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Vocal signals predict attack during aggressive interactions in black-capped chickadees

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Animals use a variety of aggressive signals to mediate territorial interactions. Often these signals can be sufficient to ward off potential rivals, thus minimizing the chance of injury due to physical encounters. Yet not all behaviours produced during territorial interactions are aggressive signals. In this investigation, we examined the aggressive signals of black-capped chickadees, *Poecile atricapillus,* by determining which signals predict attack on a competitor. We used a recently developed playback protocol involving a loudspeaker and a taxidermic mount to simulate an intruder on males' breeding territories. We examined males' behaviours prior to any physical attack on the mount, both in the minute before attack and the time period preceding this minute. In the minute before attack, we found that gargle calls consistently predicted attack. In the preceding time period, we found that high song rate predicted attack. Surprisingly, we found that attack and the behaviours associated with attack were not significantly correlated with male dominance status. We conclude that song rate and gargle calling behaviour communicate intent to attack during territorial interactions in black-capped chickadees. These results expand our knowledge of aggressive signals during territorial encounters by revealing novel vocalizations used to communicate an animal's probability to attack an opponent.

Keywords:

animal communication

black-capped chickadee

frequency matching

gargle call

overlapping

Poecile atricapillus

signal reliability

song rate

Many animals use acoustic signals to defend resources such as breeding and foraging territories. In some cases, acoustic signals are sufficient to deter rivals (Krebs 1977; Krebs et al. 1978; Yasukawa 1981; Perrill et al. 1982; Arak 1983), thereby avoiding physical conflict and preventing injury of both signaller and receiver (Maynard Smith & Price 1973). Species as diverse as gibbons (*Hylobates* spp.: Marshall & Marshall 1976), treefrogs (*Hyla* spp.: Wells & Schwartz 1984; Martins & Haddad 1988), field crickets (Gryllidae: Alexander 1961) and birds (Passeriformes: Gil & Gahr 2002) use vocalizations as a primary means of territory defence. Yet not all signals that occur in territory defence can be deemed aggressive because not all signals communicate an intention to escalate aggression (Searcy & Beecher 2009).

Songbirds present an excellent example of a complex vocal communication system that includes aggressive signals. Many territorial songbird species advertise their presence on and willingness to defend a breeding territory by broadcasting loud acoustic signals to potential rivals (reviewed in: Marler & Slabbekoorn 2004; Catchpole & Slater 2008). Experiments where males have been replaced by speakers broadcasting song demonstrate that these vocalizations alone are often sufficient to deter or delay intrusions by territorial rivals (Göransson et al. 1974; Krebs 1977; Krebs et al. 1978; Yasukawa 1981). Songbirds can also communicate their level of motivation by using various types of vocalizations in a system of graded signals. For example, song sparrows, *Melospiza melodia*, can produce a nonaggressive vocal signal by singing a song type that does not match their neighbour's songs, a moderately aggressive signal by producing a song type that is shared with their neighbour, a highly aggressive signal by matching the song type that their neighbour just produced, and their most aggressive signal by producing a quiet song that indicates imminent physical attack (Beecher & Campbell 2005; Searcy et al. 2006; Searcy & Beecher 2009).

It can be difficult for behaviourists to distinguish aggressive signals from other signals that occur during the territorial contests of male birds. Searcy & Beecher (2009) suggested three criteria for deciding whether a particular signal should be considered aggressive: (1) the context criterion (i.e. the signal increases in aggressive contexts); (2) the predictive criterion (i.e. the signal predicts attack or aggressive escalation by the signaller); and (3) the response criterion (i.e. the receiver's behaviour changes in response to the signal). Searcy & Beecher (2009) argued that a signal must fulfil all three of these criteria to be deemed an aggressive signal. Examples of signals that fulfil the context and response criteria are those that match the frequency or pattern of an opponent's signal (e.g. tits: Krebs et al. 1981; sparrows: Stoddard et al. 1992; chickadees: Shackleton & Ratcliffe 1994), those that overlap an opponent's signal in time (e.g. nightingales: Todt 1981; wrens: Hall et al. 2006; chickadees: Mennill & Ratcliffe 2004a), and those that are produced at low amplitude (e.g. blackbirds: Dabelsteen & Pedersen 1990; sparrows: Anderson et al. 2007). Although the context and receiver criteria have been studied frequently, behaviourists have only recently focused on the predictive criterion. Using a signaller's perspective playback designed by Searcy et al. (2006), researchers have recently examined the predictive criterion in four species and found that quiet vocalizations predict attack in these species (song sparrow: Searcy et al. 2006; swamp sparrow, *Melospiza georgiana*: Ballentine et al. 2008; black-throated blue warbler, *Setophaga caerulescens*: Hof & Hazlett 2010; corncrake, *Crex crex*: Ręk & Osiejuk 2011).

The experimental design presented by Searcy et al. (2006) involves looped song playback and a taxidermic mount to simulate a male intruding on another male's territory. This design creates the potential for an aggressive context by providing a simulated rival against which the subject can aggress. This design is noninteractive (i.e. the playback does not vary in response to the signals produced by the subject; Mennill & Ratcliffe 2000), allowing researchers to examine how males behave while producing a vocal signal (i.e. examine vocal behaviour from the signaller’s perspective; Vehrencamp et al. 2007). By examining the minute before the subject attacks the model, researchers can explore which of the subject’s behaviours predict attack and thereby assess the predictive criterion for aggressive signalling. In all four studies that have used this experimental design to date, quiet songs consistently predicted attack (Searcy et al. 2006; Ballentine et al. 2008; Hof & Hazlett 2010; Ręk & Osiejuk 2011). Additionally, in swamp sparrows, the visual signal of wing waving predicted attack (Ballentine et al. 2008). To date, no other vocal or visual signals have been shown to directly predict attack in birds, in spite of the wide diversity of signalling behaviours associated with territorial interactions (reviewed in Todt & Naguib 2000).

In this study, we explore aggressive signals in the territorial interactions of black-capped chickadees, *Poecile atricapillus*, using the predictive criterion framework. The singing interactions of chickadees are well studied (reviewed in Mennill & Otter 2007). In spring, males defend territories from rivals using their ‘fee-bee’ song and during these territorial interactions they often produce a variety of vocal behaviours that fulfil the context criteria of aggressive signals, including song frequency matching, when a male adjusts the frequency of his song to match that of his rival; song overlapping, when a male adjusts the timing of his song to overlap his rival’s song in time; and producing other nonsong vocalizations, such as the gargle call (Ficken et al. 1978; Shackleton & Ratcliffe 1994; Baker & Gammon 2007; Fitzsimmons et al. 2008a). Playback experiments from the receiver’s perspective revealed that male chickadees approach the loudspeaker and sing more when presented with overlapping and/or frequency-matched playback compared to nonoverlapping or nonmatching playback (e.g. Otter et al. 2002; Mennill & Ratcliffe 2004a, b; Fitzsimmons et al. 2008b; but see Searcy & Beecher 2009). Therefore, in addition to meeting the context criterion, frequency matching and, potentially overlapping, fit the response criterion. However, none of these behaviours have been examined from the predictive criterion.

We used the playback design developed by Searcy et al. (2006) to examine which behaviours of male black-capped chickadees would fulfil the predictive criterion of aggressive signals. By delivering loop playback in conjunction with a taxidermic mount, we explored behaviours that occurred in the minute preceding attack, as well as behaviours that occurred throughout the remaining portion of the experimental trials. Knowing that social status is an important influence on signalling behaviours in chickadees (reviewed in Mennill & Otter 2007), we also quantified each male's dominance, based on interactions with members of his winter flock, and compared it to his signalling behaviour and propensity to attack. This is the first study to examine countersinging behaviour using the predictive criterion (and the signaller perspective) in this well-studied temperate songbird.

METHODS

We studied a population of black-capped chickadees at Queen's University Biological Station (44°34'N, 76°19'W), north of Kingston, Ontario, Canada, between January and May 2011. This population of chickadees has been studied annually since the 1980s. For this study we banded birds with unique combinations of coloured leg bands (N = 97 birds banded), assessed birds' winter dominance status (details in Ratcliffe et al. 2007), mapped breeding-pair territories when flocks dissolved in early spring (details in Mennill et al. 2004), and examined birds' territorial singing behaviour, following previously established protocols.

Dominance

Chickadees spend the nonbreeding period in small flocks where interactions follow a stable linear dominance hierarchy (Smith 1991; Ratcliffe et al. 2007). While birds were in winter flocks, we observed pairwise social interactions at 14 feeders, dispersed throughout the study site, and tallied these interactions to determine each bird's relative dominance status (following Smith 1991; Mennill et al. 2004; Ratcliffe et al. 2007). All observations took place between January and early April, between 0700 and 1700 hours. During pairwise interactions, we scored a bird as 'dominant' when it supplanted another chickadee, resisted a supplanting by another chickadee, elicited a submissive posture from another chickadee, or fed while another chickadee waited (Smith 1991; Ratcliffe et al. 2007). Dominance data were gathered by a live observer following established protocols (see Mennill et al. 2004). We supplemented these observations with video recordings using small video cameras mounted on tripods placed 2 m from feeders (Flip MinoHD, two Kodak Play Sport Zx3's, and a Sony HDR-XR101 HandyCam; all videos recorded at 1080 pixel resolution). Videos were reviewed by the same observer that collected dominance observations in the field.

All pairwise dominance interactions observed were used to calculate a numeric rank score following Mennill et al. (2004). There were 13 males for which we had at least 10 dominance interactions and a successful playback trial. For each of these 13 males, we calculated a rank score as the number of wins (number of times the subject was scored as dominant in an interaction), divided by the total number of dominance interactions involving the subject. This generated male rank values between 0 and 1, where males with a low rank score (near 0) were more subordinate and males with a high rank score (near 1) were more dominant. Previous research confirmed that this numeric rank score provides a continuous metric that is strongly related to the nominal rank classes that have been used in previous chickadee studies (Mennill et al. 2004).

Playback with a Taxidermic Mount

In mid-April, after flocks had split up and males had begun defending breeding territories, we simulated territory intrusions using looped song playback and a taxidermic mount of a male black-capped chickadee. Some trials (N = 18) involved playback to banded males, including the 13 males whose dominance status was known. We conducted additional trials (N = 20) on unbanded males outside of our core study area. To ensure we sampled a unique individual for each trial involving an unbanded male, we used a minimum distance of 400 m between each playback site (territories in this study population are, on average, 135 m across; Mennill et al. 2004).

Playback stimuli were created from focal recordings collected in 1999 of 10 males from the study population. None of the stimulus males had been observed in our population since 2001; given the average chickadee life span of 2.5 years (Smith 1991), none of the stimulus male songs should have been familiar to the subjects. We standardized the 10 song stimuli to reduce variation between stimuli and remove differences in song characters that may be related to dominance. Using Audition software (Adobe Systems, San Jose, CA, U.S.A.), we separated the ‘fee’ and ‘bee’ notes from each of the 10 stimulus males and then recombined them to make 100 different stimulus songs. The fee notes were normalized to -6 dBFS (decibels relative to full scale), and the bee notes were normalized to -8 dBFS, a typical amplitude relationship for this species. The fee and bee notes were adjusted by inserting small intervals of silence between the two syllables and by using the ‘stretch’ function to achieve population-typical internote durations (0.124 s) and frequency intervals (fee beginning at 3814 Hz and ending at 3609 Hz; bee beginning at 3183 Hz and ending at 3295 Hz) following the population-typical values presented in Weismann et al. (1990) and Christie et al. (2004), so that all playback stimuli had the same frequency and temporal characteristics. We selected a different stimulus song for each trial and repeated the same two-note song stimulus at a rate of 14 songs per minute, which is comparable to the song rates observed in this population (Otter et al. 1997). At the start of each trial, playback amplitude was adjusted to 80 dB SPL using a 3183 Hz test tone broadcast for up to 30 s, calibrated to match the peak amplitude of the bee portion of the song stimuli. Amplitude was measured at 1 m from the speaker using an analogue sound level meter (RadioShack 33-4050; C-weighting, fast response). This amplitude approximates the natural amplitude of male song, evaluated by ear by two observers during pilot trials.

Five taxidermic mounts were created from specimens found in Ontario that were collected after window-kills or natural death. Only adult males were used because of subtle sex-based variation in plumage features (Mennill et al. 2003b); sex was confirmed by the presence of testes during specimen preparation. Since the specimens were collected opportunistically, the dominance status of each specimen was unknown. All models were positioned in the same realistic posture, perched on a birch branch that we attached to the speaker apparatus.

Following the protocol developed by Searcy et al. (2006), we played back songs at a fixed rate in conjunction with presentation of a conspecific taxidermic mount. This protocol allowed us to evaluate subjects' signalling behaviour as they approached the taxidermic mount, and to determine which signals predicted attack. The singing behaviour and territorial countersinging interactions of chickadees are different from the song sparrows studied by Searcy et al. (2006), so we modified the protocol slightly. Searcy et al. (2006) used a period of lure song, followed by a period of silence, followed by another period of playback song when the model was exposed. Our experience working with chickadees, combined with pilot trials, taught us that chickadees react to playback by singing, rapidly approaching the speaker, and sometimes landing directly on the speaker or playback apparatus. However, when playback is paused, chickadees quickly stop interacting with the loudspeaker and depart the playback area. Therefore, we exposed the taxidermic mount at the start of the trial so that subjects would not make contact with the loudspeaker before the mount was exposed. We also eliminated the silent period so that birds would not exit the playback area. Song sparrows live in open environments, so Searcy et al.'s (2006) taxidermic model had to be hidden at the start of playback. Chickadees live in visually occluded forested areas, so we ensured that subjects were out of visual range before placing the mount and commencing playback. In only one trial, the subject arrived after the mount was placed and before the playback was started, so we cancelled this trial and repeated it on another day.

The loudspeaker and taxidermic mount were set on a tripod at the approximate centre of each subject's territory. Each of the 100 fee-bee song stimuli was randomly paired with one of the five mounts, and then the stimulus/mount combinations were selected just prior to the start of each trial following a randomized list with no repetition. If a trial had to be repeated because we failed to attract a male to the site, a new stimulus/mount combination was used for the next trial. After scanning the area around the playback set-up and confirming that no chickadees were present, we started playback with the mount revealed. Playback of looped song continued for up to 20 min or until the subject attacked the mount, whichever came first. We considered attack to be any contact the subject made with the taxidermic mount. All attacks had a rapid onset, but contact was usually very clear, with subjects typically landing on the mount's head or shoulders and pecking vigorously at its head and/or eyes. We placed a video camera 2 m from the mount and used recordings to confirm the initial time of attack that was dictated in the field; field data and video data matched in all cases.

During playback, two observers sat 8.5 m from the mount and speaker. Using a directional microphone (Audio-technica AT8015; 40–20 000 Hz frequency response) and a solid-state digital recorder (Marantz PMD-660; WAVE format, 44.1 kHz sampling rate, 16-bit encoding), one observer recorded the subject's vocalizations and quietly dictated the subject's physical behaviours, including their distance to the mount at each perching site, each time they passed over the loudspeaker, and whether or not they attacked the mount. The other observer helped locate the subject, ensure timing of attack was correctly assessed, and swiftly removed the mount after attack to reduce any undue stress on the subject. Flags placed at 1 m, 2 m, 5 m and 10 m on either side of the mount, and 5 m behind the mount, aided the observers in judging a bird's distance to the mount.

Analysing Playback Recordings

We quantified the behaviours, detailed below, in all subjects' trials and compared birds that attacked the taxidermic mount (hereafter 'attackers') to birds that did not attack the taxidermic mount (hereafter 'nonattackers') during three time periods. First, we explored behaviours in the minute before birds attacked the mount, and a parallel minute in nonattackers. To select a parallel minute in each nonattacker, we selected the same minute relative to the subject's first song as we did for a randomly selected attacker. This selection procedure is similar to that used in Searcy et al.'s (2006) experiment and in subsequent experiments using their protocol. Second, we examined the entire trial preceding this minute-before-attack for both attackers and nonattackers. In one case, our method of selecting the time period of analysis in nonattackers gave rise to a time period that extended beyond the length of the nonattacker's trial; for this bird we shortened the comparison period to the period when the bird was actually interacting with the simulated intruder. Therefore, the parallel minute in this nonattacker became the 19th minute of the 20 min trial. Finally, we quantified all behaviours that occurred throughout a subject's full trial, from the first song to the end of the minute before attack in attackers, or a parallel time period in nonattackers.

To examine each subject’s behaviour during playback, we viewed spectrograms of the recordings collected during the trials using Syrinx-PC sound analysis software (J. Burt, Seattle, WA, U.S.A.; 1024 point FFT, Blackman window type, resulting in 43 Hz frequency resolution and 15 ms time resolution). For each trial we tallied the following variables separately during the minute before attack, the period preceding that minute, and throughout the whole trial: (1) number of fee-bee songs; (2) number of gargle calls; (3) number of times the subject’s song overlapped the playback in time; (4) the number of frequency shifts (≥80 Hz difference, after Horn et al. 1992) from the subject’s previous song; (5) the number of times the subject’s song frequency-matched the playback (≤50 Hz, after behaviours observed in Otter et al. 2002); and (6) number of passes over the mount. Number of songs, gargles and passes over the mount (variables 1, 2 and 6) were standardized by dividing them by the duration of the analysis period. We standardized our measures of overlapping and frequency matching, variables 3 and 5, by dividing the number of overlapping and frequency-matching events by the number of opportunities the subject had to overlap or frequency-match the playback (i.e. the number of subject songs). Frequency shifting, variable 4, could occur for each song the subject produced, excluding the first, and therefore we divided this variable by the number of subject songs minus one.

Previous studies using the experimental design of Searcy et al. (2006) have included quiet song as a response variable. We did not include quiet song as a variable because it was never heard during playback trials. This species is capable of producing quiet song (known as the faint fee-bee, Ficken et al. 1978), but it is produced when breeding pairs are communicating at the nest cavity, not during aggressive encounters (Smith 1991).

All methods involving animals were approved by the University of Windsor Animal Care Committee (AUPP number 09-06).

Statistical Analyses

We used multiple logistic regression with forward stepwise selection of variables (with the *P*-to-enter set at $P = 0.05$, and *P*-to-remove set at $P = 0.10$) to determine which of the 6 behavioural variables predicted attack (after Ballentine et al. 2008; Hof & Hazlett 2010). One regression was performed for the minute before attack and another for the time period preceding this minute. Because we had rank data for only a subset of males ($N = 13$), we conducted a separate logistic regression analysis to test whether rank predicted attack in those males.

To describe the sequence of events that preceded attack, including a minute-by-minute comparison of all of our response variables, we plotted the subjects' behaviour for 10 min prior to attack (this included all minutes where more than five males sang). These analyses are descriptive only; no additional statistical analyses were performed.

We examined the effect of rank on the six putative aggressive behavioural variables by performing six univariate Spearman rank correlations. We used a Bonferroni correction for multiple comparisons ($\alpha = 0.0083$ for six tests). These tests were performed on behaviours that occurred during the full trial, to include as many data points as possible. Given that rank and propensity to attack the mount were not statistically associated (see Results), we considered it appropriate to examine the entirety of the playback trials in conjunction with dominance rank.

All statistical analyses were two tailed and conducted in PASW v18 (IBM Inc., Armonk, NY, U.S.A.). All values are presented with mean ± SE.

RESULTS

We attracted territorial male black-capped chickadees to within visual range of the observer in 38 trials. Of the 38 responding males, 21 males attacked the taxidermic model within 20 min of the start of playback, whereas 17 males did not. Thirteen of the 38 males were colour-banded animals of known dominance status (i.e. we had gathered ≥10 dominance interactions during winter dominance observations).

Up to One Minute before Attack

In the time preceding the minute before attack, a high song rate predicted whether chickadees would later attack the taxidermic mount (attacks occurred on average 7.09±1.05 min from the start of playback; range 1.53–17.5 min; Fig. 1). The number of songs per minute was the only variable of the six that we measured to enter into the stepwise logistic regression model, and it significantly predicted whether the subject attacked (logistic regression: $\chi^2_1 = 5.0$, $P = 0.025$). In a jackknifed procedure, songs per minute correctly classified 81% of attackers and 58.8% of nonattackers (71% of all cases correctly classified).

One Minute before Attack

In the minute before birds attacked the taxidermic mount, the number of gargle calls predicted whether or not black-capped chickadees would attack the mount (logistic regression: $\chi^2_1 = 52.3$, $P < 0.0001$; Fig. 2). Indeed, gargle calls were a perfect predictor of attack; all males that produced a gargle call subsequently attacked the simulated intruder, whereas nonattackers never produced gargle calls.

Sequence of Behaviours in Attackers

A descriptive analysis of the sequence of behaviours that preceded attack revealed several interesting patterns (Fig. 3; data shown for $N = 21$ attackers). The singing behaviour of black-capped chickadees (e.g. song rate, overlapping, pitch shifts, frequency-matching and passes) showed different patterns during the trials. The number of gargle calls (Fig. 3b) and passes over the taxidermic mount (Fig. 3f) showed a peak only in the final minutes before attack. Song rate (Fig. 3a) and overlaps (Fig. 3c) increased slowly in the minutes preceding attack. Number of pitch shifts and frequency matches were higher 7–9 min before attack (Fig. 3 d, e).

Dominance Rank and Playback Response

The propensity for males to attack the taxidermic mount was not predicted by rank (logistic regression: $\chi^2_1 = 0.15$, $N = 13$ males whose dominance status was known, $P = 0.70$; Fig. 4). We compared the subjects' behaviour throughout the playback trials to their dominance rank score. Males with higher dominance status overlapped the playback more often (Spearman rank correlation: $r_S = 0.64$, $N = 13$, $P = 0.018$; Fig. 5). This trend, however, did not remain significant following correction for multiple comparisons (i.e. $\alpha = 0.0085$). The remaining five behaviours showed no relationship with dominance rank (all $r_S < 0.38$, $N = 13$, all Ps > 0.19).

DISCUSSION

Male black-capped chickadees showed strong territorial responses to loop playback paired with a taxidermic mount. Males sang and approached the playback area and, in 55% of the examined trials, they physically attacked the taxidermic mount. The gargle call, a nonsong vocalization, was a perfect predictor of attack; all birds that attacked the taxidermic mount produced gargle calls in the minute before attack, and nonattackers never produced gargle calls. Song rate in the time period preceding the minute-before-attack was also a significant predictor of attack; song rate was higher for attackers than nonattackers. Interestingly, neither gargle calls nor song rate were associated with dominance rank. Only one of the measured behaviours, song overlapping, showed a relationship with dominance rank, yet this relationship did not remain significant following correction for multiple comparisons. Based on the results of this experiment, gargle calls and song rate fulfil the predictive criterion of being an aggressive signal (sensu Searcy & Beecher 2009).

Overlapping, frequency matching, high song rate and gargle calls have all been documented in aggressive contexts in black-capped chickadees (Ficken et al. 1978; Mennill & Ratcliffe 2004b; Fitzsimmons et al. 2008a) and, therefore, fulfil the context criterion for being aggressive signals (Searcy & Beecher 2009). Overlapping and frequency matching are noted for occurring during diurnal song contests between neighbouring male chickadees (Fitzsimmons et al. 2008a), but did not predict attack in this study. In several bird species, males increase their song rate in response to agonistic playback (e.g. superb fairy-wrens, *Malurus cyaneus*: Cooney & Cockburn 1995; black-capped chickadees: Mennill & Ratcliffe 2004b; stripe-headed sparrows, *Peucaea ruficauda*: Illes & Yunes-Jimenez 2009; indigo buntings, *Passerina cyanea:* Beckett & Ritchison 2010; vermillion flycatchers, *Pyrocephalus rubinus*:

Rivera-Cáceres et al. 2011), although it is not a universal rule (see Yasukawa 1978; Molles & Vehrencamp 1999; Osiejuk et al. 2007). The gargle call is important in close-range interactions during dominance hierarchy establishment and often occurs immediately prior to aggressive supplants in winter flocks (Ficken et al. 1978, 1987; Baker et al. 1991). Gargle calls have also been noted for occurring when breeding males interact at their territory boundaries (Ficken et al. 1987; Baker & Gammon 2007). A recent report provides evidence of a fight between two high-ranking male black-capped chickadees that ended in one fatality; this mortal combat was preceded by a period of gargle calls (Hof & Hazlet, in press), further implicating this vocalization as a highly aggressive signal during aggressive chickadee encounters. Our playback study shows that both song rate and gargle calls are significantly higher during aggressive interactions preceding attack, adding to the evidence that these two behaviours are associated with escalated aggressive interactions.

These two behaviours, therefore, fit Searcy & Beecher's (2009) predictive criterion for aggressive signals, with both high song rate and gargle calls predicting subsequent attack. In our study, as in other studies that have followed a model presentation design, only a subset of the measured behaviours predicted attack (Searcy et al. 2006; Ballentine et al. 2008; Hof & Hazlett 2010; Ręk & Osiejuk 2011). Ballentine et al. (2008) found that soft songs and wing-waving behaviour predicted attack in swamp sparrows, whereas song matching and song type switching did not. Song matching and type switching are analogues of the frequency matching and frequency switching measures we report here, and therefore, our results agree with theirs. Ballentine et al. (2008) and Hof & Hazlett (2010) also tested nonsong vocalizations (wheezes and rasps for swamp sparrows; ctuks and sputters for black-throated blue warblers) but found that soft songs were the only vocalization that predicted attack. Therefore, black-capped chickadees are the first passerine species examined with Searcy et al.'s (2006) experimental approach where a nonsong vocalization is a signal of aggression, rather than a quiet version of male song. Moreover, black-capped chickadees stand apart from these previously studied songbirds because song rate predicted attack in our analyses but not in any other birds examined. Where the four prior studies found similar results with low-amplitude vocalizations fulfilling the predictive criterion for aggressive signals, our results suggest that other behaviours can also satisfy this criterion.

Interestingly, the two behaviours that predicted attack in chickadees did not occur in the same time period. Our evaluation of changes in behaviour over time reveals that song rate is high throughout the playback-induced interactions, while gargle calls occur only in the minute before attack on the taxidermic mount (Fig. 3). We also know from our analyses that song rate only predicts attack in the period preceding the minute before attack, not during the minute before attack, and that gargle calling only predicts attack in the minute before attack, not during the preceding time period. This ordered sequence of behaviours may indicate that chickadees have a graded signalling system, where heightened song rate is an initial signal of aggression, and production of gargle calls is an escalated signal of aggression. A graded signalling system also occurs in song sparrows, involving three different types of signal matching (reviewed in Beecher & Campbell 2005). Receiver perspective and observational studies of chickadees had previously suggested that chickadees use a graded signalling system (Otter et al. 2002; Fitzsimmons et al. 2008a), but these studies suggested that the graded signals were overlapping and then frequency matching. Our analysis of these signals in the framework of the predictive criterion (Searcy & Beecher 2009) do not support the idea that overlapping and matching are aggressive signals, although these behaviours may have other functions in agonistic signalling interactions.

Black-capped chickadees provide an interesting study system, in part because signalling behaviour can be related to male dominance status, a trait known to be a sexually selected target of female choice (Ramsay et al. 2000; Ratcliffe et al. 2007). We were surprised to find that rank was unrelated to a male's propensity to attack the taxidermic mount and his signalling behaviours, particularly since previous studies have revealed relationships between male dominance rank and male territorial singing behaviour (e.g. Ficken et al. 1987; Otter et al. 1997; Mennill et al. 2002, 2003a; Christie et al. 2004; Mennill & Ratcliffe 2004b). In our study, song overlap showed a nonsignificant tendency to be related to the dominance status of the singing male. Therefore, song overlap may signal dominance status, with higher-ranking males overlapping more playback songs than lower-ranking males. Overlapping, as well as frequency matching, also occurs during chickadee vocal interactions (Shackleton & Ratcliffe 1994; Otter et al. 2002; Mennill & Ratcliffe 2004b; Fitzsimmons et al. 2008a) but may have other nonaggressive signalling functions, such as signalling dominance status or directing competition towards a specific rival. Since the sample size was small for this part of our analyses (*N* = 13 males with known dominance status that interacted with the playback-simulated intruder), more research is necessary to examine the relationship between dominance status and singing behaviour.

Gargle calling and song rate fit both the context and predictive criteria for being aggressive signals in chickadees; however, receiver-based studies have only been performed on gargle calls. Baker et al. (1991) used playback to examine gargle calling in the nonbreeding season (i.e. a feeding context rather than a breeding context). They found that responses to gargle calls appeared dependent upon physical proximity of the opponent and familiarity with the gargle call that was played back. The infrequently heard, unfamiliar gargle calls of subordinates made dominant males averse to feeding; the proximity of a dominant male coupled with his familiar call made subordinates more averse to feeding (Baker et al. 1991). These reactions constitute a receiver response and, therefore, gargle calls satisfy

Searcy & Beecher's (2009) three criterion, confirming that they are an aggressive signal. Song rate has not been examined using receiver-based studies, although it is often seen to increase in response to playback in agonistic situations (Mennill et al. 2004). Also, receivers show a greater response to a simulated chick-a-dee call playback when a greater proportion of the simulation is vocalization versus silence (i.e. simulated signallers have higher duty cycle calls; Wilson & Mennill 2011). Further studies on song rate must be performed to confirm whether song rate fits the response criterion of an aggressive signal.

Gargle calling fits Searcy & Beecher's (2009) three criteria of aggressive signals and is a perfect predictor of attack; therefore, it can be called an aggressive signal in black-capped chickadees. Here we also demonstrated that song rate fits the context and predictive criterion of an aggressive signal and it is elevated prior to the minute before attack. These findings contribute to our overall understanding of how animals use signals to communicate aggression and, ultimately, resolve conflict without necessarily resorting to physical confrontation. Among birds, for example, there is now an emergent pattern whereby males use acoustic signals, such as soft song and gargle calls, to communicate their readiness to fight during territorial disputes (see Introduction). Similar patterns exist among other taxonomic groups. For example, many male lizards communicate aggression through visual signals, such as arm waves, pushups and headbobs (Ord et al. 2001; Van Dyk & Evans 2008), while certain male fish communicate aggression by prolonging their opercular displays (Evans 1985). Male anurans communicate aggression through a graded series of acoustic signals (Wagner 1989), and male primates accomplish this through facial expressions and gestures (Setchell & Wickings 2005). Together, these examples show that aggressive signalling has evolved in a wide range of taxonomic groups, probably as a mechanism for avoiding direct physical confrontation and the associated risk of injury or death (Maynard Smith & Price 1973).

Future research on black-capped chickadees should focus on examining song rate from the receiver’s perspective to examine the response criterion and explore the role of gargle calls during the breeding season. These signals may function as a graded signalling system, and this idea merits further investigation. Future studies should also explore the association between these two behaviours in naturally occurring countersinging interactions. Other signals that we examined here, including the number of song overlaps and frequency matching, may function to communicate status or to direct signals towards a particular opponent during agonistic song contests. By exploring chickadee signal functions through further research, we can expand our understanding of signals that occur in aggressive signalling interactions.

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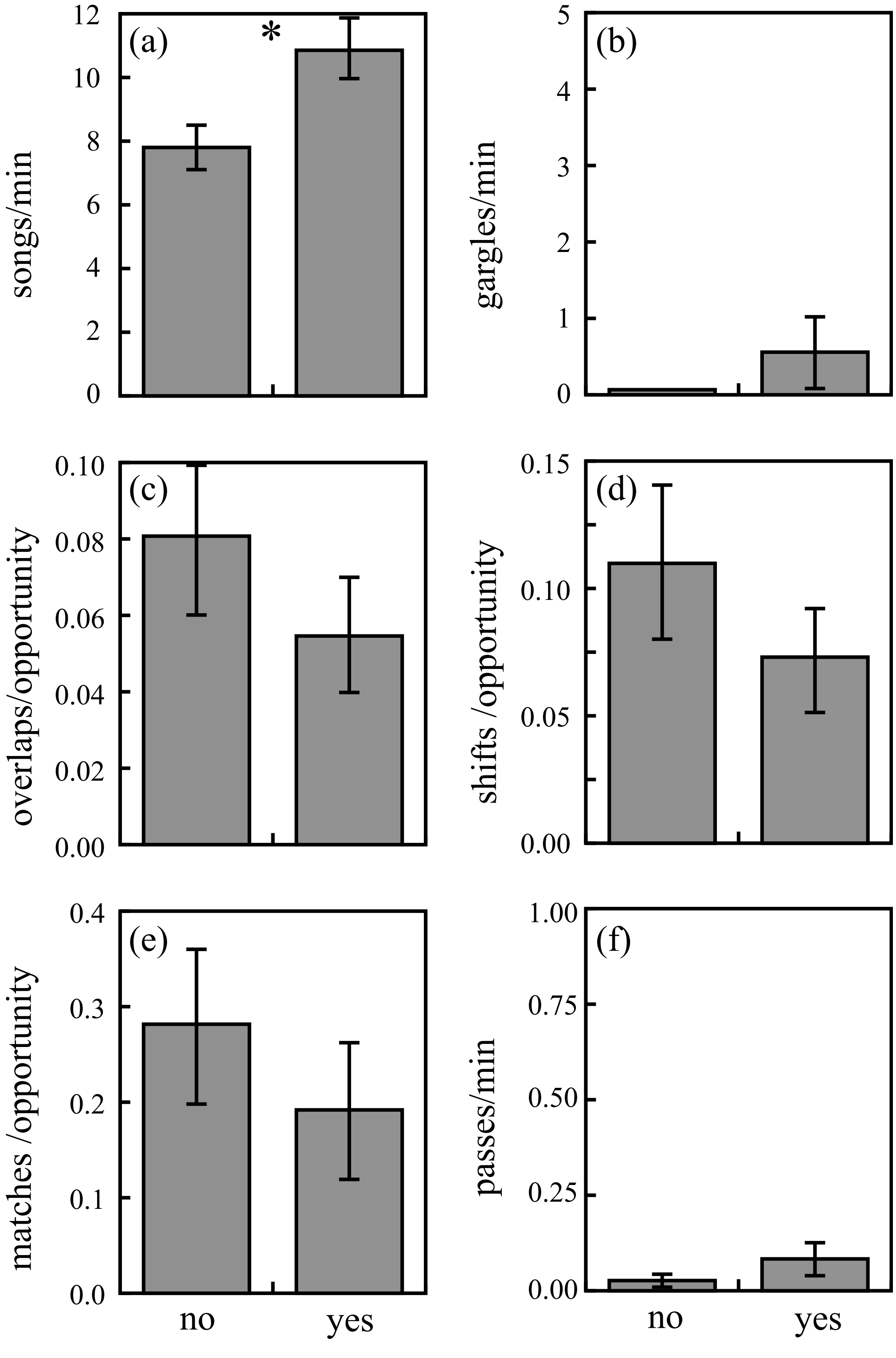
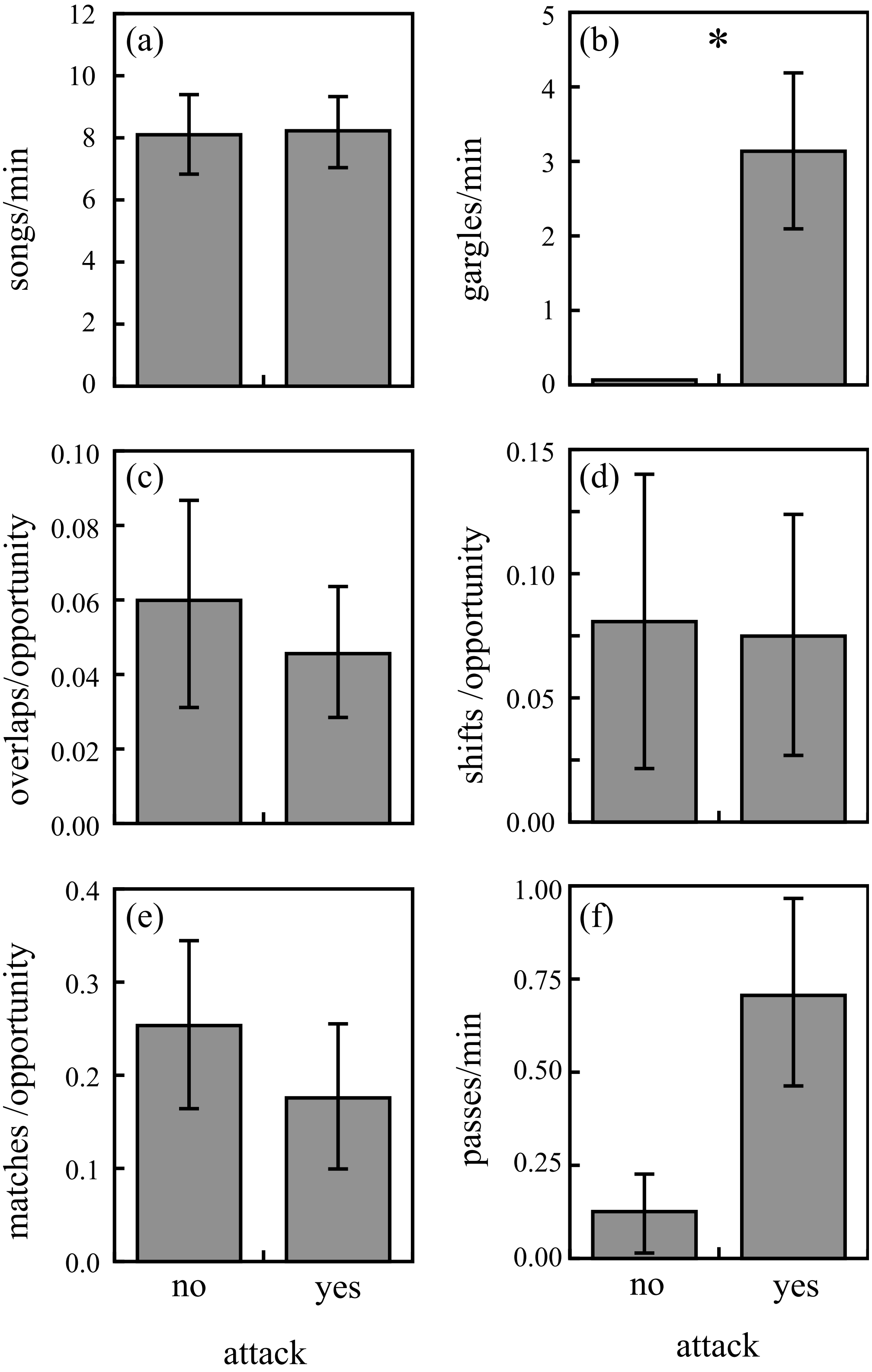
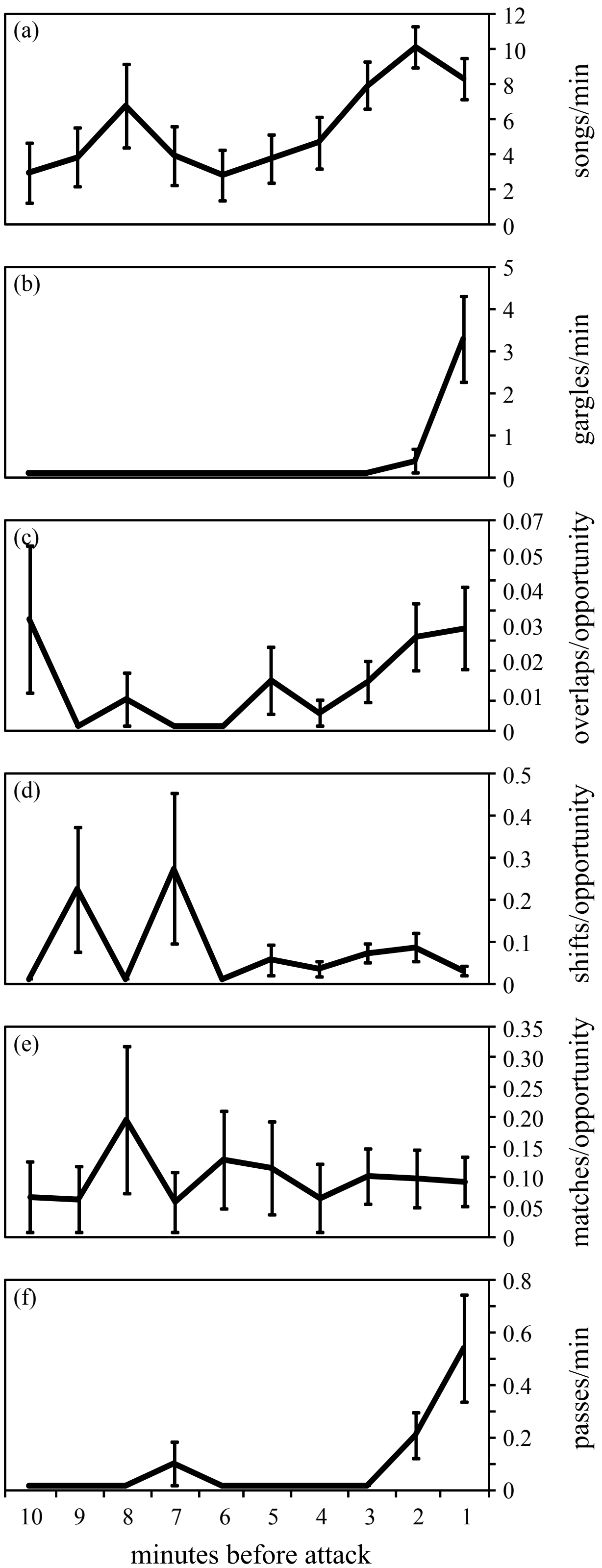

12
10
8
6
4
2
0
songs/min
(a)
*
5
4
3
2
1
0
gargles/min
(b)
0.10
0.08
0.06
0.04
0.02
0.00
overlaps/opportunity
(c)
0.15
0.10
0.05
0.00
shifts /opportunity
(d)
0.4
0.3
0.2
0.1
0.0
matches /opportunity
(e)
1.00
0.75
0.50
0.25
0.00
passes/min
(f)
no
yes
attack
no
yes
attack

Figure 1. Behavioural responses of male black-capped chickadees to playback coupled with a taxidermic mount, comparing birds that attacked the mount (*N* = 21) to birds that did not attack (*N* = 17). Data show the first portion of the playback trial, up to 1 min before birds attacked the taxidermic mount, and a parallel time period for nonattackers. Means ± SE are shown for (a) number of songs per minute, (b) number of gargle calls per minute, (c) number of overlapping songs per opportunity to overlap, (d) number of pitch shifts (≥80 Hz) per opportunity to shift, (e) number of frequency matches (≤50 Hz) per opportunity to match, and (f) number of passes over the taxidermic mount per minute. $*P < 0.05$.



(a)
songs/min
0
2
4
6
8
10
12
(b)
*
gargles/min
0
1
2
3
4
5
(c)
overlaps/opportunity
0.00
0.02
0.04
0.06
0.08
0.10
(d)
shifts /opportunity
0.00
0.05
0.10
0.15
(e)
matches /opportunity
0.0
0.1
0.2
0.3
0.4
(f)
passes/min
0.00
0.25
0.50
0.75
1.00
no
yes
attack
no
yes
attack

Figure 2. Behavioural responses of male black-capped chickadees to playback coupled with a taxidermic mount, comparing birds that attacked the mount (*N* = 21) to birds that did not attack (*N* = 17). Data show the minute before attack, or a parallel minute in nonattackers. Means ± SE are shown for (a) number of songs per minute, (b) number of gargle calls per minute, (c) number of overlapping songs per opportunity to overlap, (d) number of pitch shifts (≥80 Hz) per opportunity to shift, (e) number of frequency matches (≤50 Hz) per opportunity to match, and (f) number of passes over the taxidermic mount per minute. *$P < 0.05$.


(a)
songs/min
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2
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(b)
gargles/min
0
1
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3
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5
(c)
overlaps/opportunity
0
0.01
0.02
0.03
0.04
0.05
0.07
(d)
shifts/opportunity
0
0.1
0.2
0.3
0.4
0.5
(e)
matches/opportunity
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
(f)
passes/min
0
0.2
0.4
0.6
0.8
10
9
8
7
6
5
4
3
2
1
minutes before attack

Figure 3. Six behaviours of male black-capped chickadees in response to playback coupled with a taxidermic mount, shown as a time course for the minutes preceding attack on a taxidermic mount (*N* = 21; values are means ± SE). From top to bottom: (a) number of songs per minute, (b) number of gargle calls per minute, (c) number of overlapping songs per opportunity to overlap, (d) number of pitch shifts (≥80 Hz) per opportunity to shift, (e) number of frequency matches (≤50 Hz) per opportunity to match, and (f) number of passes over the taxidermic mount per minute. This is a descriptive depiction of the sequence of behaviours preceding attack.

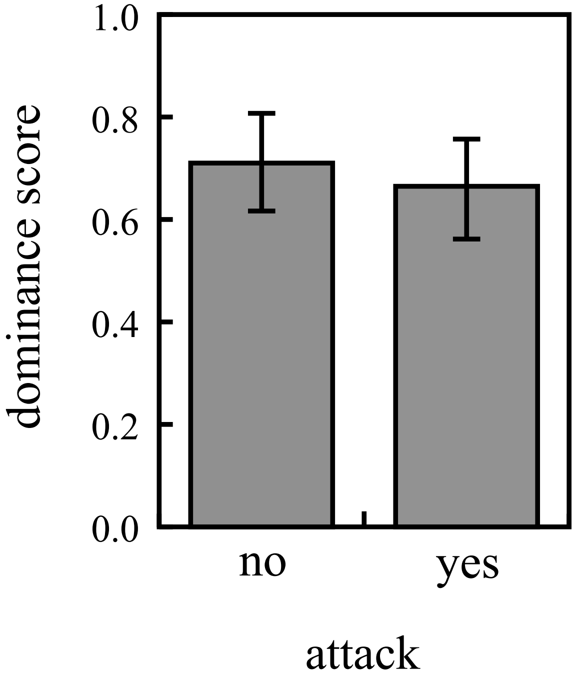

Figure 4. Dominance rank of male black-capped chickadees that attacked or did not attack a taxidermic mount coupled with playback (*N* = 13, see text).

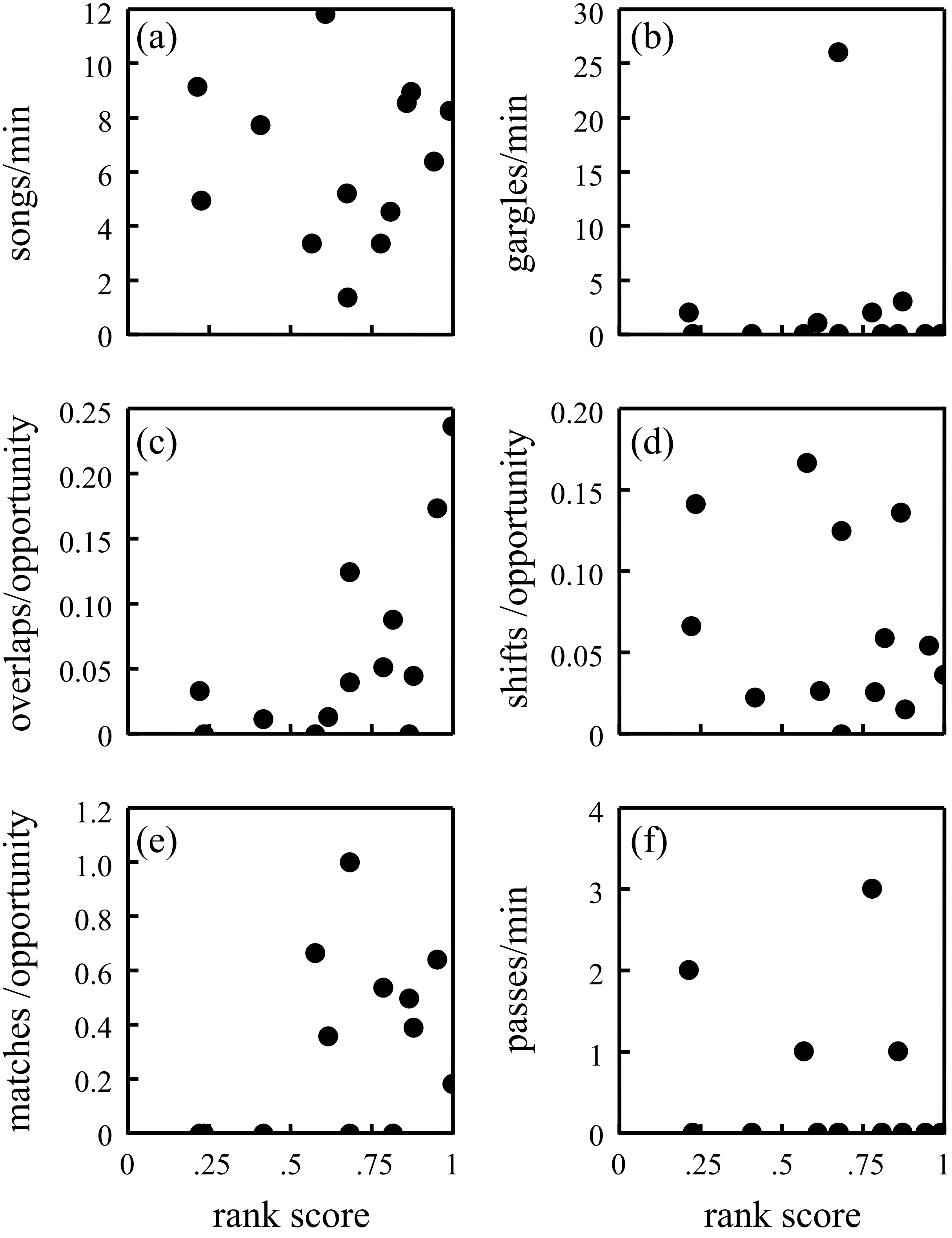
(a)
songs/min
0
2
4
6
8
10
12
(b)
gargles/min
0
5
10
15
20
25
30
(c)
overlaps/opportunity
0
0.05
0.10
0.15
0.20
0.25
(d)
shifts /opportunity
0
0.05
0.10
0.15
0.20
(e)
matches /opportunity
0
0.2
0.4
0.6
0.8
1.0
1.2
(f)
passes/min
0
1
2
3
4
0
.25
.5
.75
1
rank score
0
.25
.5
.75
1
rank score

Figure 5. Relationships between the dominance rank of male black-capped chickadees and their behaviour during the full playback trial. Comparisons are shown for (a) number of songs per minute, (b) number of gargle calls per minute, (c) number of overlapping songs per opportunity to overlap, (d) number of pitch shifts (≥ 80 Hz) per opportunity to shift, (e) number of frequency matches (≤50 Hz) per opportunity to match, and (f) number of passes over the taxidermic mount per minute.