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Female signal jamming in a socially monogamous brood parasite

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Acoustic signalling is vital to courtship in many animals, yet the role of female vocalizations is understudied. Here, we combine observational and experimental methods to assess the courtship function of the female chatter call in brown-headed cowbirds, *Molothrus ater*. While the chatter call is likely multifunctional, it is frequently used in social interactions and overlapping duets with males during the breeding season. Based on a combination of focal- and scan-sampling data from large naturalistic aviaries, we did not find support for the hypothesis that the chatter call elicits male attention or encourages continued courtship. However, we did find evidence that the chatter call plays a role in pair bond formation, as females preferentially chattered in response to songs from pair-bond males in the 2 weeks leading up to the median date of first copulation. Females were less selective in male-directed chatter use after copulations began. We also found support for the hypothesis that chatter is used to signal-jam male songs. Frame-by-frame video analysis revealed that the majority of female chatter calls were tightly time-locked to song, occurring less than 500 ms after male vocal onset. To test the effect of signal jamming on male song potency, we designed a laboratory experiment in which male song playbacks were jammed by various recorded stimuli. Natural chatter calls more effectively reduced female copulatory responses to song than high-pass filtered chatter calls, suggesting that the low frequencies in natural chatter (2–4 kHz) are important for interfering with male song and reducing its potency. Our results suggest that sexual conflict is operating in cowbird courtship, with signal jamming serving as a mechanism by which females guard, resist or select their mates. We also discuss ways in which cowbird vocal interactions may function cooperatively to coordinate reproduction or transition females into breeding condition.

Acoustic signalling is an important mode of communication for a wide range of animal taxa, particularly in the context of courtship. In birds, male song is used to defend territories, structure dominance hierarchies and attract mates (Catchpole & Slater, 2008; Dufty, 1986; Kroodsma & Byers, 1991; Rothstein, Yokel, & Fleischer, 1988). While extensive research has investigated the form and function of male songs, comparatively little is known about the role of female calls. There exists a striking diversity of female-typical calls, which have been hypothesized to serve myriad functions (Amy, Salvin, & Leboucher, 2018) including fertility advertisement (Montgomerie & Thornhill, 1989), mate quality sampling (Sæther, 2002) and reducing harassment by males (Birks & Beletsky, 1987). Given that successful courtship is ultimately the product of both male and female behaviours, investigating female contributions to courtship is essential to fully understand these complex dynamics (Riebel, Odom, Langmore, & Hall, 2019).

In some bird species, males and females vocalize in tandem – a phenomenon known as duetting (Benedict, 2008; Farabaugh, 1982; Hall, 2004; Thorpe, 1972). The utility of avian duets is likely context specific, and hypotheses for their functions range from cooperative to conflictive (Dahlin & Benedict, 2014; Hall, 2004). Cooperative duets, which may be either overlapping or antiphonal, can function in advertising coalition strength, signalling pair bond cohesion or defending joint resources and territories (Brumm & Slater, 2007; Catchpole & Slater, 2008; Dahlin & Benedict, 2014; Hall, 2004; Hall & Magrath, 2007; Marshall-Ball, Mann, & Slater, 2006). Conflictive duets, on the other hand, are often characterized by signal jamming (Grafe & Bitz, 2004; Slater, Gil, Barlow, & Graves, 2002; Tobias &

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Seddon, 2009). Signal jamming is defined as the interruption of one signaller by another, which is generally expected to result in a loss of information content (Brumm & Slabbekoorn, 2005). In Peruvian warbling-antbirds, *Hypocnemis peruviana*, for instance, mated pairs coordinate their duets when jointly defending a territory, but females jam the song of their mate in the presence of lone females to hinder extrapair mating opportunities (Tobias & Seddon, 2009). Thus, the function of duets may be flexible (Dahlin & Benedict, 2014), and female vocalizations may be co-opted for signal-jamming purposes when conflicts of interest arise.

Because the optimal reproductive strategies of males and females typically differ, sexual conflict is thought to be pervasive in nature (Parker, 1979). A wide range of taxa exhibit sexual conflict with regard to mating (Clutton-Brock & Parker, 1995; Daly, 1978), which may arise due to discrepancies in optimal mating frequency, number of partners, parental investment or mate quality (Chapman, Arnqvist, Bangham, & Rowe, 2003; Holland & Rice, 1998; Parker, 1979, 2006). As a result, sexual conflict often manifests in the context of courtship behaviour. Mate guarding, where individuals of either sex seek to limit their mate's opportunities for extrapair copulations, is one well-known example (Birkhead, 1979; Sonnenschein & Reyer, 1983). Furthermore, in species where males engage in persistent courtship, females may incur significant costs fending off unwanted suitors (Clutton-Brock & Parker, 1995; Daly, 1978), sometimes leading to evolutionary arms races (Chapman et al., 2003; Parker, 1979). Such arms races tend to be characterized by the evolution of a male trait that enables exploitation of an underlying female preference or circumvention of female choice, which in turn leads to selection for resistance mechanisms in females (Arnqvist & Rowe, 1995; Brennan et al., 2007; Holland & Rice, 1998).

Here, we explore courtship behaviour in the brown-headed cowbird, *Molothrus ater*, a brood-parasitic songbird. Despite exhibiting no parental care, cowbirds typically maintain social pair bonds throughout their breeding season (Darley, 1968, 1982; Friedmann, 1929; Laskey, 1950; Louder, Hauber, Louder, Hoover, & Schelsky, 2019; White, King, West, Gros-Louis, & Tuttle, 2010; Yokel, 1986a, 1989). Like many songbirds, male cowbirds sing to females to elicit the female copulation solicitation display (CSD), an inverted body posture that facilitates mounting and insemination by males. Laboratory experiments have demonstrated that the CSD can be elicited by auditory stimuli alone, and the songs of some males are more 'potent' than others based on their relative ability to induce this behaviour (West & King, 1986, 1988a, 1988b; King & West, 1977; Maguire, White, & Schmidt, 2013; West et al., 1979, 1981). Notably, females often appear to treat courtship as harassment, and increased courtship attention can detract from female efforts to locate and furtively parasitize nests (Yokel & Rothstein, 1991).

Female cowbirds produce a loud, broadband chatter call (Burnell & Rothstein, 1994; Friedmann, 1929; also known as a 'rattle') (Fig. 1a) that is especially prevalent during the breeding season and correlates positively with reproductive success (Kohn, 2018). As is the case for many calls (Marler, 2004), the chatter call is likely multifunctional (Burnell & Rothstein, 1994). Females often 'broadcast' their chatter calls (Friedmann, 1929), and sufficient within-population variation in the call exists that it may be useful for individual recognition (Burnell & Rothstein, 1994). There is evidence that the call attracts distant males, as chatter playback in the field results in male approach (Dufty, 1982a, 1982b; Rothstein et al., 1988). Territorial females are also attracted to chatter playback (Dufty, 1982b; Stutchbury, 1997; Yokel, 1989), and this renders the stimulus useful for luring and removing female cowbirds from the habitats of threatened songbird species (Robinson et al., 1993). In contrast to its broadcast function, the chatter call is likely antagonistic at close range (Burnell & Rothstein, 1994). Females often chatter while exhibiting threat displays towards other females in territorial interactions (Dufty, 1982a) and towards males singing

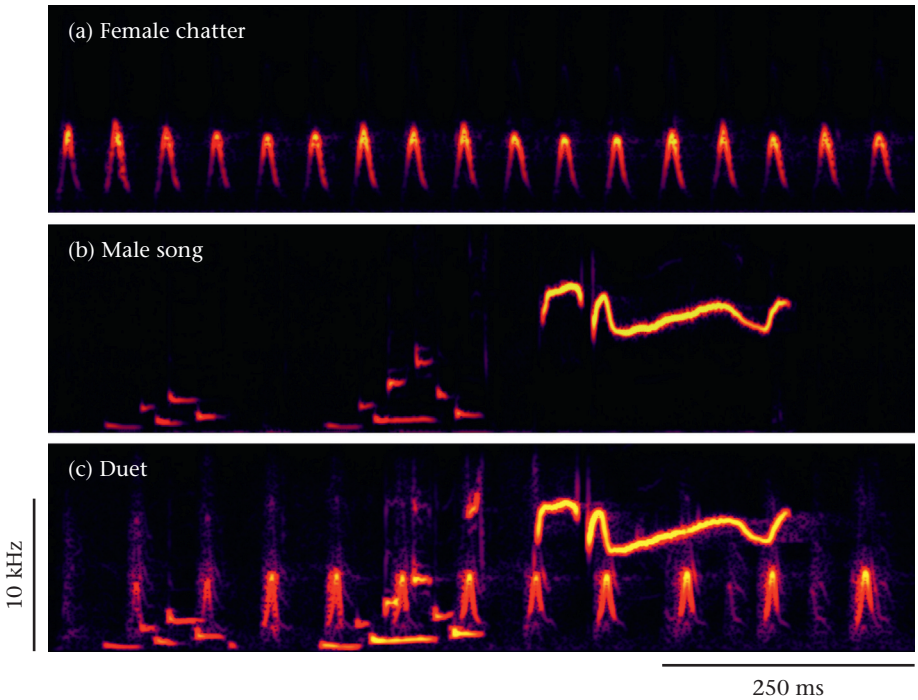

Figure 1. Acoustic structure of brown-headed cowbird chatter call, song and duet. (a) Spectrogram depicting a representative female cowbird chatter call. The call is composed of repeated, chevron-shaped syllables. (b) Spectrogram depicting a representative male cowbird song. This exemplar song contains two introductory note clusters followed by a whistle. (c) Spectrogram depicting a representative cowbird duet, comprising an overlapping male song and female chatter call.

courtship songs (Rothstein et al., 1988; Yokel, 1989). There is also evidence that the chatter call serves as a mate choice copying cue for other females: female cowbirds in laboratory playback experiments preferred male songs that had previously been associated with recorded chatter calls (Freed-Brown & White, 2009). Outside of the breeding season, the chatter call acts as a species-specific 'password', orienting young cowbirds to members of their own species after fledging the nest of a heterospecific host (Hauber, Russo, & Sherman, 2001; Louder, Balakrishnan, et al., 2019; Lynch et al., 2017). Thus, the function of the chatter call likely varies based on seasonality and social context.

During courtship, female cowbirds frequently chatter in conjunction with male song, leading some authors to consider cowbirds a duetting species (Benedict, 2008; but see Odom, Omland, & Price, 2015). Cowbird duets have previously been suggested to function in maintaining the pair bond (Kohn, 2018; Maguire et al., 2013), and similar hypotheses have also been proposed to explain female vocalizations in other icterids (Beletsky, 1982, 1985). However, given that these duets are not precisely coordinated and often overlap in time (Benedict, 2008), we hypothesized that the chatter call may instead be functioning as a jamming signal. According to this hypothesis, the broadband chatter vocalization interferes with male song to impede its transmission. Female signal jamming could function in mate guarding, as other females would be less able to eavesdrop on the courtship songs of paired males. Alternatively, signal jamming could provide a flexible mechanism for reducing male signal potency, which could allow females to control the outcome of a mating interaction.

To investigate the role of the female chatter call in cowbird courtship, we set out to test the nonexclusive hypotheses that chatter calls function to (1) elicit or sustain male courtship attention, (2) facilitate pair bond formation or maintain pair bond cohesion or (3) signal-jam the songs of males. First, we characterized the ethological context of chatter use by conducting focal video sampling of female cowbirds as they interacted with other flock members in large naturalistic aviaries. Second, we analysed scan-sampling observational data across multiple aviaries and years to determine whether female chatter use towards pair-bond and non-pair-bond males varies over the course of a breeding season. Third, we used frame-by-frame video analysis to precisely quantify the temporal dynamics of cowbird duetting and determine the prevalence of signal jamming. Finally, we conducted a laboratory playback experiment to test the prediction that jamming male song with chatter reduces male song potency.

METHODS

Study System

Brown-headed cowbirds are generalist, obligate brood parasites found throughout much of North America. Social monogamy is the predominant mating system both in aviaries and in the wild (Darley, 1968, 1982; Friedmann, 1929; Laskey, 1950; Louder, Hauber, et al., 2019; White et al., 2010; Yokel, 1986a, 1989), although some males maintain multiple pair bonds (Yokel, 1986a, 1986b) and certain ecological or demographic conditions may lead cowbirds to be more promiscuous (Elliott, 1980; Strausberger & Ashley, 2003; White, King, & West, 2002). Many cowbird pairings are also genetically monogamous – virtually exclusively so in aviaries (White et al., 2010) and frequently so in the wild (Louder, Hauber, et al., 2019; Woolfenden, Gibbs, & Sealey, 2002; Yokel, 1986a).

Sexual conflict in cowbirds cannot arise over allocation of parental care. However, females alone invest considerable time and energy prospecting for host nests (Friedmann, 1929; Hann, 1941; Norman & Robertson, 1975; Yokel & Rothstein, 1991), which parallels the asymmetrical investment that occurs in female-biased parental care. Female cowbirds lay an estimated 30–40 eggs per year on average (Fleischer, Smyth, & Rothstein, 1987; Scott & Ankney, 1980), although the output of the most fecund females may be substantially higher (Jackson & Roby, 1992). While males reportedly exhibit mate-guarding behaviour (Dufty, 1982a), no definitive evidence for direct benefits to females (e.g. vigilance against predators, access to resources, protection from extrapair courtship harassment) has been found in cowbirds (Hauber & Dearborn, 2003; Yokel & Rothstein, 1991). Thus, male genetic quality is thought to be the primary driver of female mate choice (Yokel & Rothstein, 1991).

During the breeding season, male cowbirds produce stereotyped songs that function to both attract mates and maintain male dominance hierarchies (Dufty, 1986; Rothstein et al., 1988). Male song comprises two primary elements: (1) the introductory note clusters and (2) the high-frequency whistle (Fig. 1b). The distinctive 'liquid' quality of the introductory note clusters, attributable to rapid oscillation between low- and high-frequency notes, is generated by alternating airflow between the left and right sides of the syrinx (Allan & Suthers, 1994). This rapid syringeal switching is similar to the mechanism of high-potency syllable production in canaries (Suthers, Vallet, & Kreutzer, 2012; Vallet & Kreutzer, 1995), and previous work in cowbirds suggests that the introductory note clusters are the male song components most effective at eliciting female CSD responses (King, West, & Eastzer, 1986; West et al., 1979). Female cowbirds also attend to the visual component of male displays, as audiovisual playback of male song enhances female CSD responses compared to audio alone (O'Loghlen & Rothstein, 2010; Ronald, Zeng, White, Fernández-Juricic, & Lucas, 2017).

Aviaries and Subjects

All observations were conducted during the breeding season (March through June) in large, seminaturalistic outdoor aviaries ($18.3 \times 6.1 \times 3.7$ m) in Philadelphia, Pennsylvania, U.S.A. (2006, 2007, and 2010) and West Flamborough County, Ontario, Canada (2017 and 2018). Aviaries were outfitted with shrubs, trees and additional branches for perching. We also placed artificial nests, containing variable numbers of wooden 'host' eggs, around the aviaries and monitored their activity with a motion-activated surveillance system (Geovision GV-1480 surveillance system, Irvine, CA, U.S.A.). Subjects were wild-caught brown-headed cowbirds (*M. a. ater*), which were trapped on site using funnel traps and marked with a unique series of coloured leg bands for identification. Birds were provided with vitamin-treated water and a mixture of millet, canary seed, and a modified version of the Bronx Zoo diet for omnivorous birds ad libitum. Female reproductive output was scored by tallying the total number of eggs produced by a given female (based on surveillance footage) throughout the course of a breeding season.

Focal Sampling

To gain insight into chatter use during courtship, we used focal video sampling to track the behaviours of female cowbirds ($N = 12$) as they freely interacted in one of two aviaries during the 2018 breeding season. We captured and characterized the social context of every chatter produced by a focal female during a given sampling block. The two aviaries contained mixed-sex flocks totalling 21 and 24 birds (Aviary 1: 10 males, 11 females; Aviary 2: 9 males, 15 females), respectively.

Focal video samples were collected during the 2018 breeding season, between 23 May and 29 June, using a Canon XA11 Pro Camcorder (Canon Inc., New York, NY, U.S.A.). All videos were taken between 0600 and 1200 hours when the birds are most active in

courtship. Each focal-sampling block lasted 10 min. We ensured that all birds were sampled equally at different times of the morning and never more than once per day. Post hoc video analyses were conducted in Adobe Premiere Pro CC v.13.0 (Adobe, San Jose, CA, U.S.A.) to annotate behaviours and ensure that all individuals were accurately identified. During video analysis, we tracked female behaviours including chatters, leaves (i.e. flying away from a male within 1 s of song or approach), lunges (i.e. thrusting or pecking in the direction of an adjacent male), copulation solicitation displays (CSDs) and copulations. We also noted the context in which each of the aforementioned behaviours occurred (e.g. before/after male song, before/after male approach, etc.) and recorded the number of songs a female received from individual males.

For the purposes of a concurrent investigation, six of the females in each of the 2018 aviaries had lesions targeted to HVC, a brain region known to be involved in song production in males and potentially involved in courtship behaviour in females. Because these individuals have been demonstrated to exhibit subtle changes in social behaviour (Maguire et al., 2013), none of the lesioned females were chosen for focal sampling in this study. Furthermore, we refrained from drawing aviary-level conclusions about mating dynamics in the 2018 aviaries, and instead supplemented the 2018 focal sampling of nonlesioned females with aviary-wide song, chatter and reproductive success data obtained via scan sampling in four previous years (see Scan Sampling below).

Scan Sampling

Scan-sampling observations were conducted in 10 aviaries in Philadelphia, Pennsylvania, U.S.A., during the 2006, 2007 and 2010 breeding seasons and in two aviaries in West Flamborough County, Ontario, Canada, during the 2017 breeding season. During scan sampling, trained observers monitored the behaviours of all birds interacting within an aviary. Following a song or chatter, observers shifted their attention to the location of the event and noted the identity of both the vocalizing individual and the recipient. Observers also recorded a variety of nonvocal behaviours, including leaves, lunges and copulations. These data were collected in real time using speech-to-text software, as detailed in previously published methods (White et al., 2002). Prior work demonstrates that scan sampling is the most powerful method for gathering data about all individuals in an aviary and yields a more comprehensive account of total aviary interactions compared to focal sampling (White & Smith, 2007). However, because our investigation focused on a specific female vocal behaviour, we supplemented the scan-sampling data with focal sampling of individual females as outlined above.

Chatter Use towards Pair-bond versus Non-pair-bond Males

A female's pair bond was defined as the male that sang the greatest number of songs to her over the course of a breeding season. This pair bond definition reliably predicts copulations (Freeberg, 1996; West, King, & Freeberg, 1996), and song from pair bonds typically constitutes a large majority of the total songs a given females receives (Maguire et al., 2013). While it is common for some males to form multiple pair bonds, females in aviaries rarely form more than one (White et al., 2010). Female cowbirds chatter more to their pair bond in total (Kohn, 2018) — likely because they receive the vast majority of courtship songs from him — but it is not clear whether females chatter proportionately more to pair bonds compared to non-pair bonds. Thus, we aggregated scan-sampling data across the 12 aviaries to determine whether females are more likely to chatter to the song of their pair-bond (PB) male compared to the songs of non-pair-bond (NPB) males.

Patterns of Chatter Use Across the Breeding Season

We tested whether patterns in chatter use to pair-bond versus non-pair-bond males varied over the course of the breeding season by aggregating scan-sampling data across 12 aviaries. This data set spanned four breeding seasons and contained 94 595 total observations, 196 females and 119 males. The duration of data collection in the aviaries varied between years (Appendix, Fig. A1), and not all aviaries were represented for the entire breeding season. To mitigate the potential for small sample sizes to drive any observed patterns, we only included dates for which there were data from at least three aviaries (9 March through 10 June). We split the breeding season into two major stages, separated by the median date of first copulation across the 4 years (20 April). This date offered a rough estimate of when female cowbirds move into breeding condition and mating begins.

Duet Timing Analysis

To investigate the temporal dynamics of cowbird duetting, we extracted duet interactions (i.e. instances where both sexes vocalized) from the 2018 focal-sampling videos. For each of these duets, we calculated the latency between the onset of male song and the onset of female chatter using frame-by-frame video analysis in Adobe Premiere Pro CC (frame rate = 29.97 frame/s; frame duration = 33.37 ms). In some cases, both male and female beaks were visible during vocalizations and the moment of beak opening by each individual could be used as an indicator of song and chatter onset, respectively. In videos where one or both beaks were obscured, we combined audio and visual information to estimate the onset of vocalization. For males, the point of song onset was estimated by combining information from the waveform audio with the trajectory of a bowing display performed by males known as the 'song spread', which occurs in a stereotyped manner in concert with male song (Cooper & Goller, 2004; O'Loghlen & Rothstein, 2010). For females, the point of chatter onset was estimated by combining assessment of the waveform audio with the point at which her body began to visibly vibrate during call production. By combining these visual and audio components, we are confident that our estimates of vocal onset are accurate to within two frames (i.e. <66.73 ms).

Playback Jamming Experiments

In response to high-quality male songs, female cowbirds demonstrate a rapid and robust copulation solicitation display (CSD). The CSD can be induced by playback of recorded male song to laboratory-housed female cowbirds, and variations on this method have long been used to assay female preference and male signal efficacy (Freed-Brown & White, 2009; King & West, 1977; Maguire et al., 2013; O'Loghlen & Rothstein, 1995, 2002, 2003; West & King, 1986, 1988a, 1988b; West et al., 1981; White et al., 2002). The 'potency' of male song is defined by its probability of eliciting CSD across females during playback experiments, and there tends to be independent consensus among females about which songs are the most attractive (Freeberg, King, & West, 1995; Maguire et al., 2013; West et al., 1981, 1996). In our experiments, we do not use exogenous hormone treatments and female responses correspond to song preferences in aviaries (West et al., 1981).

To test the effect of female signal jamming on male song potency, we devised a laboratory experiment to artificially jam male song playbacks using female chatter playbacks. We set up four

speakers (Presonus E3.5–3.5" 2-way near field studio monitors, PreSonus Audio Electronics, Inc., Baton Rouge, LA, U.S.A.): two speakers (hereafter, the 'song speakers') faced towards the cages of nine singly housed female cowbirds, and the other two speakers (hereafter, the 'jamming speakers') faced in the opposite direction, back towards the song speakers (see Results, Fig. 7a). The song speakers were placed 1.8 m away from the jamming speakers, and the jamming speakers were placed either beside (experiment 1) or just in front of (experiment 2) the female cages at the same height as the song speakers. The speaker orientation in this experiment was intended to mimic the directionality that often occurs naturally in male–female duets.

During the experiments, female cowbirds ($N = 9$) were placed into separate wire cages on a 3 × 3 cage rack. Individuals in adjacent cages were in view of one another. The cages (56 × 56 × 46 cm) contained food, water, cuttlebones and perching sites, and birds were monitored daily by researchers or animal care staff. At 90 min intervals between 0700 and 1900 hours, the song speakers automatically played one of five male songs. During control trials, the song speakers played a male song stimulus alone. During experimental trials, which differed between the two experiments (see below), the jamming speakers played a stimulus simultaneously with the onset of the male song recording. The male song recordings represented a range of potencies, as determined by their use in previous playback investigations with other females (Maguire et al., 2013). All trials were captured on video by webcams, and the playback speakers and webcams were automated and synchronized using a custom Python script. A blinded experimenter manually reviewed webcam videos to score female responses (i.e. complete CSD = 1, partial CSD = 0.5, no CSD = 0). A complete CSD was scored if a female adopted the full copulatory posture (i.e. arched back, tail raised, cloacal and wing feathers spread, head lifted). A partial CSD was scored if a female produced a brief or reduced version of the posture in which the tail did not reach full extension. We conducted two similar playback experiments, outlined below, using these general methods.

Experiment 1

The first playback experiment took place between 6 June and 21 June 2019. For this experiment, the same chatter recording (obtained by recording a female cowbird housed with a male conspecific) was used for all jamming trials, allowing us to determine whether a single chatter could effectively jam multiple songs. Thus, there were 10 stimuli (i.e. five male songs alone plus the same five songs jammed by chatter), and all of these stimuli together constituted a playback block. Stimuli were presented in a pseudorandom order (i.e. the stimuli within a playback block were randomized, and all 10 stimuli were played once before moving to the next randomized block). Prior to their use in playback, all song and chatter sound files were normalized by root mean square (RMS) amplitude using Audacity v.2.3.1 recording and editing software (https://audacityteam.org/), and the peak amplitudes of all sound files were between 76 and 80 dB at the cages.

Prior to the experiment, females were housed with a mixed-sex flock in a large indoor flight cage (2.4 × 1.8 × 2.4 m) and exposed to short-day light cycles (8:16 h light:dark) starting in mid-December. Beginning in February, light schedules were set to match the sunrise and sunset times of Philadelphia, Pennsylvania, and were updated weekly. Thus, birds were gradually transitioned to long days (14:10 h light:dark) that replicated breeding light conditions by the start of the experiment.

During the experiment, females were in natural breeding condition and received no additional hormonal treatments to facilitate copulatory responses. For the purpose of another study, five of these individuals had previously received electrolytic microlesions unsuccessfully targeted to nucleus RA. However, extensive prior analysis demonstrated that these birds did not differ from intact birds in song preferences, selectivity or responsiveness (A. Perkes, personal observation), and thus they were included in the present study. Over the 16-day period, a total of 120 trials were conducted comprising 12 playbacks of each song type with and without concurrent chatter calls. Two females were ultimately excluded from analysis because they did not respond to any playbacks.

Experiment 2

We conducted the second playback experiment between 9 July and 23 July 2020. To add additional controls, we used the following jamming stimuli: natural female chatter ($N = 6$), high-passed (>4 kHz) female chatter ($N = 6$) and low-passed (<4 kHz) white noise ($N = 1$) (see Results, Fig. 7b). Chatter recordings were obtained from the Xeno-canto bird sound database (Xeno-canto Foundation, www.xeno-canto.org) and selected according to clarity and minimal ambient noise. To create high-passed chatter stimuli, we applied a custom high-pass filter (>4 kHz) (created using the 'Filter Curve' equalization tool in Audacity) to the six natural chatter recordings. The high-pass filter was specifically designed to exclude the frequencies in natural chatter that coincide with the frequencies of the male introductory notes, which are thought to be the song component primarily responsible for eliciting CSDs in cowbirds (King et al., 1986; West et al., 1979). Thus, we predicted that high-pass (HP) chatter calls would be less effective at jamming male song than natural chatter calls. Finally, we created a low-pass white noise stimulus that included all frequencies between 100 Hz and 4 kHz. We expected this to be the most effective jamming stimulus, as it was designed to completely mask the frequencies in the male introductory notes responsible for eliciting CSDs. Prior to their use in playback, all song and chatter sound files were normalized by RMS amplitude using Audacity v.2.4.2 recording and editing software. Measured at the cages, the peak amplitude of song stimuli was 85 dB (as per Freed-Brown & White, 2009; West et al., 1996; White et al., 2002), and jamming stimuli peaked at 85 dB (white noise) or 95 dB (natural and HP chatter). All playback stimuli were approximately 1 s in duration. The 70 playback conditions (i.e. five songs alone, five songs jammed by six natural chatters, five songs jammed by six HP chatters and five songs jammed by white noise) were presented pseudorandomly using an automated playback system as in experiment 1.

Prior to the experiment, females were overwintered in a vivarium with exposure to males. Females were housed in cages (56 × 56 × 46 cm) either singly or with another female. Birds were exposed to short-day light cycles (10:14 h light:dark) beginning in mid-November. Due to the COVID-19 pandemic, we were unable to conduct experiments in the facility and thus did not resume long-day cycles (14:10 h light:dark) until 26 May in an effort to postpone the breeding season. The delayed onset of summer light cycles extended the period that females were responsive to playbacks further into the summer. Although overall female responsiveness was lower than in previous experiments, patterns of selectivity remained normal. No hormonal manipulations were used to facilitate female copulatory responses.

Two cohorts of females participated in experiment 2. The first cohort ($N = 9$) received playbacks from 9 July to 16 July. On the afternoon of 16 July, the birds were removed from the experimental setup and replaced by a second cohort of females ($N = 9$). We resumed playbacks on 17 July and continued until 23 July. Each cohort received 60 playback trials comprising three presentations of each song–treatment combination. Five females were ultimately excluded from analysis because they did not respond to any playbacks.

Ethical Note

The wild female cowbirds that participated in playback experiments ($N = 27$) were caught at the Marshak Dairy Farm (Kennett Square, PA) using a baited funnel trap (2.4 × 1.2 × 2.4 m). Birds are attracted to the presence of food or other cowbirds inside the trap, but they are unable to find a way out after entering through the funnel (Robinson et al., 1993). The trap was checked each afternoon and provisioned with ample food, water and natural perches at all times. Trapping was approved by the Pennsylvania Game Commission (Special Use permit no. 34866) and the U.S. Fish and Wildlife Service (permit no. MB63583B-0). Birds were transported from the trapping site in an air-conditioned vehicle inside plastic dog kennels. Kennels measured 71 × 50 × 50 cm and were outfitted with natural perches and thoroughly cleaned between each use. Fewer than 10 birds (including no more than one male) were transported in each kennel, and birds spent fewer than 2 h inside the kennels. Upon arriving at the research facility, birds were quarantined in either an outdoor aviary (2.4 × 1.2 × 2.4 m) or in cages (56 × 56 × 46 cm) in a separate vivarium space for at least 2 weeks prior to introduction to other birds. During this time, we performed tests to screen for common diseases. The vivarium space was kept at 24 °C and 30% humidity. Through the use of multiple lighting sources, light transitions in the vivarium occurred over a 45 min period to more closely match natural crepuscular transitions. Birds were provided with perches, enrichment and ad libitum water and food (modified Bronx Zoo diet for omnivorous birds) at all times. Animal care staff or researchers checked on all birds daily for any signs of illness, injury or distress. All protocols were compliant with ASAB/ABS guidelines and approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania (IACUC protocol numbers 806007, 806651 and 806727) and the University Animal Research Ethics Board of Wilfrid Laurier University (UAREB proposal no. 17000).

Statistical Analyses

To analyse the results of the first playback jamming experiment, we developed a generalized linear mixed-effects model (GLMM) using the 'lme4' package in R (Bates, Mächler, Bolker, & Walker, 2015). This package allows for incorporation of a binomial error structure (possible female responses were between 0 and 1) and multiple random effects. The full model included the following: CSDs elicited per playback as the response variable; jamming treatment (i.e. control or chatter), song, and their interaction as fixed effects; female identity as a random slope; and playback date and time as random intercepts. An optimizer (BOBYQA) was used to facilitate model convergence. We validated the model using the R package 'DHARMa', which allows for intuitive visualization of lme4 residuals (Hartig, 2017). A similar GLMM was used to analyse the data from experiment 2. The full model included the following: CSDs elicited per playback as the response variable; jamming treatment (i.e. control, natural chatter, HP chatter or white noise) and song as fixed effects; and female identity, playback date, playback time and jamming stimulus (i.e. to account for different chatter and HP chatter recordings) as random intercepts. The random effect of jamming stimulus was ultimately excluded because it did not account for any of the variance in the model. We conducted post hoc pairwise Tukey tests using the 'emmeans' package in R (Lenth, Singmann, Love, Buerkner, & Herve, 2018) to compare the effects of the three jamming treatments. All descriptive statistics are presented as means ± 1 SEM unless stated otherwise.

RESULTS

Chatter Use Varies Among Females

Over a period of 38 days during the 2018 breeding season, we collected a median of five 10 min focal observation blocks per female (mean number of blocks = 4.8 ± 0.2; minimum = 3, maximum = 6) from each of the two aviaries. Among the 12 females, we observed a total of 588 chatters, 199 leaves and 40 lunges across 570 min of observation. There was considerable variability in chatter use among females. The highest-chattering female produced 165 chatter calls across five 10 min observation blocks (33.0 ± 8.1 calls/block; rate = 198 calls/h), while the lowest-chattering female produced just one chatter call across five 10 min observation blocks (0.2 ± 0.2 calls/block; rate = 1.2 calls/h). The average number of chatter calls produced per observation block was 10.7 ± 3.2, yielding an average rate of 63.9 ± 18.9 calls/h across females.

Chatter Is the Most Common Female Response to Male Song

Following male song, females chattered 43.5 ± 8.5% of the time (maximum = 80.8%, minimum = 6.3%), left (i.e. flew away) 19.7 ± 3.3% of the time (maximum = 38.4%, minimum = 3.3%) and lunged 9.7 ± 3.5% of the time (maximum = 31.3%, minimum = 0.0%). In contrast, females exhibited no observed response to male song 28.7 ± 5.3% of the time (maximum = 61.6%, minimum = 4.2%). Thus, it is notable that females, on average, were more likely to respond to male song with chatter than with any other behaviour.

Male-directed Chatter Is Primarily a Response Vocalization

If chatter functions to elicit male attention (Burnell & Rothstein, 1994; Rothstein, Yokel, & Fleischer, 1988), we should expect males to approach or sing to females soon after they chatter. However, based on aviary focal sampling, females were

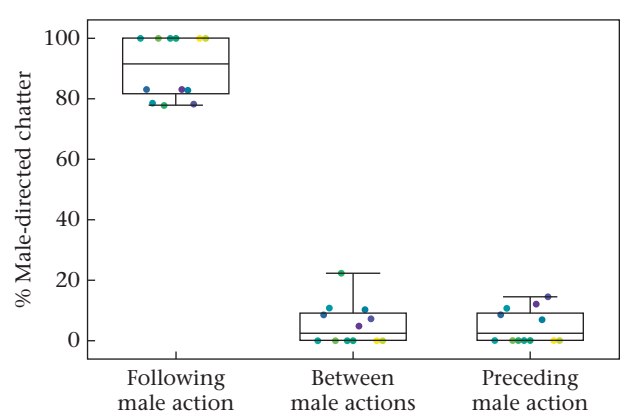

Figure 2. Percentage of male-directed female chatter calls observed immediately following a male action (e.g. song, approach, fly-by, or flight whistle), between two male actions (i.e. following one male action and preceding a second male action) and immediately preceding a male action. Coloured points represent average values for individual females ($N = 12$) across focal observation blocks. Box plot lines (bottom to top) represent minimum, first quartile, median, third quartile and maximum values across all females.

Table 1
Ethological context of observed chatter calls during focal sampling

Chatter context	% All chatters observed, averaged across females	% Male-directed chatters observed, averaged across females
Following male song	31.2 (± 7.0)	42.2 (± 7.4)
Following male approach	29.6 (± 7.0)	45.0 (± 8.0)
Following male fly-by	1.4 (± 0.6)	2.4 (± 1.1)
Following distant male flight whistle	0.4 (± 0.2)	0.7 (± 0.5)
Between male actions	3.9 (± 1.8)	5.3 (± 2.0)
Preceding male song	1.1 (± 0.6)	1.9 (± 1.0)
Preceding male approach	1.2 (± 0.6)	2.1 (± 1.0)
Preceding male pursuit	0.2 (± 0.1)	0.4 (± 0.2)
Unclear	28.7 (± 6.2)	–
Following female action	2.4 (± 1.3)	–

Values are means ± SE.

significantly more likely to produce chatter calls following male actions than preceding them (Wilcoxon signed-ranks test: $V = 78$, $N = 12$, $P = 0.002$). Among the chatter calls used in interactions with males ($N = 360$), an average of 90.3 ± 3.0% occurred immediately (approximately 1 s) following a male action, while only 4.4 ± 1.6% occurred immediately preceding a male action (Fig. 2, Table 1). Finally, an average of 5.3 ± 2.0% of chatter events occurred between two male actions (e.g. a male approached a female, she chattered, and the male sang to the female following the chatter). We also note that, in some instances, females chattered for reasons that were unclear (Table 1). These events are likely consistent with the previously noted 'broadcast' use of female chatter (Burnell & Rothstein, 1994; Friedmann, 1929). However, these chatters did not have clear ties to courtship, nor did they discernibly precede actions from males or other females. In summary, we did not find support for the hypothesis that chatter is used to elicit male attention or courtship behaviour, and we conclude that male-directed chatter calls are primarily response vocalizations (Fig. 2).

Chatter Does Not Elicit Sustained Courtship from Males

Although chatter did not seem to initiate interactions with males (Fig. 2), we hypothesized that chatter may encourage a male to continue courting a female within a given interaction. To address this, we used time-stamped scan-sampling data across the 12 aviaries to determine whether a male that received a chatter call in response to his initial song was more likely to sing to a female again in the following 30 s. We found that males were no more likely to repeatedly sing to females that chattered compared to females that exhibited no response (Fig. 3). This effect held true for both pair-bond males (Welch's t test: $t_{112.87} = 0.85$, $P = 0.40$) and non-pair-bond males ($t_{84.79} = 0.30$, $P = 0.76$). Conversely, males were significantly less likely to sing again to a female that left in response to his initial song (pair-bond males: $t_{111.41} = -8.79$, $P < 0.00001$; non-pair-bond males: $t_{79.42} = -3.69$, $P = 0.0004$). Being that males have the option to pursue females that leave in response to song, this result is not necessarily obvious. Therefore, while chattering does not appear to influence male courtship persistence or encourage continued singing, leaving has a strong inhibitory effect on male courtship (Fig. 3).

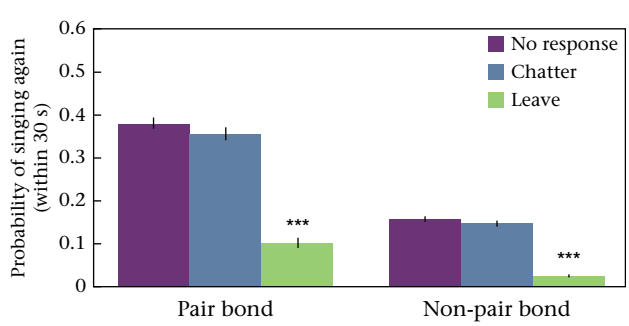

Figure 3. Probability of pair-bond and non-pair-bond males singing to a given female again based on her response to their initial song (i.e. no response, chatter, or leave). ***$P < 0.001$. Values are means ± 2 SEM.

Female Chatter Use Varies Across the Breeding Season

We used aggregated scan-sampling data across 12 aviaries and four breeding seasons to test the hypothesis that chatter functions in pair bond formation. We found that female cowbirds produced very few response chatters during March and early April (Fig. 4a) despite receiving song from both pair-bond (PB) and non-pair-bond (NPB) males during this period (Fig. 4b). However, in the 2 weeks leading up to the median date of first copulation, females became significantly more likely to chatter in response to their PB male's song compared to songs from NPB males (Fig. 4a). One week after the increase in female chatter probability towards PBs, pair-bond male song rate began to significantly exceed the combined song rate of all NPB males ($t_{493.03} = 3.33$, $P = 0.0009$) (Fig. 4b). The observed pattern of chatter use towards pair-bond males just prior to the onset of mating may indicate a role for chatter in the pair bond formation process.

If chatter functions in maintaining the pair bond, we should expect females to preferentially chatter towards pair-bond males after these relationships have been formed and mating is occurring (i.e. after the median date of first copulation). However, females exhibited a marked increase in their probability of chattering in response to NPB song the week after the start of copulations in the aviaries (Fig. 4a). During the weeks of 27 April, 11 May and 18 May, females chattered in response to PB and NPB songs with similar probability (Fig. 4a). This pattern was not driven by changes in the frequency of NPB singing, as NPB song rates remained relatively constant throughout the course of the breeding season (Fig. 4b). Unlike PB song rates, which were significantly higher during the copulation stage of the breeding season than during the pre-copulation stage ($t_{4403.11} = 21.36$,

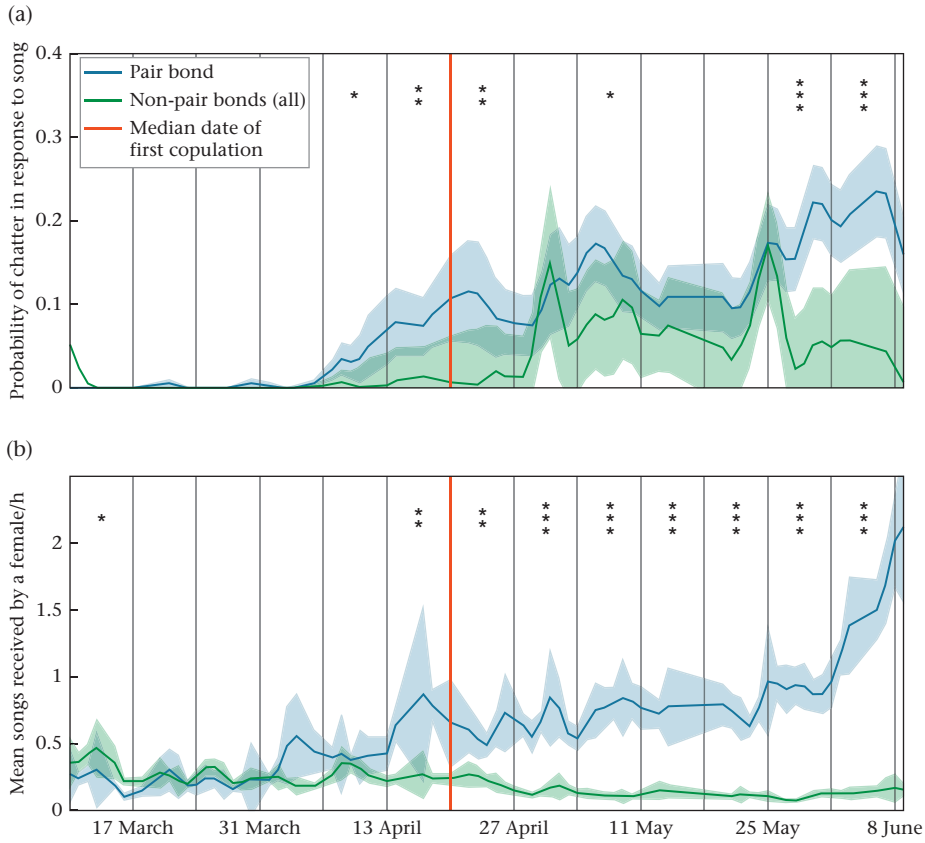

Figure 4. (a) Average probability of female chattering in response to songs of pair-bond and non-pair-bond males across the breeding season. Asterisks denote weeks in which females were significantly more likely to chatter in response to songs from pair-bond males than in response to songs from non-pair-bond males (Welch's t test: $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$). (b) Average number of songs females received/h from pair-bond and non-pair-bond males across the breeding season. Asterisks denote weeks in which the average number of songs females received/h significantly differed between pair-bond and non-pair-bond males (P values as above).

$P < 0.0001$), NPB song rates did not differ between the two major stages of the breeding season ($t_{6520.47} = 0.35$, $P = 0.73$). We suspect that variation in chatter selectivity may be linked to changes in female behavioural state over the course of the breeding season. Given the similar probability of response chattering towards PB and NPB males during several weeks in May, chatter likely serves functions other than pair bond maintenance at some stages of the breeding season.

Females Chatter with High Temporal Precision in Response to Male Song

Frame-by-frame analysis of focal video data revealed that female cowbirds tend to chatter immediately following male song, with 60.6% of duet chatters ($N = 109/180$) occurring in the first 500 ms after song onset (Fig. 5). In another 13.3% of cases ($N = 24/180$), chatter onset pre-empted male song onset. Chatters beginning prior to male song typically continued throughout the song, thereby jamming it in its entirety. Given that most male and female vocalizations last about 1 s (Fig. 1), the observed timing of female chatter onset indicates that cowbird duets are generally characterized by signal jamming (i.e. maximization of overlap) rather than jamming avoidance (i.e. minimization of overlap).

We also tested whether the timing of female response chatters differed when chatters were used in duets with pair-bond versus non-pair-bond males. To account for nonindependence in the data set (i.e. the same females were represented multiple times), we calculated the average response time for each female in response to pair-bond and non-pair-bond males, respectively. Females that were not observed duetting with both types of males were excluded from the analysis. We then performed a paired Wilcoxon signed-ranks test comparing a female's average chatter latency in duets with her pair-bond male compared to her average latency in duets with non-pair-bond males. While females chattered earlier in duets with pair-bond males (0.14 ± 0.11 s) than in duets with non-pair-bond males (0.44 ± 0.13 s), the difference in means was marginally nonsignificant (Wilcoxon signed-ranks test: $V = 25$, $N = 7$, $P = 0.08$). However, it is notable that nearly all chatters occurring prior to or synchronously with male song onset occurred in duets with pair-bond males.

Chatter is Negatively Correlated with Leaving

We hypothesized that individual females use either chattering or leaving (i.e. flying away from a male) as alternative strategies for dealing with undesired male courtship attention. This hypothesis

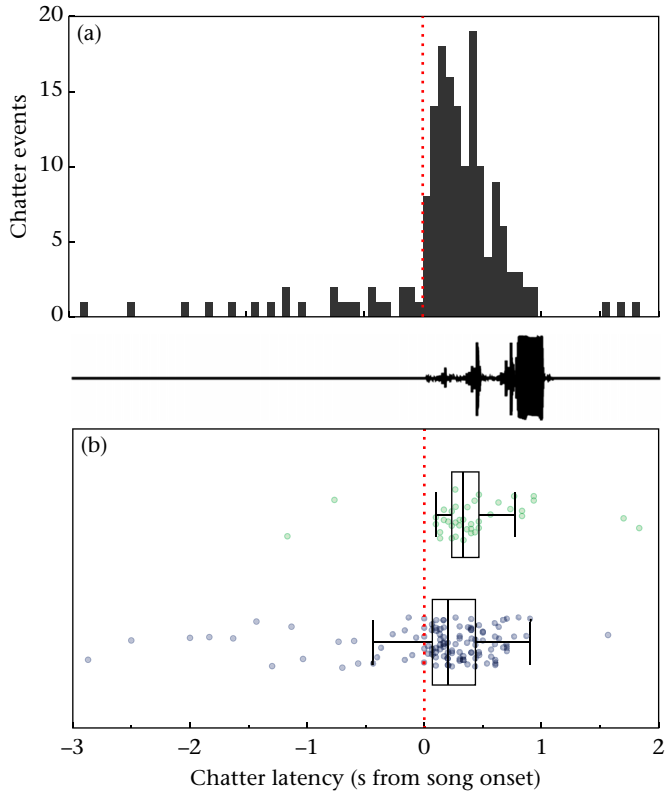

Figure 5. Latency of female chatter calls in relation to male song onset (dotted red line represents male song onset). (a) Onset of female chatter calls relative to male song onset during all duets. Bars represent the frequency of chatter onset per 33 ms time bin (equal to the frame rate of the camera). (b) The timing of female chatters in duets with pair-bond males (blue circles) and non-pair-bond males (green circles). A waveform of an exemplar male song is included in the centre for reference. Box plots (left to right) represent minimum, first quartile, median, third quartile and maximum values. Points outside the range of the minimum and maximum values are outliers.

was supported, as females with high chatter counts (summed across all observation blocks) had low corresponding leave counts, and vice versa (Pearson correlation: $r_{10} = -0.67$, $P = 0.02$; Fig. 6). Only chatters ($N = 360$) and leaves ($N = 199$) that were observed in interactions with males were included in this analysis, and

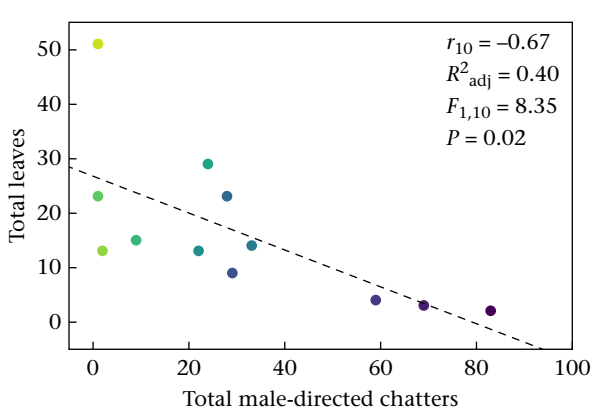

Figure 6. Individual females' ($N = 12$) propensity to chatter versus leave in response to male actions during focal observations. Coloured points represent individual females and correspond to Fig. 2.

instances where females chattered and left in the same interaction ($N = 61$) were excluded. To examine this negative relationship further, we generated a linear regression using the 'lm' function in R to model total observed leaves by a given female as a function of her total observed chatters. Based on the model, male-directed chatter count significantly predicted total leave count ($R^2_{adj} = 0.40$, $F_{1,10} = 8.35$, $P = 0.02$). Thus, our results suggest that individual females vary in their propensity to chatter or leave, and these actions may constitute alternative strategies for evading male courtship attempts.

Chatter Reduces Song Potency in Playback Jamming Experiments

If signal jamming via chatter effectively reduces the potency of male song, playback of female chatter calls concurrent with playback of song should reduce female CSD responses. In the first set of playbacks, females ($N = 7$) produced significantly fewer CSDs when chatter was played concurrently with song compared to when the same song was played alone (GLMM: $z = -4.52$, $P < 0.0001$; Fig. 7c; Appendix, Table A1). These results indicate that the same chatter vocalization can effectively jam a variety of different songs, which is ethologically relevant given that a single female may receive song from multiple males. We also observed a significant interaction effect between potency and signal jamming (likelihood ratio test: $\chi^2_4 = 33.66$, $P < 0.0001$). While this suggests that signal jamming is disproportionately effective at neutralizing the effect of high-potency songs, the detected interaction may also be the product of a floor effect on the low-potency songs.

In the second playback experiment, we presented females ($N = 13$) with the same song stimuli but varied the acoustic nature of the jamming stimulus. The three jamming stimuli (Fig. 7b) included pre-recorded chatter calls from six different females, low-pass filtered (100 Hz–4 kHz) white noise and high-pass filtered (>4 kHz) chatter calls that were specifically designed to avoid overlapping with the frequency range of the male introductory note clusters. While all jamming treatments significantly reduced song potency relative to the control stimuli (i.e. songs alone), natural chatter calls had the greatest negative effect on female CSD responses (Fig. 7d; Appendix, Table A2). These results demonstrate that different chatter vocalizations are similarly effective at jamming male song. Furthermore, HP chatter was significantly less effective than natural chatter (Tukey post hoc test: z ratio $= -3.03$, $P = 0.01$), consistent with the prediction that low frequencies in the chatter call (i.e. <4 kHz) are important in reducing song potency. The effect of white noise did not significantly differ from natural chatter (z ratio $= -1.11$, $P = 0.68$) or HP chatter (Tukey post hoc test: $z = 2.06$, $P = 0.17$).

DISCUSSION

In this study, we aimed to elucidate the function of the female chatter call during cowbird courtship, hypothesizing that it may play a role in (1) attracting or sustaining male attention, (2) forming or maintaining pair bonds or (3) signal-jamming the songs of males. We did not find support for the hypothesis that chatter elicits male attention, as the vast majority of male-associated chatter calls immediately followed rather than preceded male actions (Fig. 2). In addition, chattering did not appear to encourage continued courtship, as response chatters did not increase the likelihood that males would sing to a female again (Fig. 3). In contrast, we did find support for the hypothesis that early-season chatter is involved in pair bond formation. Females preferentially

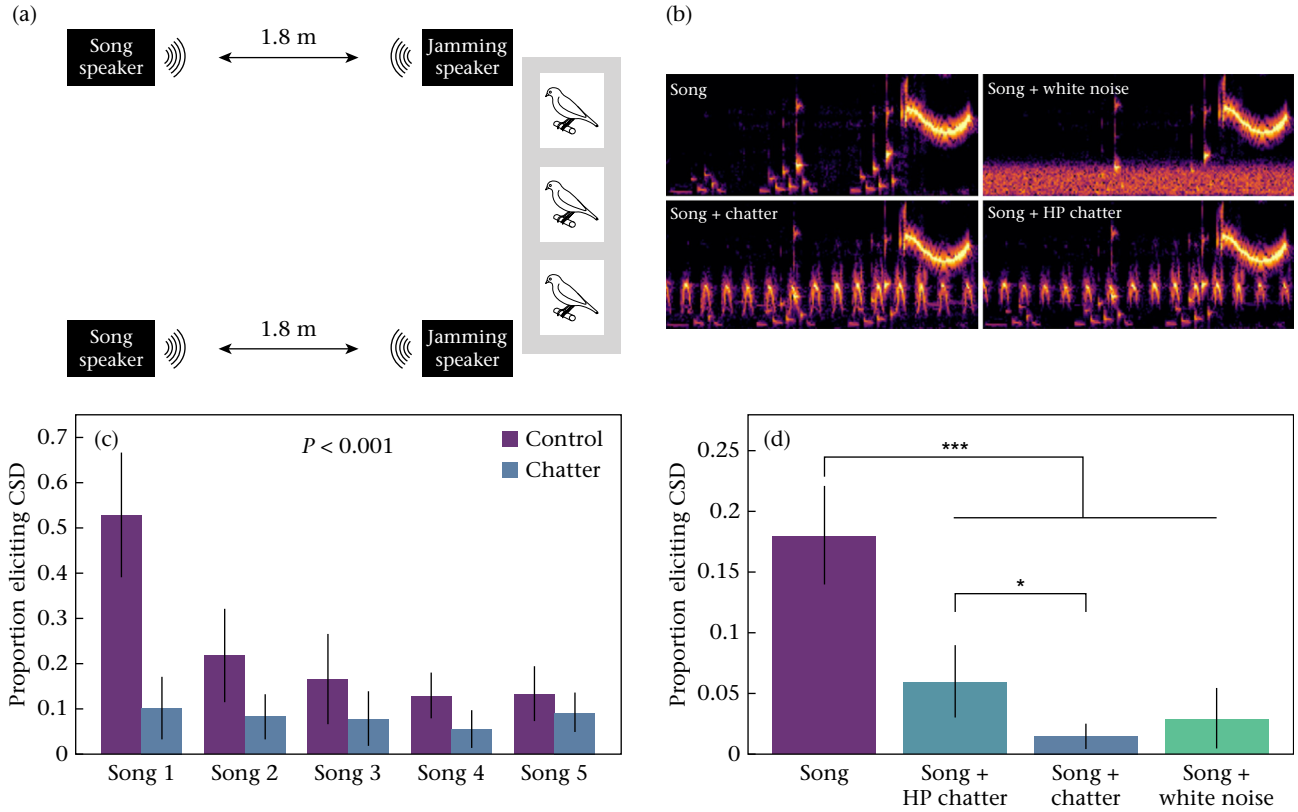

Figure 7. (a) Set-up of the playback jamming experiments (see Methods for details). (b). Examples of stimuli used in experiment 2. Clockwise from top left: song alone (control), song jammed by low-pass filtered white noise (100 Hz–4 kHz), song jammed by high-pass filtered (HP) chatter (>4 kHz) and song jammed by natural chatter. Stimuli were presented pseudorandomly at 90 min intervals. (c) Proportion of control and jammed song stimuli eliciting female copulation solicitation displays (CSDs) in experiment 1. (d) Proportion of control and jammed song stimuli eliciting female CSDs in experiment 2. Error bars represent ± 1 SEM. $*P = 0.01$; $***P < 0.0001$.

chattered in response to songs from pair-bond males in the weeks leading up to the start of copulations. However, during the middle of the breeding season, females exhibited similar probabilities of chattering towards pair-bond and non-pair-bond males (Fig. 4a). The distinct phenological pattern of chatter use towards pair-bond and non-pair-bond males suggests that female calls may serve different functions at different stages in the breeding season.

We also found support for the signal jamming hypothesis. Consistent with recent evidence that call timing is under tight neural control (Benichov et al., 2016), female chatter calls were time-locked to male vocalizations, frequently occurring within the first 500 ms of male song onset (Fig. 5). Because the typical durations of song and chatter are similar (Fig. 1), chattering just before or after male song reliably jams the majority of the song. In addition to its temporal characteristics, the acoustic structure of chatter is consistent with a signal jamming function. The chatter call's highest spectral power (2–5 kHz) specifically coincides with the frequency of male song introductory note clusters (Fig. 1), which are the components thought to be primarily responsible for inducing female copulatory responses in cowbirds (King et al., 1986; West et al., 1979). The playback jamming experiments provide support for this interpretation, as natural chatter reduced male song potency more effectively than did high-pass chatter (>4 kHz) (Fig. 4d) despite retaining the same amplitude and duration. Signal jamming via chatter may reduce song potency by increasing the entropy of incoming song syllables, as increased entropy is a predictor of reduced song potency in cowbirds (Gersick & White, 2018).

While the timing, structure and effect of female cowbird vocalizations are consistent with a jamming function, the motivation for this behaviour remains uncertain. Signal jamming results in a loss of information content (Brumm & Slabbekoorn, 2005) and likely incurs an energetic cost to the initial signaller (Eberhardt, 1994; O'Loghlen & Rothstein, 2010), and thus tends to occur in cases of conflict (Corcoran & Conner, 2014; Corcoran, Barber, & Conner, 2009; Grafe & Bitz, 2004; Tobias & Seddon, 2009). Intersexual conflict in cowbirds may arise over optimal number of mates, mate quality or mating frequency (Louder, Hauber, et al., 2019; Rice & Holland, 1997). Below, we suggest three potential functions of female signal jamming in cowbird courtship – mate guarding, female resistance and attending to more reliable indicators of mate quality – all of which are driven by sexual conflict. Finally, we also discuss the possibility that cowbird vocal interactions may be inherently cooperative.

Acoustic Mate Guarding

As has been proposed in other species that exhibit interruption during duets (Grafe & Bitz, 2004; Slater et al., 2002; Tobias & Seddon, 2009), acoustic mate guarding is a potential explanation for the vocal behaviour of female cowbirds. Signal jamming via chatter reduces the perceived potency or attractiveness of male song (Fig. 7), potentially inhibiting a male's ability to attract or copulate with other females. Early in the breeding season, when pair bonds are forming but copulations have not yet begun to occur (Fig. 4a), females may increase response chatter rates to restrict the ability of preferred males to court and pair with other females. Other females may also avoid pairing with a male that is actively being guarded (Slater et al., 2002). Later in the season, when pair

bonds have been solidified and copulations are occurring (Fig. 4a), mate guarding via signal jamming could ensure that females have unrestricted access to their pair-bond males.

In cowbirds, mate-guarding behaviour may be driven by sexual conflict over the optimal number of mates. Recent genetic evidence indicates that cowbirds conform to Bateman's principle: male fitness tends to increase with the number of mating partners whereas female fitness does not (Bateman, 1948; Louder, Hauber, et al., 2019). Thus, males may be incentivized to seek extrapair copulations at the expense of their pair-bond female. While female cowbirds apparently do not gain any direct benefits from their pair bond (Hauber & Dearborn, 2003; Yokel & Rothstein, 1991), they may benefit from retaining exclusive access to their preferred male for breeding. The pair bond relationship may increase female fecundity by ensuring continued reproductive stimulation via courtship (explored below), potentially providing insight into why a brood-parasitic species without parental care should exhibit a socially monogamous mating system.

The finding that females often chattered earlier in duets with their pair bonds (Fig. 5b) also lends support to the mate-guarding interpretation. Although the difference in mean timing between pair-bond and non-pair-bond males was marginally nonsignificant, the relationship warrants future investigation using larger sample sizes. More precise time-locking or earlier interruption should result in improved signal jamming, and females would be expected to preferentially guard their own mates over other males. The marginal difference in female duet timing may also be a product of greater familiarity with the cadence of the pair-bond male's stereotyped song spread display (Cooper & Goller, 2004), thereby allowing females to better predict their mates' song onset and time their chatters accordingly. Alternatively, it is possible that females are more stimulated by song of their pair-bond males compared to the songs of other males, on average, and thus it may be more important to jam the songs of their pair-bond males as a resistance mechanism (explored below).

Female Resistance

The chase-away model of sexual selection (Holland & Rice, 1998) is a theoretical positive feedback loop wherein males evolve increasingly stimulatory displays to the detriment of female fitness ('antagonistic seduction'), which in turn leads females to evolve ever greater resistance to such stimulation to the detriment of male fitness. While many discussions of female resistance have invoked the evolution of preference thresholds and sensitivities relative to male signals (Gavrilets, Arnqvist, & Friberg, 2001; Holland & Rice, 1998; Rowe, Cameron, & Day, 2005), a female's behavioural state at a given point in time may be more important than male signal potency per se in determining whether this threshold is reached (Perkes, Badger, Pfrommer, Daniilidis, & Schmidt, 2020). Thus, flexible vocal mechanisms (e.g. signal jamming) that mediate female responses to stimulating courtship displays may provide a novel mode of resistance to male coercion, allowing females to maintain control over reproductive outcomes even in heightened motivational states. Notably, the increase in female response chatter probability roughly coincides with the time that females move into breeding condition (Fig. 4a) and become more sexually responsive to male songs. Furthermore, females used response chatters less selectively in the middle of the breeding season (Fig. 4a), which may reflect the need to jam songs from all males (both pair-bond and non-pair-bond alike) during this period.

If male songs have the potential to be coercive – either in terms of eliciting the CSD response itself, or in stimulating a female to invest in a given male beyond what is optimum for her own fitness – females would benefit from signal jamming by retaining control over mate choice, regulating mating frequency or assessing a male's persistence as a proxy for quality (Arnqvist, 1992; Byers, Hebets, & Podos, 2010; Holland & Rice, 1998). Employing the general strategy of resisting copulation attempts via signal jamming may enable females to select for the most persistent and viable males, as these are the only individuals that can successfully elicit a copulation. Indeed, courtship persistence is the strongest predictor of copulation success in male cowbirds (White et al., 2010).

Intrasexual competition has been suggested to drive the evolution of coercive and harmful male mating traits (Brennan & Prum, 2012) and could result in the evolution of more potent or stimulatory vocalizations. The male-biased sex ratio and lack of male territoriality in cowbirds results in intense male–male competition (Yokel, 1989; Yokel & Rothstein, 1991), and male dominance hierarchies are determined and maintained through countersinging bouts (Rothstein et al., 1988). Thus, it is possible that intrasexual selection on males is the primary driver for increased song potency but also renders songs hyperstimulatory in courtship contexts. Consistent with this scenario, male cowbird song has among the highest frequency range, frequency maximum and modulation rate of any known songbird (Greenewalt, 1968; West et al., 1979). Increases in song potency via intrasexual selection could in turn lead to sexually antagonistic coevolution between male and females, as females would be under selection to jam coercive signals, and males would be under selection to evade the jam.

Attending to Visual Display Components

Across taxa, successful courtship displays rely on multimodal components (Mitoyen, Quigley, & Fusani, 2019). In male cowbirds, song production occurs concurrently with a bowing display known as the 'song spread' (Cooper & Goller, 2004; Friedmann, 1929). This visual component of the male cowbird display is known to be a target of female preference (O'Loghlen & Rothstein, 2010, 2012), and the iridescence of male plumage reflects male nutritional status during moult (McGraw, Mackillop, Dale, & Hauber, 2002). Furthermore, song potency alone (as measured by the laboratory CSD assay) does not always correlate with male reproductive success in aviaries (White et al., 2010), implying that females may attend to other characteristics (e.g. body size, plumage quality, bow intensity) when choosing a mate.

While there is evidence that the iridescent plumage of male cowbirds is an honest signal of condition (McGraw et al., 2002), song may not be a reliable signal of male quality. Because any juvenile male raised without aggressive adult males in his social rearing environment will develop a superpotent song (King & West, 1977, 1988b; West & King, 1980; White et al., 2002), cowbird song quality does not appear to be indicative of 'good genes'. Rather, the honesty of the male signal is likely regulated by the social environment (West & King, 1980), as a male must be sufficiently dominant to defend singing a potent song (White et al., 2010). However, potent songs may be wielded dishonestly. Males can modulate their song potency based on social context (Gersick & White, 2018), and thus subordinate males could sing highly potent songs when dominant males are not in the immediate vicinity. The flexibility of male acoustic signals may lead to selection on females to account for other characteristics in mate choice, and signal jamming offers a potential mechanism to facilitate female evaluation of multimodal male traits.

In other species, such as the deafeningly loud white bellbird, *Procnias albus*, females balance the risks of approaching a male (i.e. potential hearing damage) with the apparent benefits of assessing male quality from close range (Podos & Cohn-Haft, 2019). Female cowbirds may face similar risks, as male song potency increases with proximity to the singer (King, West, Eastzer, & Staddon, 1981).

Thus, signal jamming may allow females to assess the visual components of a male display at close range while remaining (1) unaffected by the acoustic component that is responsible for driving the CSD response or (2) unbiased by a potentially dishonest signal. Furthermore, males that are worthy of closer inspection may be more likely to have their songs jammed by chatter calls, which may offer an explanation for why – in the absence of other information – chatter is used as a mate choice copying cue by other females (Freed-Brown & White, 2009).

Sexual Cooperation

While we have thus far interpreted signal jamming as an indicator of sexual conflict, it is also possible that cowbird duets are inherently cooperative. Although cowbirds do not jointly defend resources or territories, duetting may function to coordinate reproduction or signal receptivity to courtship. For instance, increases in response chattering may serve as an indicator that females are transitioning into breeding condition (Fig. 4a), and this cue could then stimulate pair-bond males to subsequently increase their courtship efforts (Fig. 4b).

Another possibility is that male courtship motivates a female to chatter (Fig. 2) but the chatter call itself is self-stimulatory. Similar mechanisms operate in ring dove, *Streptopelia capicola*, courtship, where male song stimulates the female to produce a nest coo, but her own vocalization transitions her into a reproductive state (Cheng, 1992). The role of vocal self-stimulation in modulating endocrine physiology may be a widespread phenomenon (Ball & Balthazart, 2009). Because oestrogen can act directly on the auditory forebrain to enhance auditory responses (Remage-Healey et al., 2008, 2010), it is also possible that the act of chattering increases the perceived potency of a male's song over time, eventually culminating in copulation. In addition, signals that originate from conflict may evolve towards cooperation in pair-bonding species, and a signal that initially caused the female to overinvest may become necessary for baseline reproductive stimulation (Servedio, Powers, Lande, & Price, 2019).

Recent work has demonstrated a positive correlation between female chatter frequency and reproductive output (Kohn, 2018), which may warrant future research into a mechanistic link between chattering and egg laying in cowbirds. In contrast to chattering, female leaving in response to male song correlates negatively with reproductive output (D. J. White., personal observation), likely because it discourages subsequent male courtship attention (Fig. 3) and reduces opportunities for female reproductive stimulation. Furthermore, females that leave rather than chatter (Fig. 6) may guard their mates less effectively and incur costs associated with nonexclusive access to their pair-bond male. While the precise time-locking of female jamming suggests a conflictive dynamic, we acknowledge that the hypotheses outlined here are not mutually exclusive and may act synergistically in the context of courtship.

Conclusions

We have presented evidence that female cowbirds use the chatter call to signal-jam male songs during courtship, which effectively reduces male song potency. While the function of signal jamming remains unclear, we suggest that it may allow females to acoustically guard their mate, resist coercive male signals or more accurately assess multimodal display traits. In addition to conflictive explanations for the observed duets, male and female cowbirds may also act cooperatively to coordinate reproduction. Patterns of female chatter use varied across the breeding season, and increased response chatters may play a role in pair bond formation in the weeks leading up to the onset of copulations. Whether an increase in chatter frequency is the product or the driver of a female's transition to a reproductive state remains to be tested. Future research is necessary to untangle the possible explanations for the observed patterns of female vocalizations in cowbirds, which could serve both conflictive and cooperative functions.

Because this study was conducted in aviaries, we acknowledge that we were likely unable to capture all uses of the cowbird chatter call. For instance, chatter may be more frequently used to summon males in the wild (Rothstein et al., 1988), where cowbirds span wider areas (Rothstein, Verner, & Stevens, 1984) and pairs may be located further apart from one another. Moreover, we only observed a small number of instances where females chattered towards another female (Table 1). This may be an artefact of the aviary environment, as female-directed chatter is suspected to mediate territorial interactions in the wild (Dufty, 1982a). Nevertheless, our study provides insight into the close-range function of female chatter during courtship, revealing its apparent function in signal jamming male song. In this case, conducting observations in naturalistic aviaries allowed us to obtain a sample size and degree of resolution that would have been difficult to achieve in a field study. The multiple hypotheses for the function of cowbird chatter need not be mutually exclusive, and the role of chatter is likely dependent upon social context, seasonality and female behavioural state.

In addition to demonstrating a pattern of courtship signal jamming in a brood-parasitic species, this paper provides a conceptual framework that may have applications in other contexts. Females of other avian species – including the closely related red-winged blackbird, *Agelaius phoenicius*, and the distantly related house sparrow, *Passer domesticus* – use similar broadband chatter vocalizations during courtship (Beletsky, 1985; Nivison, 1978), raising the possibility that similar dynamics are at play. We stress the need for continued research into female vocalizations to test the hypotheses presented in this discussion, and further studies of this kind will provide insight into the complex interplay between sexual signals and incentives during courtship.

Author Contributions

H.L.A. conceived focal hypotheses, collected focal-sampling videos, designed and performed focal-sampling analyses, co-designed and co-implemented playback experiments, analysed playback experiment data, co-designed the figures and wrote the paper. A.P. designed and performed scan-sampling analyses, co-designed and co-implemented playback experiments, and co-designed and created the figures. J.S.G. reviewed focal-sampling videos and playback experiment videos. H.B.D. set up aviaries and provided manuscript feedback. D.J.W. conceived aviary study methods, contributed historical scan-sampling data and provided manuscript revisions. M.F.S. co-designed playback experiments, co-designed figures and provided manuscript revisions.

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Appendix

Table A1
Output of the generalized linear mixed model for experiment 1

lme4 Model
glmer(femaleResponse ~ Treatment*Song + (Treatment | Female) + (1 | Date) + (1 | Time), family = binomial), weights = possible, data = df, control = glmerControl (optimizer = "bobyqa"))

Fixed effects	Estimate	SE	*z*	*P*
(Intercept)	−1.03	0.63	1.62	0.11
TreatmentChatter	−4.86	1.08	−4.52	6.25e-06***
Song 2	−2.37	0.31	−7.60	3.35e-14***
Song 3	−3.38	0.35	−9.63	<2e-16***
Song 4	−3.12	0.36	−8.59	<2e-16***
Song 5	−2.76	0.33	−8.43	<2e-16***
TreatmentChatter: Song 2	1.74	0.51	3.39	0.0007***
TreatmentChatter: Song 3	3.07	0.54	5.64	1.73e-08***
TreatmentChatter: Song 4	2.08	0.58	3.62	0.0003***
TreatmentChatter: Song 5	2.05	0.54	3.79	0.0002***
Random effects	**Variance**	**SD**	***N***	**Correlation**
Female (Intercept)	2.21	1.49	7	–
Date (Intercept)	0.24	0.49	16	–
Time (Intercept)	0.21	0.46	8	–
Female (TreatmentChatter)	5.53	2.35	9	-0.01

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table A2
Output of the generalized linear mixed model for experiment 2

lme4 Model:
glmer(femaleResponse ~ Treatment + Song + (1 | Female) + (1 | Date) + (1 | Time), family = binomial, weights = possible, data = df2, control = glmerControl(optimizer = "bobyqa"))

Fixed effects	Estimate	SE	*z*	*P*
(Intercept)	−1.10	0.53	−2.09	0.0367*
TreatmentChatter	−3.45	0.50	−6.84	7.99e-12***
TreatmentWhiteNoise	−2.82	0.43	−6.48	9.03e-11***
TreatmentHPChatter	−1.88	0.32	−5.87	4.42e-09***
Song 2	−1.03	0.37	−2.76	0.0058**
Song 3	−1.64	0.42	−3.92	9.03e-05***
Song 4	−1.09	0.39	−2.76	0.0059**
Song 5	−1.70	0.45	−3.78	0.0002***
Random effects	**Variance**	**SD**	***N***	–
Female (Intercept)	1.50	1.22	13	–
Date (Intercept)	0.98	0.99	15	–
Time (Intercept)	0.10	0.31	9	–

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

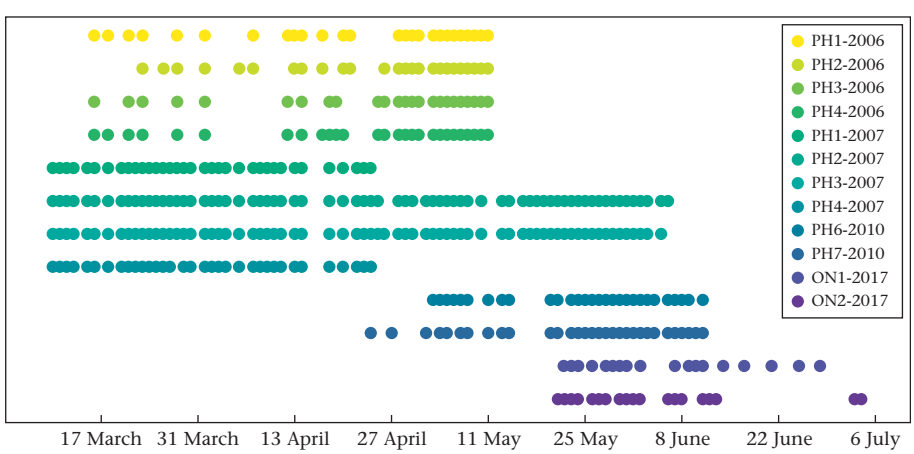

Figure A1. Number of days where scan-sampling data were collected in each of the 12 aviaries. For our analyses, we only included dates for which at least three aviaries were represented (9 March – 10 June). Codes in the figure legend represent location (either Philadelphia, PA, U.S.A., or West Flamborough County, ON, Canada) and year.