/10.1163/156853905774405308

Wang, L., & Anderson, D. J. (2010). Identification of an aggression-promoting pheromone and its receptor neurons in Drosophila. *Nature*, *463*(7278), 227–231. https://doi.org/10.1038/NATURE08678

Wertheim, B., Allemand, R., Vet, L. E. M., & Dicke, M. (2006). Effects of aggregation pheromone on individual behaviour and food web interactions: a field study on Drosophila. *Ecological Entomology*, *31*(3), 216–226. https://doi.org/10.1111/J.1365-2311.2006.00757.X

Wheeler, B. C. (2008). Selfish or altruistic? An analysis of alarm call function in wild capuchin monkeys, Cebus apella nigritus. *Animal Behaviour*, *76*(5), 1465–1475. https://doi.org/10.1016/J.ANBEHAV.2008.06.023

White, D. J., King, A. P., Cole, A., & West, M. J. (2002). Opening the social gateway: Early vocal and social sensitivities in brown-headed cowbirds (Molothrus ater). *Ethology*, *108*(1), 23–37. https://doi.org/10.1046/J.1439-0310.2002.00755.X

Whiten, A. (2021). The burgeoning reach of animal culture. *Science*, *372*(6537). https://doi.org/10.1126/SCIENCE.ABE6514/ASSET/BC7A4602-DD0B-4278-A7DC-C4B94E902B88/ASSETS/GRAPHIC/372_ABE6514_FA.JPEG

Wiley, R. H. (1983). Evolution of Communication. *Animal Behaviour*, *2*, 156–189.

Yurkovic, A., Wang, O., Basu, A. C., & Kravitz, E. A. (2006). Learning and memory associated with aggression in Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(46), 17519–17524. https://doi.org/10.1073/PNAS.0608211103/SUPPL_FILE/INDEX.HTML

Zuberbühler, K. (2008). Audience effects. *Current Biology*, *18*(5), R189–R190. https://doi.org/10.1016/j.cub.2007.12.041