Aggressiveness, reliable signaling and survival in a wild songbird

Çağlar Akçay[1,2], S. Elizabeth Campbell[1], Michael D. Beecher[1,3]

1. Department of Psychology, University of Washington, Seattle, WA 98195
2. Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061
3. Department of Biology, University of Washington, Seattle, WA 98195

Author for correspondence:

Çağlar Akçay
Department of Biological Sciences
Virginia Tech
Blacksburg, VA 24061
caglar@vt.edu

Keywords: honest signaling, aggression, territoriality, personality, song sparrows, survival, soft songs

Running title: *aggression, honesty and survival*

Access to data is available through the supplementary materials.

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1195v2 | CC-BY 4.0 Open Access | rec: 7 Sep 2015, publ: 7 Sep 2015

Abstract

The evolution and maintenance of honest or reliable signaling has been a major question in evolutionary biology. The question is especially puzzling for a particular class of signals used in aggressive interactions: threat signals. Here we report a study on song sparrows (*Melospiza melodia*) in which we assayed males with playbacks in their territories to quantify their aggressiveness (flights, and close proximity) and aggressive signaling levels (rates of soft song, a close range signal reliably predicting attack) and asked whether these traits affect individuals' survival on territory. We found that the effect of aggressive signaling via soft song interacted with aggressive behaviors such that there was a negative correlational selection: among males with low aggression, those males that signaled at higher levels (over-signalers) had higher survival whereas among males with high aggression those that signaled at low levels (under-signalers) survived longer. In other words, males who deviate from reliable signaling have a survival advantage. These results, along with previous research that suggested most of the deviation from reliable signaling in this system is in the form of under-signaling (high aggression males signaling at low levels) pose a puzzle for future research on how this reliable signaling system is maintained.

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1195v2 | CC-BY 4.0 Open Access | rec: 7 Sep 2015, publ: 7 Sep 2015

Introduction

In signaling interactions between two unrelated individuals, the interests of signalers and receivers rarely coincide perfectly, which poses the question of how animal signals can evolve to be reliable (Maynard Smith and Harper 2003; Searcy and Nowicki 2005). This question is particularly prominent in the case of threat signals used in aggressive situations, when the interests of signalers and receivers are opposed to each other. Some threat signals reveal traits like physical size or strength of the signaler in a way that cannot be cheated (e.g. parallel walk in red deer that emphasizes the body size; Clutton-Brock et al. 1979) but many threat signals are not intrinsically tied to an easily observable and costly trait. These signals, termed "conventional signals" because of the arbitrary relationship between the signal structure and signal message (Guilford and Dawkins 1995), are seemingly easy to cheat and therefore their reliability represents an empirical and theoretical puzzle (Maynard Smith and Harper 2003). Although conventional signals were initially thought to be unreliable and non-predictive in contests over all but trivial resources (Maynard Smith 1974; Dawkins and Krebs 1978; Caryl 1979; Maynard Smith 1979; Maynard Smith et al. 1988), recent research has shown many such signals to be at least partially reliable in predicting further escalation in recent research (Waas 1991; Searcy et al. 2006; Laidre 2009; Akçay et al. 2013).

Although conventional signals can be statistically reliable in predicting an escalation, the reliability is often imperfect (Searcy et al. 2013). Furthermore, signaling strategies of at least some species appear to be individually consistent over time, i.e., some individuals signaling consistently at high levels and others consistently at low levels, even though conventional signals are generally assumed to be flexible behaviors. For example we recently showed that male song sparrows (*Melospiza melodia*) show individually consistent signaling strategies in repeated aggression assays even after accounting for their aggression levels (Akçay et al. 2014a), e.g., some low-aggression individual consistently signal higher than expected, and some high-aggression individuals lower than expected. These results suggest that at least part of the variation present in imperfectly reliable signals are correlated with consistent individual differences (Botero et al. 2010).

The last decade has seen a proliferation of studies focused on consistent individual differences, sometimes termed animal personality (Gosling 2001; Sih et al. 2004; Bell et al. 2009; Dingemanse and Wolf 2010). These consistent individual differences often affect fitness (Dingemanse et al. 2004; Dingemanse and Réale 2005; Smith and Blumstein 2008; Seyfarth et al. 2012). Aggressiveness in particular has been subject of a number of studies (Smith and Blumstein 2008; Bell et al. 2009). The costs and benefits of aggressive signaling however has only been studied in the short-term by looking at immediate receiver responses (e.g. Templeton et al. 2012; Anderson et al. 2013). To the best of our knowledge no prior study has examined the long-term fitness consequences of individual differences in variation in aggressive signaling together with aggressiveness in a wild animal.

In this paper we report aggression and aggressive signaling measures from a population of male song sparrows that were assayed multiple times over a period of several years. Our previous studies found that both aggressive behaviors and aggressive signaling were individually repeatable over this time period as well as positively correlated with each other (Akçay et al. 2014a). Crucially, when aggressive behavior levels were controlled for residual signaling was also repeatable, suggesting individually consistent signaling strategies (Akçay et al. 2014a). Here we ask whether these deviations from reliability have fitness consequences and whether the selective forces can explain the maintenance of the reliability of aggressive signaling.

Methods

Study site and subjects: We studied male song sparrows breeding in Discovery Park, Seattle, WA. The song sparrow population has been subject of a long term field study since 1986 (Stoddard et al. 1988; Beecher et al. 1994). Each male was banded with a US Fish and Wildlife Service aluminum band and a unique combination of 3 color bands for individual identification in the field. Subjects were randomly selected 69 males that held territories in June 2009 which was a little over half the breeding males in our study area (n=123, Akçay et al. 2014b).

Aggression and aggressive signaling assays: We carried out repeated playbacks to simulate territorial intrusions and assay aggressive behaviors and aggressive signaling. The details of the protocol have been reported by Akçay and colleagues (2014a). Briefly, we placed a speaker (Pignose Inc.) at the territory center connected to an iPod (Apple Inc.) with a 20 m audio cable. We played each subject two of his own songs (self song) that had been recorded earlier using a Marantz PMD 660 digital recorder and a Sennheiser ME66/K6 directional microphone. Each song was played for 5 minutes at a rate of 1 song every 15 seconds for a total 10-minute trial. The amplitude song amplitude was approximately 80 dB SPL measured at 1 m (Radio Shack 33-2055 sound meter). In previous research, song sparrows have shown no behavioral differences in response to stranger song and self song (Searcy et al. 1982; Stoddard et al. 1992).

The playbacks were carried out in September and October 2009 and January, February and May 2010, and we attempted to test each male once in each of these months for a total of five trials. The achieved sample sizes varied due to disappearance of subjects, either temporarily or permanently. Eleven subjects were tested once, 13 subjects were tested twice, 12 subjects 3 times, 19 subjects 4 times, and 14 subjects all 5 times (mean+SD trials per subject: 3.17+1.38, total number of trials: 219).

During each trial two investigators stood at about 20 m from the speaker and observed the subject. One of the observers also recorded the trial using the same recording gear as above. We noted verbally the following behavioral measures by narrating the behaviors as they occurred: flights, distance from the speaker at each flight, loud songs, soft songs, wing waves. Although the amplitude variation in song sparrow songs is continuous, an experienced observer can reliably classify loud songs and soft songs in the field (Anderson et al. 2008). The trial recordings were later viewed and annotated using Syrinx (John Burt, Seattle, WA; www.syrinxpc.com).

Behavioral measures: We extracted the following behaviors as our measure of aggression from each trial: rate of flights (per minute), proportion of time spent within 5m of the speaker, and closest approach to the speaker during the trial. These three measures were highly correlated with each other and therefore entered into a principal component analysis (PCA). The first component of the PCA explained 67.9% of the variance and was taken as the aggression score. We found found that the average aggression scores from these trials in 2009-2010 predicted whether the birds would attack a taxidermic mount in Spring 2011(Akçay et al. 2014a). Additionally, a similar PCA score in another study with a taxidermic mount was able to predict attackers and non-attackers with 92% accuracy, whereas individual variables that went into the PCA were able to predict attacks and non-attacks 77 to 81% of the time. (Akçay et al. 2013). Therefore the PCA aggression scores capture most of the important variation in aggressive behavior.

We use the definition of a signal proposed by Otte (1974): “behavioral, physiological, or morphological characteristics fashioned or maintained by natural selection because they convey information to other organisms” (p. 738). This definition explicitly excludes behaviors that may yield information to receivers but are selected primarily for other purposes. The behaviors we are classifying as aggressive fall into this latter category. Although flying at and staying close to a receiver may convey information to him, under this definition they don’t qualify as aggressive signals, since flying towards the receiver and staying close have likely evolved for physically dealing with an intruder rather than because of their signal value. Previous work on song sparrows showed that soft songs (low amplitude songs given at close distance) and wing waves (rapid fluttering of the wings without taking off) are reliable signals on average that predict attack on a taxidermic mount (Searcy et al. 2006; Akçay et al. 2013; Searcy et al. 2014). These behaviors have no intrinsic function in a physical fight, with their only function in the aggressive interactions stemming from the fact that they are selected to be a reliable indicator of (i.e. correlated with) an impending attack. Our definition of the signals are explicitly *a priori* but not arbitrary as it draws upon extensive observational and experimental work on the natural history of this species (Nice 1943; Arcese et al. 2003 ; Akçay et al. 2013; Searcy et al. 2014). It is also worth noting that because we are interested in studying the evolution and maintenance of signal reliability, we need to define signals and the non-signaling behaviors they predict in an *a priori* manner, independent of the correlation between them. Nevertheless, we also demonstrate that the putative signaling behaviors and aggressive behaviors correspond to separate behavioral modules using a confirmatory factor analysis (CFA), as suggested by Araya-Ajoy and Dingemanse (2014). We ran two separate CFA models using the package “sem” in R (Fox et al. 2013), one with a single latent variable explaining variation in five behavioral measures (the three aggressive behaviors and two signaling behaviors), and another with two latent variables one explaining variation in the aggressive behaviors and the other explaining variation in the signaling behaviors. The second model had a lower AIC value (ΔAIC of the single latent variable model: 9.55) indicating significantly higher support for two behavioral modules as opposed to one behavioral module.

We extracted the number of soft songs and wing waves and converted these to rates per minute to account for unequal durations of observation due to different latencies of first response. Note that although we also counted loud songs, loud songs in song sparrows consistently have been shown not to predict attack nor to correlate with the signals that do predict attack, soft songs and wing waves (Searcy and Beecher 2009; Akçay et al. 2013; Searcy et al. 2014). Therefore loud songs were not included as an aggressive signal. The same goes for type matching (replying to the playback with the same song type), which has been shown to predict attack early in a sequence of escalation in this population (Akçay et al. 2013) but not when playbacks are only done at the territory center (Akçay et al. 2011; Searcy et al. 2014). We focus on soft songs as the representative signaling behavior in the analyses below as it is the most reliable close-range signal in this and several other songbird species (Akçay et al. 2015), and is highly correlated with wing waves.

Survival: We censused the study area at least once every two weeks in between January 2010 and February 2015 except in November and December of each year to determine whether subjects were still alive on territory. Survival on territory is a major component of male fitness (Smith 1988), as territory ownership is a prerequisite to both being paired with a female and having any extra-pair copulations (Sardell et al. 2010; Hill et al. 2011). Each territory was searched extensively, using playback as necessary. In cases, when a new male was detected, we continued to check the territory as well as the neighboring territories for the former owner. We considered a subject to have disappeared permanently if the subject was not seen on his territory or any of the nearby territories (within two territories of the original one) and the area was being actively defended by a new male. This operational measure of survival is based on 25 years of continuous study of our study population, validated by studies in other populations (Smith 1988; Arcese 1989; Hughes and Hyman 2013). Two of the subjects held territories that were adjacent to an army base where we could not engage in extensive searching and so were excluded from the analyses, as we could not be sure whether they had disappeared or simply made a short move (short moves are not uncommon). In the analyses below we consider years survived past 2009 as our response variable. Of our 67 subjects, 7 did not survive past 2009 (were gone in January 2010), 34 survived only to 2010, 10 survived to 2011, 4 survived to 2012, 7 survived to 2013 and 5 survived to 2014. All of the original subjects had disappeared by January 2015.

Data Analysis: The repeatabilities for aggression scores (r= 0.48, 95% CI: 0.35, 0.62; p< 0.0001, n=219 trials, 69 subjects), soft song rates (r=0.31, 95% CI: 0.18, 0.46, p< 0.0001, n=219 trials, 69 subjects) and wing wave rates (r=0.50, 95% CI: 0.36, 0.63, p < 0.0001, n= 219 trials, 69 subjects) were all highly significant (Akçay et al. 2014a). Here we focus on average aggression scores and average soft song rates across all trials for a given subject.

Our main analysis was the phenotypic selection analysis proposed by Lande and Arnold (1983). The phenotypic selection analysis yields selection gradients that are readily comparable across different studies and different analyses and is therefore a valuable way of quantifying selection. We standardized the aggression scores and soft song and wing wave rates (taking their z-scores), and took the relative fitness of individuals by dividing the number of years a bird survived by the average number of years survived for the entire sample. We then ran a linear regression model on the relative fitness scores with the predictor variables standardized aggression scores, standardized soft song rates, the quadratic terms of the two variables and the cross-products. These correspond to directional, non-linear (stabilizing or disruptive) and correlational selection. We report the selection gradients, which are equal to the regression coefficients in the Lande-Arnold regression, except for the quadratic terms for which the coefficients and their standard errors are doubled to get the selection gradients (Stinchcombe et al. 2008). We also drew a selection surface using the coefficients from the full model to visualize the nature of selection (Sinervo and Svensson 2002). All analyses were carried out in R (R Core Team 2012). In the supplementary materials we report additional analyses on the effect of age that show that age does not interact with either aggression scores or soft song in determining survival.

Results

In the phenotypic selection analysis with aggression scores and soft songs, two selection coefficients were significant: the quadratic term for soft songs with a negative coefficient indicating stabilizing selection and the interaction term again with a negative coefficient, indicating negative correlational selection (Table 1). The selection surface (Figure 1) indicates that selection favors males with high aggression scores and low rates of soft song (under-signalers) and males with low aggression scores and high rates of soft song (over-signalers). The stabilizing selection is harder to discern from this angle but see Figure S1 in supplementary materials. A similar pattern of results held in the subset of subjects with age data (supplementary materials). Looking at the survival (in years) of subjects as a function of aggression scores and soft song rates (Figure 2) one sees that males that are closer to the back of the 3-D plot (high aggression/low signaling) tend to have higher survival, i.e. under-signalers are favored. Note also that there are few if any males on the closer corner of the plot (low aggression/high signaling, or over-signalers)

Discussion

In this study we examined the effects of aggression and aggressive signaling on an important component of fitness, survival on territory. We found evidence of stabilizing selection on soft songs on the one hand and a negative correlational selection on the other. The latter finding suggests that males that deviate from reliable signaling have a survival advantage and the correlation between the signal and the aggressive behaviors (i.e. reliability) will tend to decrease over time in the absence of a counteracting selection effect (assuming heritability of these behaviors).

Correlational selection, behavioral syndromes and evolution of reliability

Our main questions in this study were (1) what are the fitness consequences of variation in aggressive signaling and aggression and (2) does selection explain the evolution and maintenance of reliability? In recent years, the evolution of behavioral syndromes (correlations between suites of individually consistent behaviors, sometimes in different contexts (Sih et al. 2004)) has attracted a great deal of research effort. The evolution of reliability in individually consistent signaling behaviors can be viewed as an analogous problem in which individually consistent levels of signaling and aggressive behaviors are correlated with each other, although in this case, the behaviors are given in the same context (aggression). Behavioral traits can become correlated with each other to yield behavioral syndromes either through pleiotropic effects of a genetic, hormonal or developmental pathway or through correlational selection that creates linkage disequilibrium in the genes controlling the traits (Ketterson and Nolan 1999; Dingemanse and Réale 2005; Bell 2007).

In this context the most interesting finding from the perspective of honest signaling is the significant correlational selection between the two signaling behaviors on the one hand and aggressive behaviors on the other. Although we also found evidence of stabilizing selection on soft song, the selection surface indicates that the negative correlational effect may have a more significant influence compared to the stabilizing selection. Interestingly, the direction of this correlational selection is not what would be expected from the hypothesis that correlational selection underlies the evolution of reliability. Multiple previous studies had shown that soft songs reliably predict attack in this and several other species (Searcy et al. 2006; Akçay et al. 2013; Searcy et al. 2014; Akçay et al. 2015), but our results show significant *negative* correlational selection that would tend to disrupt the reliability of soft songs in the long term (assuming a non-zero heritability of signaling and aggression). These results therefore rule out correlational selection as the ultimate cause of the reliability between signaling and aggressive behavior.

The above conclusion is also consistent with a handful of previous studies which considered correlational selection as a possible cause in the emergence of behavioral syndromes but failed to support that hypothesis (Bell and Sih 2007; Adriaenssens and Johnsson 2013; Han and Brooks 2013). These previous studies and our study differ significantly however in that the former have detected no significant correlational selection either way whereas we detected a significant correlational selection, but the direction of the selection is opposite of what one would expect based on the reliability of the system.

Ruling out correlational selection leaves open the question of how signaling reliability is maintained. Previous behavioral studies have suggested a social cost of using soft songs in the form retaliation from the receivers (Anderson et al. 2012; Templeton et al. 2012; Anderson et al. 2013). It's not clear however, how this short-term cost translates to long-term fitness and the present results should sound a cautionary note on extrapolating from short-term costs to long term fitness. Another hypothesis is that variation in threat signals is dependent on a pleiotropic effect of a genetic locus or loci or a hormonal pathway (Sinervo and Svensson 2002; Bell 2007; McGlothlin and Ketterson 2008). It is also possible that the negative correlational selection on survival on territory may be offset by an opposing selective gradient on reproductive success. The positive correlation between signals and aggressive behaviors may also come about through shared developmental pathways.

It is worth noting that the effect of the negative correlational selection on reliability may not be as disruptive as the selection gradients suggest. First we do not yet know the heritability of signaling and aggression in this species although it is likely to be greater than zero (Turkheimer 2000). Second, selection acts on existing variation. Previous research by our group and others have shown that most deviations from reliability is in the form of under-signalers, males that signal at low levels but attack a taxidermic mount with very few instances of actual over-signalers, males that signal at high levels but don't attack (Akçay et al. 2013; Searcy et al. 2013). Specifically, in an experiment in which 31 out 48 subjects attacked the mount, rates of soft songs correctly predicted 88% of non-attacks (i.e. these non-attackers sang soft songs at low levels, and only 12% of non-attackers were over-signalers) but only 55% of attacks (i.e. 45% of attackers also sang soft songs at low levels and thus were under-signalers). (Akçay et al. 2013). Figure 2 also shows a similar pattern in the current dataset (more under-signalers than over-signalers).

Over-signaling has been long recognized as a problem for the evolution and maintenance of reliability, e.g. (Maynard Smith 1979; Rowell et al. 2006). However the effect of under-signalers on the maintenance of reliability has only been considered by a few models (reviewed in (Searcy et al. 2013)). It is possible that under-signalers could co-exist in an otherwise reliable signaling system: a male who attacks without signaling may not be a major problem for reliability as long as males who do signal also end up attacking. In other words, as long as signalers end up attacking at higher than chance levels, receivers will keep paying attention to the signals, even if some opponents do not signal and still attack.

Another question posed by our results is why the under-signalers and over-signalers seem to have a fitness benefit in terms of survival to start with. We provide two hypotheses, one for under-signaling and one for over-signaling. First, an over-signaling low aggression male might be more effective in holding on to his territory without paying the cost of physical fights if they are rare enough that receivers still pay attention to the signals. This is the standard argument that signaling systems can carry a limited amount of “cheaters” and be stable (Johnstone and Grafen 1993; Adams and Mesterton-Gibbons 1995; Rowell et al. 2006). The fact that over-signalers seem to be rare in our population would fit these models.

The fitness benefit to under-signalers may stem from the fact that signaling often exposes the signalers to risk of predation, a common cause of territory loss. Predation risk tends to be especially increased during aggressive encounters (Jakobsson et al. 1995). In our population, Cooper’s hawks (*Accipiter cooperii*) are a major predator of song sparrows, hunting from concealed perches through surprise attacks. Recently we showed that most (although not all) males cease singing (and wing waving) when the presence of a hawk is simulated through playbacks of hawk calls in the midst of a simulated intrusion by a male song sparrow (Akçay et al. in review). This result implies that aggressive signals, even soft song with its low amplitude, may be intercepted by the hawks and increase predation risk. Such risk may be especially pronounced males who respond with high aggression to intruders, as one of our trials in the present experiment illustrated when a subject was almost taken by a Cooper's hawk during a very close approach to the playback speaker in the middle of the trial (we aborted the trial and to our relief, the hawk "missed"). If signaling increases the risk of predation for aggressive males, then under-signalers may benefit from not signaling and avoiding the unwanted attention of eavesdropping predators while at the same time dealing with intruders effectively via high levels of aggression. This hypothesis, along with the hypothesis in the previous paragraph still needs to be tested in the field for this species.

In summary our study indicates that there may be opposing selective forces on reliable signaling just as theory to date has suggested (Maynard Smith and Harper 2003). Given the correlational nature of the current dataset, and the current lack of information on heritability, it is hard to gauge what the response to selection will be in this system but we can rule out current correlational selection as the cause of maintenance of reliability in this honest signaling system. The present study is a first step in understanding the role of individually consistent variation in aggression and aggressive signaling in the evolution and maintenance of reliable communication systems and suggests a multitude of hypotheses and new questions for future research as discussed above. Studies of long-term fitness consequences of variation in signaling phenotypes will be a fruitful avenue for further research.

Acknowledgements

We thank Veronica Reed, Mari Tom and Dylan Holmes for assistance in the field, Erol Akcay for comments on the manuscript and Shannon Pittman for statistical advice. As always, special thanks to Discovery Park for hosting our research. This research was funded by NSF Research Grant IOS-0733991 to MDB and NSF Dissertation Improvement Grant IOS-1009567 to ÇA and was conducted in accordance with the ABS/ASAB Guidelines for the Treatment of Animals in Behavioral Research and Teaching, with approval from the University of Washington IACUC (no. 2207-03) and USFWS bird banding permit (no. 20220).

Literature cited

Adams, E. S., and M. Mesterton-Gibbons. 1995. The cost of threat displays and the stability of deceptive communication. Journal of Theoretical Biology 175:405-421.

Adriaenssens, B., and J. I. Johnsson. 2013. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. Ecology Letters 16:47-55.

Akçay, Ç., R. C. Anderson, S. Nowicki, M. D. Beecher, and W. A. Searcy. 2015. Quiet threats: soft song as an aggressive signal in birds. Animal Behaviour http://dx.doi.org/10.1016/j.anbehav.2015.03.009.

Akçay, Ç., S. E. Campbell, and M. D. Beecher. 2014a. Individual differences affect honest signaling in a songbird. Proceedings of the Royal Society, B 281:20132496.

Akçay, Ç., S. E. Campbell, V. A. Reed, and M. D. Beecher. 2014b. Song sparrows do not learn more songs from aggressive tutors. Animal Behaviour 94:151-159.

Akçay, Ç., A. N. Clay, S. E. Campbell, and M. D. Beecher. in review. The sparrow and the hawk: aggressive signaling under risk of predation. Behavioral Ecology.

Akçay, Ç., M. E. Tom, S. E. Campbell, and M. D. Beecher. 2013. Song type matching is an honest early threat signal in a hierarchical animal communication system. Proceedings of the Royal Society, B 280:20122517.

Akçay, Ç., M. E. Tom, D. Holmes, S. E. Campbell, and M. D. Beecher. 2011. Sing softly and carry a big stick: signals of aggressive intent in song sparrows. Animal Behaviour 82:377- 382.

Anderson, R., A. DuBois, D. Piech, W. Searcy, and S. Nowicki. 2013. Male response to an aggressive visual signal, the wing wave display, in swamp sparrows. Behavioral Ecology and Sociobiology 67:593-600.

Anderson, R. C., W. A. Searcy, M. Hughes, and S. Nowicki. 2012. The receiver-dependent cost of soft song: a signal of aggressive intent in songbirds. Animal Behaviour 83:1443-1448.

Anderson, R. C., W. A. Searcy, S. Peters, and S. Nowicki. 2008. Soft song in song sparrows: Acoustic structure and implications for signal function. Ethology 114:662-676.

Araya-Ajoy, Y. G., and N. J. Dingemanse. 2014. Characterizing behavioural ‘characters’: an evolutionary framework. Proceedings of the Royal Society of London B: Biological Sciences 281:20132645.

Arcese, P. 1989. Territory acquisition and loss in male song sparrows. Animal Behaviour 37:45-55.

Arcese, P., M. K. Sogge, A. B. Marr, and M. Patten. 2003 The Song Sparrow *in* A. Poole, and F. Gill, eds. The Birds of the North America. Philadelphia Academy of Natural Sciences, Philadelphia, PA.

Beecher, M. D., S. E. Campbell, and P. K. Stoddard. 1994. Correlation of song learning and territory establishment strategies in the song sparrow. Proceedings of National Academy of Sciences 91:1450-1454.

Bell, A., S. J. Hankison, and K. L. Laslowski. 2009. The repeatability of behavior: a meta-analysis. Animal Behaviour 77:771-783.

Bell, A. M. 2007. Future directions in behavioural syndromes research.

Bell, A. M., and A. Sih. 2007. Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). Ecology Letters 10:828-834.

Botero, C. A., I. Pen, J. Komdeur, and F. J. Weissing. 2010. The evolution of individual variation in communication strategies. Evolution 64:3123- 3133.

Caryl, P. G. 1979. Communication by agonistic displays: what can game theory contribute to ethology? Behavior 68:136-169.

Clutton-Brock, T. H., S. D. Albon, R. M. Gibson, and F. E. Guinness. 1979. The logical stag: adaptive aspects of fighting in red deer (Cervus elaphus l.). Animal Behavior 27:211-225.

Dawkins, R., and J. R. Krebs. 1978. Animal Signals: information or manipulation? Pp. 282-309 *in* J. R. Krebs, and N. B. Davies, eds. Behavioral Ecology. Blackwell, Oxford.

Dingemanse, N. J., C. Both, P. J. Drent, and J. M. Tinbergen. 2004. Fitness consequences of avian personalities in a fluctuating environment. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 271:847-852.

Dingemanse, N. J., and D. Réale. 2005. Natural selection and animal personality. Behaviour 142:9-10.

Dingemanse, N. J., and M. Wolf. 2010. Recent models for adaptive personality differences: a review. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3947-3958.

Fox, J., Z. Nie, J. Byrnes, M. Culbertson, S. DebRoy, M. Friendly, R. H. Jones, A. Kramer, G. Monette, and M. J. Fox. 2013. Package ‘sem’.

Gosling, S. D. 2001. From mice to men: what can we learn about personality from animal research? Psychological bulletin 127:45- 86.

Guilford, T., and M. S. Dawkins. 1995. What are conventional signals? Animal Behavior 49:1689-1695.

Han, C. S., and R. C. Brooks. 2013. Correlational selection does not explain the evolution of a behavioural syndrome. Journal of Evolutionary Biology 26:2260-2270.

Hill, C. E., Ç. Akçay, S. E. Campbell, and M. D. Beecher. 2011. Extrapair paternity, song and genetic quality in song sparrows. Behavioral Ecology 22:73-81.

Hughes, M., and J. Hyman. 2013. Should I stay or should I go now: late establishment and low site fidelity as alternative territorial behaviors. Ethology.

Jakobsson, S., O. Brick, and C. Kullberg. 1995. Escalated fighting behaviour incurs increased predation risk. Animal Behaviour 49:235-239.

Johnstone, R. F., and A. Grafen. 1993. Dishonesty and the handicap principle. Animal Behaviour 46:759-764.

Ketterson, E. D., and V. Nolan. 1999. Adaptation, exaptation, and constraint: A hormonal perspective. American Naturalist 154:S4-S25.

Laidre, M. E. 2009. How often do animals lie about their intentions? An experimental test. American Naturalist 173:337-346.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution:1210-1226.

Maynard Smith, J. 1974. The theory of games and the evolution of animal conflicts. Journal of Theoretical Biology 47:209- 221.

Maynard Smith, J. 1979. Game theory and the evolution of behaviour. Proceedings of Royal Society of London, B. 205:475- 488.

Maynard Smith, J., and D. Harper. 2003. Animal signals. Oxford University Press, Oxford.

Maynard Smith, J., D. G. C. Harper, and J. F. Y. Brookfield. 1988. The Evolution of Aggression: Can Selection Generate Variability? [and Discussion]. Philosophical Transactions of the Royal Society of London B: Biological Sciences 319:557-570.

McGlothlin, J. W., and E. D. Ketterson. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Philosophical Transactions of Royal Society of London B. 363:1611-1620.

Nice, M. M. 1943. Studies in the life history of the song sparrow II. The behavior of the song sparrow and other passerines. Trans. Linn. Soc. NY 6:1-328.

Otte, D. 1974. Effects and functions in the evolution of signaling systems. Annual Review of Ecology and Systematics 5:385-417.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rowell, Jonathan T., Stephen P. Ellner, and H. K. Reeve. 2006. Why Animals Lie: How Dishonesty and Belief Can Coexist in a Signaling System. Am. Nat. 168:E180-E204.

Sardell, R. J., L. F. Keller, P. Arcese, T. Bucher, and J. M. Reid. 2010. Comprehensive paternity assignment: genotype, spatial location and social status in song sparrows. Mol Ecol 19:4352-4364.

Searcy, W. A., Ç. Akçay, S. Nowicki, and M. D. Beecher. 2014. Aggressive Signaling in Song Sparrows and Other Songbirds. Advances in the Study of Behavior 46:89-125.

Searcy, W. A., R. C. Anderson, B. Ballentine, and S. Nowicki. 2013. Limits to reliability in avian aggressive signals. Behaviour.

Searcy, W. A., R. C. Anderson, and S. Nowicki. 2006. Bird song as a signal of aggressive intent. Behavioral Ecology and Sociobiology 60:234-241.

Searcy, W. A., and M. D. Beecher. 2009. Song as an aggressive signal in songbirds. Animal Behaviour 78:1281-1292.

Searcy, W. A., P. D. McArthur, S. S. Peters, and P. Marler. 1982. Response of Male Song and Swamp Sparrows to Neighbour, Stranger, and Self Songs. Behaviour 77:152-163.

Searcy, W. A., and S. Nowicki. 2005. The evolution of animal communication. Princeton University Press, Princeton

Seyfarth, R. M., J. B. Silk, and D. L. Cheney. 2012. Variation in personality and fitness in wild female baboons. Proc. Natl. Acad. Sci. USA 109:16980-16985.

Sih, A., A. Bell, and J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372- 378.

Sinervo, B., and E. Svensson. 2002. Correlational selection and the evolution of genomic architecture. Heredity 89:329-338.

Smith, B. R., and D. T. Blumstein. 2008. Fitness consequences of personality: a meta-analysis. Behavioral Ecology 19:448-455.

Smith, J. N. M. 1988. Determinants of lifetime reproductive success in the song sparrow *in* T. H. Clutton-Brock, ed. Reproductive Success. University of Chicago Press, Chicago.

Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution 62:2435-2440.

Stoddard, P. K., M. D. Beecher, S. E. Campbell, and C. L. Horning. 1992. Song-type matching in the song sparrow. Canadian Journal of Zoology 70:1440-1444.

Stoddard, P. K., M. D. Beecher, and M. S. Willis. 1988. Response of territorial male song sparrows to song types and variations. Behavioral Ecology and Sociobiology 22:124-130.

Templeton, C. N., Ç. Akçay, S. E. Campbell, and M. D. Beecher. 2012. Soft song is a reliable signal of aggressive intent in song sparrows. Behavioral Ecology and Sociobiology:1-7.

Turkheimer, E. 2000. Three laws of behavior genetics and what they mean. Current Directions in Psychological Science 9:160-164.

Waas, J. R. 1991. Do little blue penguins signal their intentions during aggressive interactions with strangers. Animal Behaviour 41:375-382.

Table 1. Selection gradients from Lande-Arnold phenotypic selection analysis. Note that the coefficients and the standard errors for the quadratic terms are doubled.

variable	**Coefficient ±SE**	***p***
Aggression scores	-0.04 ± 0.16	0.78
Soft song rates	0.13± 0.11	0.23
Aggression scores^2	0.34 ± 0.18	0.15
Soft song rates^2	**-0.70 ± 0.27**	**0.01**
Aggression*soft song rates	**-0.79 ± 0.21**	**0.0003**

Figure 1. Selection surface as a function of soft songs and PCA aggression scores (both as z-scores). The arrows indicate z-scores getting larger. There are two fitness peaks, one for under-signalers (high aggression, low signaling) and another for over-signalers (low aggression, high signaling), with a valley in between them.

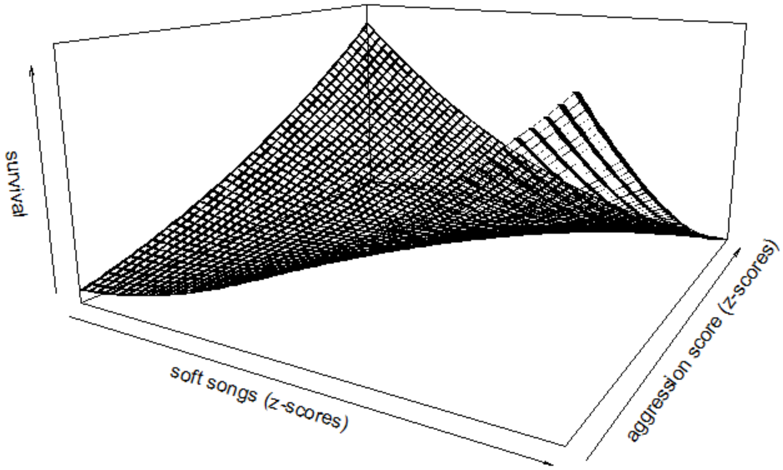
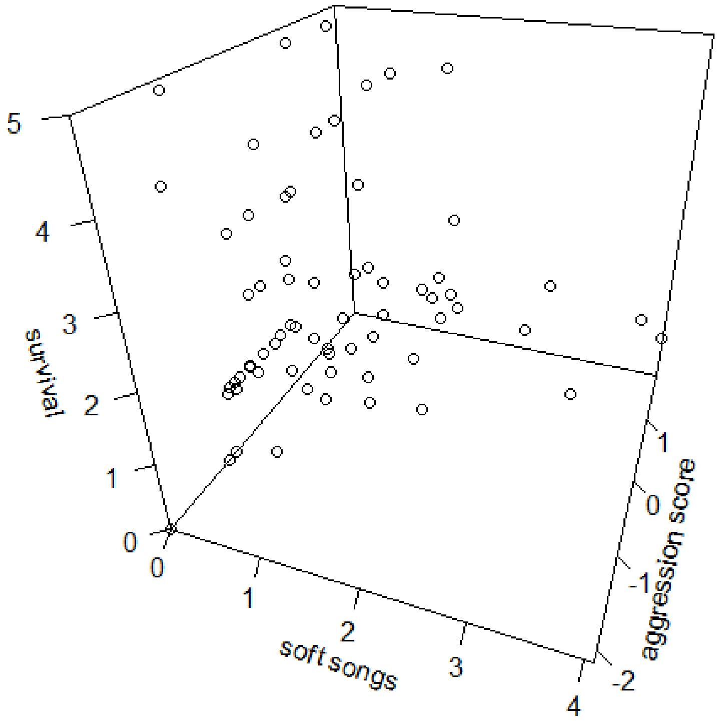


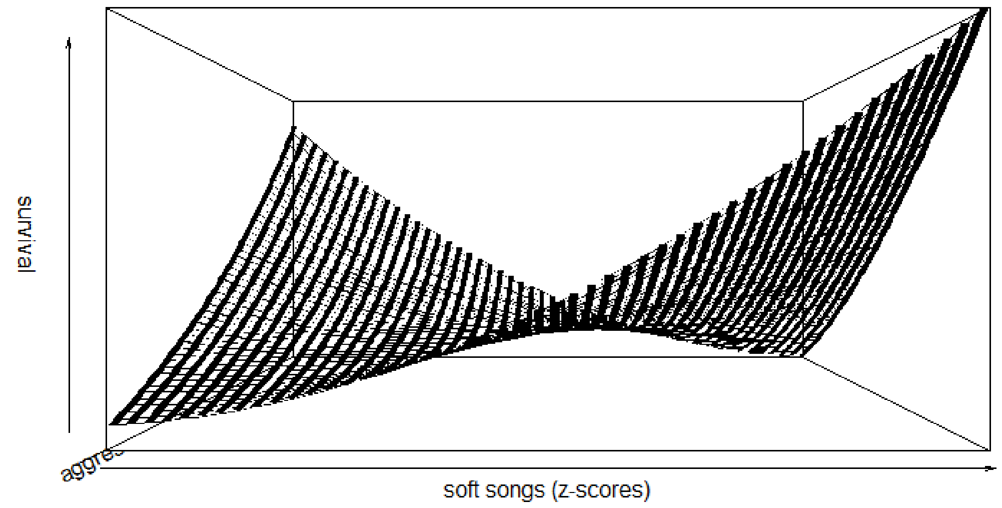
Figure 2. 3-D scatterplot of survival in years as a function of rates of soft songs and aggression scores. Note the lack of over-signalers but the relative abundance of under-signalers (towards the upper back corner of the plot).



Supplementary Material for

Akcay, C. Campbell, S. E., Beecher, M. D.. *Aggression, reliable signaling and survival in a wild songbird* (in review)

Figure S1. Selection surface viewed from a different angle (aggression scores increase towards the back of the plot). The stabilizing selection on soft songs can be seen from this angle as an increase in average fitness towards the middle of the range of soft songs.



Lande-Arnold phenotypic selection analyses on rates of soft songs and aggressive behaviors.

The tables below report coefficients from the summary output from R-code (attached as supplementary material). Note that the quadratic term coefficients and SEs need to be doubled for selection gradients. See main text for the details of the Lande-Arnold phenotypic selection analysis, as well as Lande and Arnold (1983).

Table S1: rates of soft songs (zsoft) and time spent within 5m (ztime5).

```
             Estimate Std. Error t value Pr(>|t|)
zsoft         0.05828    0.15833   0.368  0.71410
ztime5       -0.07348    0.11598  -0.634  0.52872
I(zsoft^2)    0.27887    0.13478   2.069  0.04279 *
I(ztime5^2)   0.01822    0.11983   0.152  0.87966
zsoft:ztime5 -0.61414    0.19957  -3.077  0.00313 **
Residual standard error: 0.7611 on 61 degrees of freedom
Multiple R-squared:  0.1783,   Adjusted R-squared:  0.111
```

```
F-statistic: 2.647 on 5 and 61 DF,  p-value: 0.03131
```

Table S2: rates of soft songs (zsoft) and rate of flights (zflight).

```
              Estimate Std. Error t value Pr(>|t|)
zsoft         -0.05585    0.16742  -0.334   0.7398
zflight        0.07824    0.15412   0.508   0.6135
I(zsoft^2)     0.19924    0.13068   1.525   0.1325
I(zflight^2)   0.01580    0.09493   0.166   0.8684
zsoft:zflight -0.44943    0.17007  -2.643   0.0104 *
Residual standard error: 0.7816 on 61 degrees of freedom
Multiple R-squared:  0.1333,   Adjusted R-squared:  0.06227
F-statistic: 1.877 on 5 and 61 DF,  p-value: 0.1116
```

Table S3: rates of soft songs (zsoft) and closest approach (zclosest).

```
               Estimate Std. Error t value Pr(>|t|)
zsoft           0.27626    0.15272   1.809  0.07539 .
zclosest        0.33676    0.17127   1.966  0.05383 .
I(zsoft^2)      0.07529    0.09117   0.826  0.41211
I(zclosest^2)   0.05559    0.07402   0.751  0.45550
zsoft:zclosest  0.68638    0.22862   3.002  0.00388 **
Residual standard error: 0.7598 on 61 degrees of freedom
Multiple R-squared:  0.1809,   Adjusted R-squared:  0.1138
F-statistic: 2.695 on 5 and 61 DF,  p-value: 0.02892
```

Survival and age: For 33 of the 67 subjects for whom we have survival information, we also have information on the exact age of the male because they were banded in the nest, in their juvenile plumage (before their first September) or singing plastic song during their first fall. In previous analyses, reported in Akçay et al. (2014a), we did not find an effect of age on aggression scores or aggressive signaling scores. To ask whether the effects of the predictor variables on survival could be attributed to age we ran a separate set of analyses with the subset of subjects for whom we had age data by adding the age as a covariate in a Cox regression (Cox 1972) along with the same predictor variables as in the phenotypic selection model. The Cox regression was carried out with the package ‘survival’ in R. (Therneau and Lumley 2014). We then model-averaged the resulting model by taking every model within 2 ΔAIC of the best model. The model averaged results show a significant effect of age (birds that were older in 2009 were less likely to survive longer, which is somewhat trivial) and crucially, a significant interaction of soft songs and aggression scores, indicating negative correlational selection.

Table S4: Model selection table for Cox-Regression analysis. Full model contains the terms Age in 2009, Aggression Scores (linear and quadratic), Soft song rates (linear and quadratic) and Aggression scores*Soft song rates (interaction term). See the R-code for more detail.

Model Number	**Intercept**	**Age in 2009**	**Aggression Scores**	**Soft song rates**	**Soft song rates^2**	**Aggression *Soft Song**	**df**	**logLik**	**AICc**	**delta**	**weight**
2	+	0.2171					1	-82.588	167.3	0	0.277
44	+	0.2894	-0.01214	-0.488		0.4363	4	-79.281	168	0.68	0.197
26	+	0.2463		-0.9587	0.2964		3	-80.914	168.7	1.35	0.141
1	+						0	-84.361	168.7	1.42	0.137
4	+	0.2396	0.1851				2	-82.183	168.8	1.46	0.134
10	+	0.2234		-0.1498			2	-82.337	169.1	1.77	0.114
Models ranked by AICc(x)											

Table S5: Averaged model for the Cox-regression.

Variable	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Age in 2009	**0.2427**	**0.12**	**2.007**	**0.0448**
Aggression Scores	0.0676	0.247	0.273	0.785
Soft song rates	-0.5493	0.47	1.167	0.2432
Aggression*Soft Song	**0.4363**	**0.192**	**2.270**	**0.0232**
Soft song rates^2	0.2964	0.162	1.820	0.0688

References

Cox, D. 1972. Regression Models and Life-Tables. Journal of the Royal Statistical Society. Series B (Methodological) 34:187-220.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution:1210-1226.

Therneau, T. M., and T. Lumley. 2014. Package ‘survival’.

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1195v2 | CC-BY 4.0 Open Access | rec: 7 Sep 2015, publ: 7 Sep 2015