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Males adjust their signalling behaviour according to experience of male signals and male–female signal duets

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Abstract

Sexual signals are conspicuous sources of information about neighbouring competitors, and species in which males and females signal during pair formation provide various sources of public information to which individuals can adjust their behaviour. We performed two experiments with a duetting vibrational insect, *Enchenopa binotata* treehoppers (Hemiptera: Membracidae), to ask whether males adjust their signalling behaviour according to (1a) their own experience of competitors' signals, (1b) how females adjust their mate preferences on the basis of their experience of male signals (described in prior work), and/or (2) their own experience of female response signals to competitors' signals. We presented males with synthetic male signals of different frequencies and combinations thereof for 2 weeks. We recorded males a day after their last signal exposure, finding that (1a) male signal rate increased in response to experience of attractive competitors, but that (1b) male signal frequency did not shift in a manner consistent with how females adjust their mate preferences in those experience treatments. Second, we presented males with different male–female duets for 2 weeks, finding that (2) male signal length increased from experience of female duets with attractive competitors. Males thus make two types of adjustment according to two sources of public information: one provided by experience of male signals and another by experience of female responses to male signals. Signalling plasticity can generate feedback loops between the adjustments that males and females make, and we discuss the potential consequences of such feedback loops for the evolution of communication systems.

Introduction

A distinctive feature of sexual and social selection is the occurrence of feedback loops between the causes and targets of selection. These feedbacks arise because, in competition with conspecifics for mates and other resources, the behaviour of conspecifics constitutes the selective environment (Darwin, 1871; West-Eberhard, 1983, 2014; Andersson, 1994; Wolf *et al.*, 1999; Lyon & Montgomerie, 2012; Prum, 2012; Tobias *et al.*, 2012). As a result, changes in behaviours that aid individuals in competition also alter the selective environment, which in turn favours further changes in behaviours that subsequently alter the selective environment, and so on (West-Eberhard, 1983, 2014). Feedback not only occurs on evolutionary timescales, but also during interactions between individuals, because behaviours are highly plastic and adjustable to social settings (Danchin *et al.*, 2004; Foster, 2013; Zuk *et al.*, 2014). This interplay between individuals is likely to have important consequences: many aspects of the social environment are easily detected (e.g. conspicuous mate-attraction displays) and compensated for with real-time responses, and these responses are a main determinant of individual fitness (Darwin, 1871; West-Eberhard, 1983, 2014; Andersson, 1994; Kokko *et al.*, 2006; Taborsky & Oliveira, 2012). Indeed, feedback

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between the causes and targets of selection increases the rate of evolution (Darwin, 1871; West-Eberhard, 1983, 2014; Andersson, 1994; Moore *et al.*, 1997; Wolf, 2003), helps maintain selectable variation (Harris *et al.*, 2008; Wolf *et al.*, 2008), and promotes the evolution of extravagant traits such as showy displays and extreme altruism (Darwin, 1871; West-Eberhard, 1983, 2014; Andersson, 1994; Wolf *et al.*, 1999).

With the great variety of interactions that take place in social environments, individuals may be selected to adjust their behaviour according to different features of their surroundings, or according to what those features convey about the selective context. In competition for mates, for example, a male may adjust his behaviour according to his perception of the density and types of competitors that are present (Bretman *et al.*, 2011). Additionally, females too may adjust their mate choice decisions on the basis of their social surroundings and ecological conditions (Lesna & Sabelis, 1999; Pfennig, 2007; Chaine & Lyon, 2008; Hebets & Sullivan-Beckers, 2010; Verzijden *et al.*, 2012; Rodríguez *et al.*, 2013), and males may in turn be selected to adjust their behaviour according to those female adjustments (Kahn *et al.*, 2013). In other words, by eavesdropping on the signals of competitors, a male may garner information about both sides of the mate choice equation – who he is up against, and what females do in the presence of such competitors. Further, female behaviour often offers direct indications of their likely decisions, ranging from subtle cues present in their posture to overt signals used to interact with males along the reproductive process, and males may adjust their behaviour on the basis of such cues and signals (Rodríguez & Barbosa, 2014; Rodríguez, 2015).

In other words, social environments present multiple avenues for animals to monitor public information, allowing them to adjust their behaviour to sexual and social competition. Consequently, understanding sexual and social selection requires understanding the back-and-forth that occurs between the behaviours of different individuals in social environments.

Here, we ask three questions that analyse whether adjustment in male signalling behaviour follows public information derived from the signals of male competitors (which is informative about competitors and about the mate choice behaviour of females with experience of competitors) and/or from the signals of females interacting with competitors (which are informative about the expression of the females' mate preferences).

Some of the clearest examples of the potential for public information on both sides of mate choice interactions occur when males and females use overt signals during pair formation. In many insects and spiders, for example, males and females engage in signal exchanges (duets) that facilitate pair formation (Bailey, 2003; Cocroft & Rodríguez, 2005; Sullivan-Beckers & Hebets, 2011), and female mate preferences are expressed as selective duetting with males (Bailey, 2003; Cocroft & Rodríguez, 2005; Rodríguez *et al.*, 2012).

We worked with a duetting insect, a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Enchenopa* are plant-feeding insects that communicate with plant-borne vibrational signals. Males produce advertisement signals, and if a female finds the signal attractive, she responds with her own signal. The established duet continues and prompts the male to search locally until he locates the female and copulation begins (Rodríguez *et al.*, 2004; Rodríguez & Cocroft, 2006; Cocroft *et al.*, 2008). Thus, female responses to male signals increase the likelihood of mating with a given male. *Enchenopa* females have strong mate preferences according to variation in the features of those signals, particularly frequency and length (Rodríguez *et al.*, 2004, 2006), with the former being the most divergent adult trait among members of the complex (Cocroft *et al.*, 2010). Male mating signals are thus a strong determinant of mating success in *Enchenopa* (Sullivan-Beckers & Cocroft, 2010), and evolve under strong sexual selection due to mate choice. Plasticity in male signalling behaviour is thus likely to have important consequences for male reproductive success and subsequent evolution.

We experimentally manipulated *Enchenopa* males' experience of social signalling environments over a span of 2 weeks beginning at adult eclosion (the extent of their adult life before they begin to search and compete for mates) to ask the following questions:

(1a) Do males adjust their signalling behaviour according to their experience of their competitors in the social environment? There is broad evidence that males adjust their signalling according to immediate risk or intensity of competition (e.g. Callander *et al.*, 2013; Höbel, 2015) as well as to longer term mean risk or intensity perceived by social signalling environments (e.g. Bailey *et al.*, 2010; Kasumovic *et al.*, 2011; Bertram *et al.*, 2013; Rebar *et al.*, 2016). Asking this question for *Enchenopa* offers a background for the following questions. In principle, any signal trait might be subject to adjustment in this context, but a common finding in species that use acoustic signals is that males increase their signalling rates in the presence of competitors (Gerhardt & Huber, 2002; Bertram *et al.*, 2013; Callander *et al.*, 2013). We extend this finding to a vibrational insect. We predicted that males would increase their signal rates when given the experience of competitors, especially with experience of attractive rather than unattractive competitors.

(1b) Do males adjust their signalling behaviour according to adjustments that females make on the basis of their (females') perception of the social signalling environment? There is broad evidence that

females adjust various aspects of their mate preferences and mate choice behaviour according to their experience of the abundance and types of mating partners present in the social environment (e.g. Hebets, 2003; Hebets & Sullivan-Beckers, 2010; Bretman *et al.*, 2011; Bailey & Zuk, 2012; Rodríguez *et al.*, 2013). Thus, social signalling environments may offer males not only public information about their competitors, but also indirect information about the adjustments that females make on the basis of male signals in the environment. Prior work has shown that *Enchenopa* females become more selective (narrowing and strengthening their preferences for the population mean signal frequency) when they have had prior experience of that preferred signal, especially if those signals are mixed with nonpreferred signals (Fowler-Finn & Rodríguez, 2012a,b). We therefore ask whether males shift their signal frequency closer to the preferred value when given experience of competitors with signals is closer to that preferred value. Male treehoppers produce signals through thoracic muscle contractions, and individuals generate different signal types of varying frequencies depending on the context (Cocroft & Rodríguez, 2005). Thus, males could adjust the frequency of their advertisement signals, but whether they are selected to adjust to such predictable female adjustments remains unknown.

(2) Do males adjust their signalling behaviour according to their perception of female duetting with other males? Female duetting signals offer direct public information about expressed female mate preferences. There is evidence that courting males that attend to the feedback present in female behaviour benefit by increasing their reproductive success (Patricelli *et al.*, 2002, 2006; Peretti *et al.*, 2006). In *Enchenopa*, there is evidence that males attend to female cues present in their duetting signals and modify their signalling behaviour accordingly, but whether they benefit from it is unknown (Rodríguez *et al.*, 2012). Here, we ask whether males eavesdrop on this feedback from females as it is being provided to other males. The basic expectation might be for males to shift their signal frequency closer to the preferred value when given experience of females responding to competitors with signals is closer to that value, but in principle, any change that might make signals more attractive might be expected.

We asked the above questions with two experiments in which we manipulated *Enchenopa* males' experience of the social signalling environment. We used synthetic playbacks of male signals and male–female signal duets as experimental treatments, and tested for the effects of those experience treatments on male signalling behaviour. In the first experiment, we asked questions (1a) and (1b) by presenting males with treatments consisting of male signals having different signal frequencies and combinations of signal frequencies. In the second experiment, we asked question (2) by presenting males with treatments consisting of different male–female duetting interactions, wherein female responses were given to male signals having different signal frequencies and combinations of signal frequencies.

Materials and methods

Study species

There are two members of the *Enchenopa binotata* complex that live on the host plant *Viburnum lentago* (Caprifoliaceae) at our field site (Tendick Nature Park, Saukville, WI, USA). Aggregations of nymphs and adults of these two species can be found on the same plant, but they are also found on transects that are principally one species or the other. While many of the species of this complex have not yet been formally described (Hamilton & Cocroft, 2009), male signal frequency is the most divergent adult trait among species, ranging from 100 Hz to 500 Hz, and can thus be reliably used to identify each species (Rodríguez *et al.*, 2004; Hamilton & Cocroft, 2009; Cocroft *et al.*, 2010). The two species found on *V. lentago* at our field site differ in signal frequency by over 100 Hz, and we used the low-signal-frequency species (dominant frequency = 180 Hz). We also kept voucher specimens in 95% EtOH.

General methods

We obtained treehopper individuals by randomly cutting stems containing egg masses from various host plants along a 100-m transect known to be predominantly the low-signal-frequency species at Tendick Nature Park in February 2011 for the first experiment and in February 2013 for the second experiment. We placed each stem in a water tube in a greenhouse set at 25 °C under a 14:10 light to dark cycle in order to promote leaf budding and, thus, egg eclosion (Cocroft *et al.*, 2008). When nymphs emerged 2 weeks later, we transferred them onto several potted plant individuals at approximately the same density in the greenhouse, and reared them to adulthood.

Upon the adult moult, we transferred each male to his own potted plant. We readied these plants by potting bare root plants (~0.3 m tall) in one-gallon plastic pots with Fafard 3B potting soil mix (Conrad Fafard, Inc., Agawam, MA, USA), and brought them into the greenhouse to promote the onset of budding, matching it to the development timeline of the treehoppers. By keeping adult males singly on individual plants, we restricted their experience of signals to our experimental treatments.

We performed two experiments, each followed by recording of male signals. In the first experiment, we

manipulated the experience of variation in male signals. In the second experiment, we manipulated the experience of the responses of females to male signals.

We imparted vibrational signals onto the plants by broadcasting stimuli through a loudspeaker (Rokit 8; KRK Systems, Deerfield Beach, FL, USA) suspended above the plants in an anechoic chamber. This technique allowed us to stimulate multiple plants at the same time without contacting the substrate (Rebar *et al.*, 2012). We validated this method by showing that male and female *Enchenopa* treehoppers respond similarly to these broadcast stimuli as compared to traditional contact-based methods (Rebar *et al.*, 2012). The loudspeaker was connected to a computer (Pavilion dm4; Hewlett-Packard, Palo Alto, CA, USA) through an Edirol UA-25 USB interface (Roland Corp., Los Angeles, CA, USA). We first calibrated the amplitude of all stimuli presented in the signalling treatments each day using one plant individual that was different from any of the treatment plants. We monitored the playback stimuli with a laser vibrometer (CLV-2534; Polytec, Inc., Auburn, MA, USA). We focused the laser beam onto a small piece of reflective tape (~2 mm^2) placed on the plant stem. The laser vibrometer signal was sent through a band-pass filter (40–4000 Hz; Krohn-Hite 3202; Krohn-Hite Corporation, Brockton, MA, USA) at 60 Hz. We monitored the stimuli with a 50-MHz oscilloscope (Hameg Instruments, Mainhausen, Germany, model HM 504-2), adjusting the amplitude for all stimuli to 0.10 mm s^{-1}. We then placed our experimental plants in a circle on the floor below the loudspeaker in an anechoic chamber at 25 °C (Fig. 1a), during which time we presented those individuals to their respective treatment. Plants were equidistant from the loudspeaker to ensure that they received similar stimulation. We created and delivered all synthetic stimuli using a custom MATLAB script (R2010b; Mathworks, Inc., Natick, MA, USA).

Experiment 1: variation in experience of male signals

We randomly assigned 160 males to one of eight signalling environment treatments ($N = 20$ per treatment) that varied only in the frequency (Hz) of the male signal stimuli presented. Signalling environment treatments included the species mean (180 Hz; preferred by females; mean treatment in Table 1) to near the end of the conspecific range (160 and 200 Hz; Con Low and Con High treatments in Table 1, respectively) to beyond that range (80 Hz and 280 Hz; Het Low and Het High treatments in Table 1, respectively), corresponding to potential heterospecific signals. In addition, we included mixes of different conspecific and heterospecific signal frequencies (Con Mixed and Het Mixed treatments in Table 1, respectively); we also used a Silent treatment (Table 1). We randomized the order of the treatments every day. Males typically produce signals in a bout in which each signal is followed by a short pause. We presented signals in each experience treatment as a bout of three, the mean number of signals per bout of males from this population, and bouts were separated by 15 s of silence. We set all other features of the signals to the population mean. We presented each treatment for 1 h per day for a minimum of 14 days (range 14–15 days) beginning upon adult eclosion. In nature, males may experience signalling males for several hours per day (Wood & Guttman, 1982; Sullivan-Beckers & Cocroft, 2010), and our treatments thus reflect a natural amount of daily signal experience while allowing us to expose all males during the time of day when males normally call.

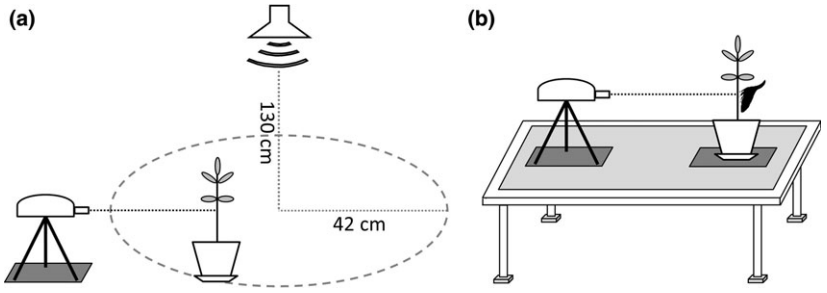

Fig. 1 Depiction of the set-ups used to create the signalling environments and record male mating signals. (a) Broadcast airborne signals were imparted onto plant stems as vibrational stimuli for treehoppers on those plants. The loudspeaker was suspended from the ceiling of an anechoic chamber, and each plant was placed equidistantly from the loudspeaker (dashed circle) so that males were subjected to a similar experience in signalling environments. All plants and thus males for each signalling environment treatment were stimulated at the same time, and the figure is merely simplified for clarity. A laser vibrometer was used to calibrate each stimulus and was isolated with sorbothane from the vibrations due to broadcast stimuli. Full details for the method are provided in Rebar *et al.* (2012). (b) Male mating signals were recorded using a laser vibrometer that was focused onto the plant stem. The entire set-up was isolated from building vibrations with vibration-dampening pads under the table legs, an iron plank on partially inflated bicycle inner tubes on top of a slate table, and sorbothane underneath both the laser vibrometer and potted plant.

Table 1 Adult males were randomly assigned to one of the signalling environment treatments in which they were exposed to stimuli for 1 h per day for a minimum of 14 days.

Treatment	Signal experience
Experiment 1: variation in experience of male signals	
Silent	No exposure to any other male signals
Het Low	Presented signals at 100 Hz below the population mean (80 Hz)
Con Low	Presented signals at 20 Hz below the population mean (160 Hz)
Mean	Presented the mean signal frequency (180 Hz) of conspecific males in the population
Con High	Presented signals at 20 Hz above the population mean (200 Hz)
Het High	Presented signals at 100 Hz above the population mean (280 Hz)
Con Mixed	Presented Con Low, Mean and Con High signals in a randomized order and in equal amounts
Het Mixed	Presented Het Low, Mean and Het High signals in a randomized order and in equal amounts
Experiment 2: variation in experience of female responses to male signals	
Duet Low	Presented Con Mixed (from above; Con Low, Mean and Con High signals) with female responses only to Con Low signals
Duet Mean	Presented Con Mixed with female responses only to Mean signals
Duet High	Presented Con Mixed with female responses only to Con High signals
Duet All	Presented Con Mixed with female responses to all signals

Experiment 1 varied in the frequency of male signal stimuli, ranging from the species mean (preferred by females) to near the end of the conspecific range to beyond that range. Experiment 2 varied in the frequency of male signal stimuli that received female response signals.

Experiment 2: variation in experience of female responses to male signals

In this experiment, we randomly placed 100 individuals into one of four treatments (N = 25 per treatment) that varied in the frequency of male signal stimuli that received female response signals. All treatments consisted of a mixture of conspecific male signal frequencies (corresponding to the Con Mixed treatment in experiment 1; Table 1), but with female responses to different signal frequencies in each treatment (Table 1). We randomized the order of treatments each day. We set the features of the female signal to the population mean. We presented each treatment for 1 h per day for a minimum of 14 days (range 14–15 days).

Signal recording and analysis

For each experiment, a single recording plant individual was used to record the mating signals of all males after they had been subjected to the experience treatments. This plant differed from the plants on which they were kept during the experience period. Using a single recording plant minimizes the potential of our measures of signal variation to be influenced by differences in plant signal-transmission features. To isolate the set-up from noise due to building vibrations, we placed vibration-dampening pads (model 3291-22-PM-50; Polymer Dynamics, Inc., Allentown, PA, USA) under the legs of a slate table (~1 × 2 m), and placed an iron plank (~135 kg) resting on partially inflated bicycle inner tubes on top of the table. We then placed the recording plant and laser vibrometer on shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY, USA) on the top of the iron plank to further isolate the entire set-up (Fig. 1b).

To record each male, we removed him from his treatment plant and placed him at a standard position on the recording plant stem (5 cm from where the laser-detected vibrations). We primed each male to signal by playing a recording of an actual male–female duet through a piezoelectric actuator attached to the stem with accelerometer wax (model AE0505D16; Thorlabs, Newton, NJ, USA). The actuator was controlled by a piezocontroller (model MDT694A; Thorlabs) from an iMac computer at an amplitude of 0.10 mm s^{-1}. We played the same priming recording for each male, and this recording differed from the experience treatments as we used only synthetic signals for those. Because all males were primed with the same male–female duet immediately before being recorded, any differences in signal traits between experience treatments reflect the treatment itself and not any immediate adjustments to the playback. Males that failed to signal in the 5 min following the recording playback were returned to their treatment plant and tested again the next day (N = 5 and 7 males in experiments 1 and 2, respectively; these males all signalled successfully on the next day). Males were tested the day after their last treatment, maintaining at least 18 h between treatment exposure and signal recording.

We recorded male signals using the same laser vibrometer set-up as above. Males were within 10 cm of the reflective tape when they signalled, and we noted the temperature at the time of recording at the position of the laser (Exp. 1 range: 22.5–24.5 °C; Exp. 2 range: 23–24 °C). The output from the band-pass filter was sent to an iMac computer through an Edirol UA-25 USB interface and recorded with the sound recording software AUDACITY (v. 1.2.5; http://audacity.soundforge.net) at a sampling rate of 44.1 kHz. All males were recorded in April 2011 for the first experiment and April 2013 for the second experiment, and each male was only used once.

Some natural and accidental death (i.e. caught in the mesh screen) occurred across treatments, resulting in an average of 16 males recorded per treatment in the first experiment (range: N = 14–18) and an average of 21 males recorded per treatment in the second experiment (range: N = 20–21).

Enchenopa males typically produce bouts of several signals (Fig. 2). We standardized our measurements of male traits by selecting the highest amplitude bout, and measured the third signal in the bout. If males produced less than three signals, we measured the last signal in the bout. Male signals consist of a whine portion followed by several pulses (Cocroft *et al.*, 2010). We analysed variation in seven signal traits that differ among species in the *E. binotata* complex. We measured the signals per bout, the signal rate within bouts, length of the whine portion (henceforth, whine length), number and length of the pulses, the pulse rate, and the dominant frequency (Fig. 2). We measured frequency from the last 10 cycles of the whine portion of the waveform because male signals are relatively pure tone. We conducted all analyses with AUDACITY.

Statistical analysis

We were interested in analysing each signal trait separately because they are associated with differently shaped female mate preference functions (Rodríguez *et al.*, 2006; Cocroft *et al.*, 2008; Sullivan-Beckers & Cocroft, 2010). Thus, different signal traits may differ in their contributions to mate choice decisions, to variation in male reproductive success, and to patterns of reproductive isolation among the members of the *E. binotata* complex (Rodríguez *et al.*, 2006; Cocroft *et al.*, 2008; Sullivan-Beckers & Cocroft, 2010). We recognize that this approach increases the risk of spurious significance for any one trait (Rice, 1989), but methods to control this risk reduce statistical power (Moran, 2003; Nakagawa, 2004). To assess the potential risk of spurious significance in our data, we estimated the strength of the correlations between signal traits. We first performed a principal component analysis (PCA) on the seven signal traits to assess the nonindependence in our data. For the first experiment, the PCA resulted in three axes with eigenvalues > 1 (1.91, 1.22 and 1.08) that only accounted for 60% of the total variation in male signals (27.3, 17.4 and 15.4%, respectively). For the second experiment, the PCA produced a similar result: three eigenvalues > 1 (1.59, 1.36 and 1.17) that explained only 59% of the total variation in male signals (22.7, 19.4 and 16.7%, respectively). The low amount of variation accounted for in each PCA suggested that the correlations between traits were generally weak. We confirmed this result by estimating Pearson product-moment correlations between the seven signal traits. For both experiments, we found that $r \leq 0.33$ for all cases except for between frequency and whine length ($r = -0.46$ and -0.34 for experiments 1 and 2, respectively). We therefore consider separate analysis of each signal trait to be warranted, as well as evolutionarily important, and we account for the known negative correlation between frequency and whine length (Cocroft *et al.*, 2008, 2010). Nonetheless, we provide the corrected significance level after adjusting for multiple comparisons using the Bonferroni method and false discovery rate (FDR) (Benjamini & Hochberg, 1995) as a point of reference.

We used a one-way ANOVA for each signal trait to address variation in that trait among signalling environment treatments. We initially included temperature as a covariate, but it was nonsignificant for all signal traits and we therefore removed it from the analyses. In instances of overall significance, we performed a *post hoc* Tukey's HSD test to determine which treatments differed from one another. We also provide a minimum and maximum effect size estimate for the variation in each signal trait between social experience treatments. Effect size was calculated as Cohen's *d*, and converted to the standardized correlation *r* in order to express effect size in terms of small ($r < 0.2$), medium ($0.2 \leq r \leq 0.5$) or large ($r \geq 0.5$) (Cohen, 1988; Nakagawa & Cuthill, 2007).

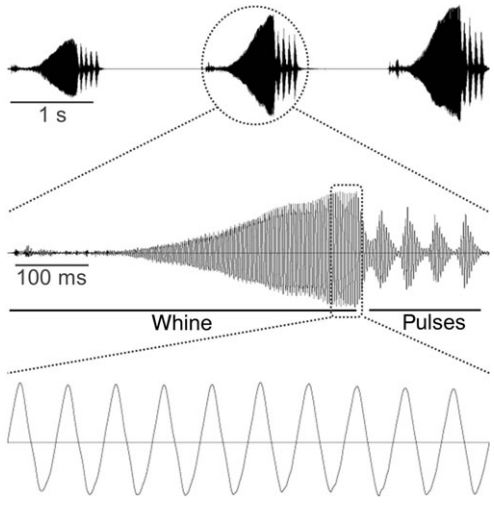

Fig. 2 An *Enchenopa binotata* male signal bout consisting of three signals that increase in amplitude. Each signal is composed of a whine portion followed by several pulses. A close-up of the waveform shows the pure-tone (sine wave) property of the signal.

Results

Experiment 1

(1a) Do males adjust their signalling behaviour according to their experience of competitors' signals?

The experience treatments affected only one male signal trait (signal rate), and there was a clear difference in statistical significance between signal trait, the trait that was influenced by experience treatments (with $P = 0.009$), and the other traits that were unaffected by experience treatments (all $P > 0.24$) (Table 2). The signal rates of males in the Con Mixed treatment significantly differed from those in the Silent and Con High

Table 2 Variation in *Enchenopa* male signal traits attributed to variation in experience with conspecific and heterospecific male signals.

Trait	d.f.	*F*	*P*	*r*
Signals in bout	7, 123	1.18	0.320	0.00–0.34
Signal rate	7, 117	**2.83**	**0.009**	**0.03–0.56**
Whine length	7, 123	1.12	0.355	0.00–0.40
Pulses	7, 123	0.17	0.991	0.00–0.20
Pulse rate	7, 120	1.33	0.242	0.00–0.48
Pulse length	7, 123	0.69	0.684	0.02–0.40
Frequency	7, 123	0.82	0.573	0.01–0.38

As a point of reference, the corrected significance level after adjusting for multiple comparisons by either the Bonferroni or FDR method is 0.0071 for the significant trait, signal rate. The range of the effect size (*r*) from all pairwise comparisons is reported. Significant tests are in bold.

treatment (Fig. 3). More specifically, males signalled faster when they experienced a range of conspecific competitors (Con Mixed; Fig. 3) and slower when they experienced either no competitors or unattractive conspecific males (Silent, Con High; Fig. 3).

(1b) Do males shift their dominant frequency to increase their attractiveness to females when they hear rival males signal at the females' preferred value?

The above effect of the experience treatments on male signal rate and the lack of effect on the other signal traits (Table 2; Fig. 3) does not suggest that males adjust their signals according to the kind of adjustments that females make when exposed to such social signalling environments, which would have involved shifts in signal frequency (Fowler-Finn & Rodríguez, 2012a,b).

Experiment 2

(2) Do males adjust their signalling behaviour according to their experience of female feedback to competitors?

These experience treatments affected only one male signal trait (whine length); as in experiment 1, there was a clear distinction between the trait that was influenced (with $P = 0.008$) and the traits that were unaffected (all $P > 0.14$) (Table 3). Whine length significantly differed between the Duet Mean and Duet All treatments (Fig. 4); that is, males produced longer whines when they experienced females who only engaged in duets with attractive competitors (stimulus at the conspecific mean frequency: Duet Mean; Fig. 4), and males produced shorter whines when they experienced females who engaged in duets with all available competitors (Duet All; Fig. 4). Because whine length and frequency are negatively correlated with one another (Cocroft

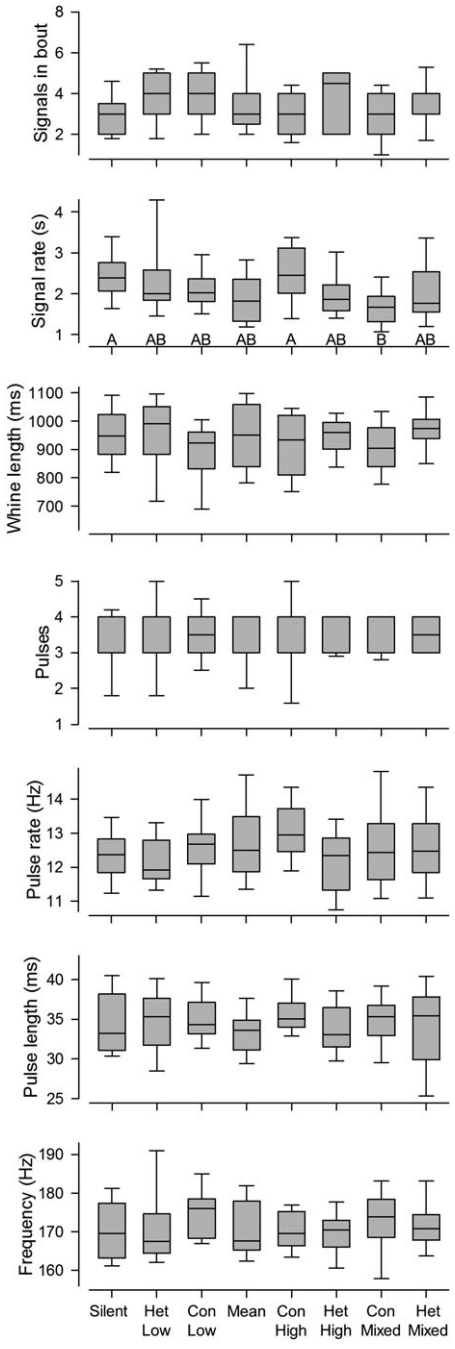

Fig. 3 Box-and-whisker plots for seven male signal traits analysed across the eight treatments that adult males experienced. The box indicates the 25th and 75th percentiles, the whiskers the 10th and 90th percentiles, and the line the median. Treatments varied in exposure to male signals, which went beyond the conspecific to potential heterospecific male signals. The *y*-axis represents the range of phenotypic variation for each male trait in the study. Treatments not sharing a letter are significantly different (*post hoc* Tukey's HSD test).

Table 3 Variation in *Enchenopa* male signal traits attributed to experience with variation in female preference for conspecific male signals.

Trait	d.f.	*F*	*P*	*r*
Signals in bout	3, 78	0.86	0.466	0.03–0.18
Signal rate	3, 73	1.13	0.341	0.02–0.30
Whine length	3, 78	**4.25**	**0.008**	**0.10–0.42**
Pulses	3, 78	1.21	0.313	0.02–0.27
Pulse rate	3, 76	0.22	0.882	0.01–0.07
Pulse length	3, 78	0.50	0.686	0.04–0.19
Frequency	3, 78	1.86	0.143	0.03–0.39

As a point of reference, the corrected significance level after adjusting for multiple comparisons by either the Bonferroni or FDR method is 0.0071 for the significant trait, whine length. The range of the effect size (*r*) from all pairwise comparisons is reported. Significant tests are in bold.

et al., 2010), and because for question (1b) there was the potential for an effect on signal frequency, we were concerned that the differences in whine length we detected here might be a by-product of differences in mean signal frequency between treatments. However, the difference in frequency was nonsignificant (Table 2; Fig. 3), and its small magnitude (3 Hz) would only predict a corresponding difference in whine length of 23 ms (given the slope of the frequency~whine length relationship, which was −7 ms per Hz in the current data set), which is well below the detected difference of ca. 100 ms. We therefore interpret this result as an active adjustment of whine length by males on the basis of their experience, rather than as by-product of trait correlations.

Discussion

We demonstrate that adult males adjust their signalling behaviour in response to different sources of public information in their social environment. By manipulating experience with male signals, we found that: (1a) males increased their signalling rate as a response to experience of attractive competitors, particularly when accompanied by unattractive conspecifics. However, (1b) males did not adjust signal frequency in accordance with the adjustments that females make on the basis of their experience of signalling males. Then, by manipulating experience with female responses to

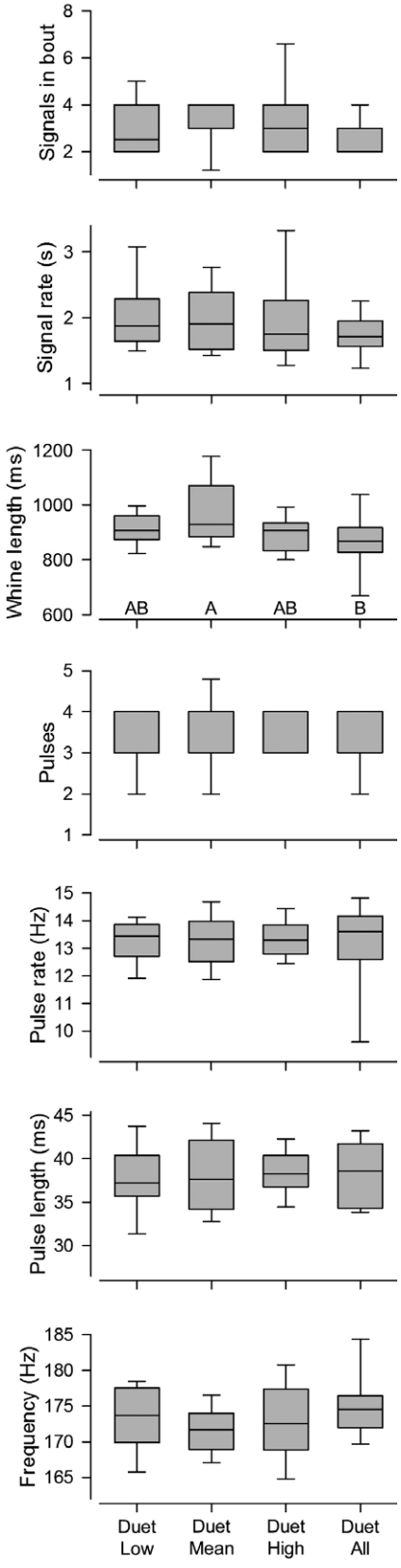

Fig. 4 Box-and-whiskers plots for seven male signal traits analysed across the four treatments that adult males experienced. The box indicates the 25th and 75th percentiles, the whiskers the 10th and 90th percentiles, and the line the median. Treatments varied in the responses of females to conspecific male signals. The *y*-axis represents the range of phenotypic variation for each male trait in the study. Treatments not sharing a letter are significantly different (*post hoc* Tukey's HSD test).

conspecific male signals, we found that: (2) males increased whine length according to perceived female feedback to attractive competitors. This opens the possibility for socially plastic and evolutionary feedback loops between the adjustments that males and females make in response to each other, which may alter the dynamics of sexual selection.

The consequences of these feedback loops for sexual selection will depend on a number of factors, including on the nature of male adjustments according to the competitors in social environments, on how females adjust their mate preferences in such contexts, on the cues available for males about those adjustments, and on whether males attend to them (Rodríguez, 2015). This is because the composition of available males in the social environment influences the shape of female mate preferences, and those preferences are a cause of sexual selection on males (Jennions & Petrie, 1997; Brooks & Endler, 2001; Chaine & Lyon, 2008; Verzijden *et al.*, 2012). Conversely, the composition of competitors in the social environment and feedback from females influence the distribution of male traits, influencing the form of selection exerted by female mate preferences. Here, we take a step towards understanding such feedback loops by comparing the adjustments made by males and females in response to variation in social experience via signal playbacks.

Our findings suggest that feedback loops based on social plasticity in signals and mate preferences are complex. For example, in response to similar changes in their experience of male signals in the social environment, males adjust signal rate while females adjust preference selectivity for signal frequency. And males adjust whine length when they perceive females as being selective regarding signal frequency. Moreover, the potential for complex feedback dynamics is greater than hinted at by our experiments. Not only do we not yet know whether and how females adjust their preferences for signal rate or length, but a previous experiment that manipulated the composition of social groupings detected strong effects of social neighbours not only on preference selectivity but on preferred signal values (Rebar & Rodríguez, 2013).

These different feedback loops may have important consequences for divergence. *Enchenopa* treehoppers, for instance, develop entirely on the host plant where their mothers laid their eggs (Wood, 1993), and the composition of nymph groups on the plant, along with the host plant itself, influences the sexual traits of individuals as adults. Specifically, the genetic makeup of social neighbours as nymphs has been shown to shift the peak preferences and selectivity of adult *Enchenopa* females (Rebar & Rodríguez, 2013). Furthermore, the genetic make-up of the host plant has been shown to shift both male signals (including signal frequency and whine length) and female peak preferences (Rebar & Rodríguez, 2014a,b), with surprisingly strong correlated shifts in male signal frequency and female peak preference (Rebar & Rodríguez, 2015). This phenotypic signal–preference covariance may lead to assortative mating, which may generate a runaway process among environments (Bailey & Moore, 2012). Further, such assortative mating may establish direct genetic covariance between signals and preferences and kick-start the Fisherian runaway process (Bailey & Moore, 2012; Drown & Wade, 2014; Rebar & Rodríguez, 2015).

In conclusion, our results provide clear evidence of male signal behaviour adjustments in response to various types of public information, giving insight into how social environments can influence the dynamics of sexual selection. The ability of males to be plastic to social cues can generate complex feedback loops between male signals and female preferences. These feedback loops, in turn, may promote or constrain the evolution of communication systems.

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References

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.

Bailey, W.J. 2003. Insect duets: underlying mechanisms and their evolution. *Physiol. Entomol.* **28**: 157–174.

Bailey, N.W. & Moore, A.J. 2012. Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the Fisher process. *Evolution* **66**: 2674–2684.

Bailey, N.W. & Zuk, M. 2012. Socially flexible female choice differs among populations of the Pacific field cricket: geographical variation in the interaction coefficient psi (Ψ). *Proc. R. Soc. B* **279**: 3589–3596.

Bailey, N.W., Gray, B. & Zuk, M. 2010. Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets. *Curr. Biol.* **20**: 845–849.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* **57**: 289–300.

Bertram, S.M., Harrison, S.J., Thomson, I.R. & Fitzsimmons, L.P. 2013. Adaptive plasticity in wild field cricket's acoustic signaling. *PLoS ONE* **8**: e69247.

Bretman, A., Gage, M.J.G. & Chapman, T. 2011. Quick-change artists: male plastic behavioural responses to rivals. *Trends Ecol. Evol.* **26**: 467–473.

Brooks, R. & Endler, J.A. 2001. Female guppies agree to differ: phenotypic and genetic varaition in mate-choice behavior and the consequences for sexual selection. *Evolution* **55**: 1644–1655.

Callander, S., Kahn, A.T., Hunt, J., Backwell, P.R.Y. & Jennions, M.D. 2013. The effect of competitors on calling effort and life span in male field crickets. *Behav. Ecol.* **24**: 1251–1259.

Chaine, A.S. & Lyon, B.E. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science* **319**: 459–462.

Cocroft, R.B. & Rodríguez, R.L. 2005. The behavioral ecology of insect vibrational communication. *Bioscience* **55**: 323.

Cocroft, R.B., Rodríguez, R.L. & Hunt, R.E. 2008. Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. In: *Evolution of populations and species* (K.J. Tilmon, ed.), pp. 88–100. University of California Press, Berkeley, CA.

Cocroft, R.B., Rodríguez, R.L. & Hunt, R.E. 2010. Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biol. J. Linn. Soc.* **99**: 60–72.

Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Lawrence Erlbaum Associates Inc, Hillsdale, NJ.

Danchin, E., Giraldeau, L., Valone, T. & Wagner, R. 2004. Public information: from nosy neighbors to cultural evolution. *Science* **305**: 487–491.

Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. J Murray, London.

Drown, D.M. & Wade, M.J. 2014. Runaway coevolution: adaptation to heritable and nonheritable environments. *Evolution* **68**: 3039–3046.

Foster, S.A. 2013. Evolution of behavioural phenotypes: influences of ancestry and expression. *Anim. Behav.* **85**: 1061–1075.

Fowler-Finn, K.D. & Rodríguez, R.L. 2012a. Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* **66**: 459–468.

Fowler-Finn, K.D. & Rodríguez, R.L. 2012b. The evolution of experience-mediated plasticity in mate preferences. *J. Evol. Biol.* **25**: 1855–1863.

Gerhardt, H.C. & Huber, F. 2002. *Acoustic Communication in Insects and Anurans*. University of Chicago Press, Chicago.

Hamilton, K.G.A. & Cocroft, R.B. 2009. Establishing the identity of existing names in the North American Enchenopa binotata species complex of treehoppers (Hemiptera: Membracidae). *Entomol. News* **120**: 554–565.

Harris, W.E., McKane, A.J. & Wolf, J.B. 2008. The maintenance of heritable variation through social competition. *Evolution* **62**: 337–347.

Hebets, E.A. 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. *Proc. Natl. Acad. Sci. USA* **100**: 13390–13395.

Hebets, E. & Sullivan-Beckers, L. 2010. Mate choice and learning. In: *Encyclopedia of Animal Behavior* (M.D. Breed & J. Moore, eds), pp. 389–393. Elsevier BV, Amsterdam.

Höbel, G. 2015. Socially mediated plasticity of chorusing behavior in the gladiator frog *Hypsiboas rosenbergi*. *Acta Ethol.* **18**: 145–152.

Jennions, M.D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev.* **72**: 283–327.

Kahn, A.T., Dolstra, T., Jennions, M.D. & Backwell, P.R.Y. 2013. Strategic male courtship effort varies in concert with adaptive shifts in female mating preferences. *Behav. Ecol.* **24**: 906–913.

Kasumovic, M.M., Hall, M.D., Try, H. & Brooks, R.C. 2011. The importance of listening: juvenile allocation shifts in response to acoustic cues of the social environment. *J. Evol. Biol.* **24**: 1325–1334.

Kokko, H., Jennions, M.D. & Brooks, R. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Evol. Syst.* **37**: 43–66.

Lesna, I. & Sabelis, M.W. 1999. Diet-dependent female choice for males with "good genes" in a soil predatory mite. *Nature* **401**: 581–584.

Lyon, B.E. & Montgomerie, R. 2012. Sexual selection is a form of social selection. *Philos. Trans. R. Soc. B Biol. Sci.* **367**: 2266–2273.

Moore, A.J., Brodie, E.D. III & Wolf, J.B. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution* **51**: 1352–1362.

Moran, M.D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**: 403–405.

Nakagawa, S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav. Ecol.* **15**: 1044–1045.

Nakagawa, S. & Cuthill, I.C. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev.* **82**: 591–605.

Patricelli, G.L., Uy, J.A.C., Walsh, G. & Borgia, G. 2002. Sexual selection: male displays adjusted to female's response. *Nature* **415**: 279–280.

Patricelli, G.L., Coleman, S.W. & Borgia, G. 2006. Male satin bowerbirds, *Ptilonorhynchus violaceus*, adjust their display intensity in response to female startling: an experiment with robotic females. *Anim. Behav.* **71**: 49–59.

Peretti, A., Eberhard, W.G. & Briceño, R.D. 2006. Copulatory dialogue: female spiders sing during copulation to influence male genitalic movements. *Anim. Behav.* **72**: 413–421.

Pfennig, K.S. 2007. Facultative mate choice drives adaptive hybridization. *Science* **318**: 965–967.

Prum, R.O. 2012. Aesthetic evolution by mate choice: Darwin's really dangerous idea. *Philos. Trans. R. Soc. Lond. B* **367**: 2253–2265.

Rebar, D. & Rodríguez, R.L. 2013. Genetic variation in social influence on mate preferences. *Proc. R. Soc. B* **280**: 20130803.

Rebar, D. & Rodríguez, R.L. 2014a. Genetic variation in host plants influences the mate preferences of a plant-feeding insect. *Am. Nat.* **184**: 489–499.

Rebar, D. & Rodríguez, R.L. 2014b. Trees to treehoppers: genetic variation in host plants contributes to variation in the mating signals of a plant-feeding insect. *Ecol. Lett.* **17**: 203–210.

Rebar, D. & Rodríguez, R.L. 2015. Insect mating signal and mate preference phenotypes covary among host plant genotypes. *Evolution* **69**: 602–610.

Rebar, D., Höbel, G. & Rodríguez, R.L. 2012. Vibrational playback by means of airborne stimuli. *J. Exp. Biol.* **215**: 3513–3518.

Rebar, D., Barbosa, F. & Greenfield, M.D. 2016. Acoustic experience influences male and female pre- and postcopulatory behaviors in a bushcricket. *Behav. Ecol.* **27**: 434–443.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

Rodríguez, R.L. 2015. Mating is a give-and-take of influence and communication between the sexes. In: *Cryptic Female Choice in Arthropods* (A. V Peretti & A. Aisenberg, eds), pp. 479–496. Springer International Publishing, Switzerland.

Rodríguez, R.L., Barbosa, F. 2014. Mutual behavioral adjustment in vibrational duetting. In: *Studying Vibrational Communication* (R.B. Cocroft, M. Gogala, P.S.M. Hill, & A. Wessel, eds), pp. 147–169. Springer-Verlag, Berlin, Heidelberg.

Rodríguez, R.L. & Cocroft, R.B. 2006. Divergence in female duetting signals in the Enchenopa binotata species complex of treehoppers (Hemiptera: Membracidae). *Ethology* **112**: 1231–1238.

Rodríguez, R.L., Sullivan, L.E. & Cocroft, R.B. 2004. Vibrational communication and reproductive isolation in the Enchenopa binotata species complex of treehoppers (Hemiptera: Membracidae). *Evolution* **58**: 571–578.

Rodríguez, R.L., Ramaswamy, K. & Cocroft, R.B. 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. *Proc. R. Soc. B* **273**: 2585–2593.

Rodríguez, R.L., Haen, C., Cocroft, R.B. & Fowler-Finn, K.D. 2012. Males adjust signaling effort based on female mate-preference cues. *Behav. Ecol.* **23**: 1218–1225.

Rodríguez, R.L., Rebar, D. & Fowler-Finn, K.D. 2013. The evolution and evolutionary consequences of social plasticity in mate preferences. *Anim. Behav.* **85**: 1041–1047.

Sullivan-Beckers, L. & Cocroft, R.B. 2010. The importance of female choice, male-male competition, and signal transmission as causes of selection on male mating signals. *Evolution* **64**: 3158–3171.

Sullivan-Beckers, L. & Hebets, E.A. 2011. Modality-specific experience with female feedback increases the efficacy of courtship signalling in male wolf spiders. *Anim. Behav.* **82**: 1051–1057.

Taborsky, B. & Oliveira, R.F. 2012. Social competence: an evolutionary approach. *Trends Ecol. Evol.* **27**: 679–688. Elsevier Ltd

Tobias, J.A., Montgomerie, R. & Lyon, B.E. 2012. The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philos. Trans. R. Soc. B Biol. Sci.* **367**: 2274–2293.

Verzijden, M.N., ten Cate, C., Servedio, M.R., Kozak, G.M., Boughman, J.W. & Svensson, E.I. 2012. The impact of learning on sexual selection and speciation. *Trends Ecol. Evol.* **27**: 511–519.

West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Q. Rev. Biol.* **58**: 155–183.

West-Eberhard, M.J. 2014. Darwin's forgotten idea: the social essence of sexual selection. *Neurosci. Biobehav. Rev.* **46**(Pt 4): 501–508.

Wolf, J.B. 2003. Genetic architecture and evolutionary constraint when the environment contains genes. *Proc. Natl. Acad. Sci. USA* **100**: 4655–4660.

Wolf, J.B., Brodie, E.D. III & Moore, A.J. 1999. Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. *Am. Nat.* **153**: 254–266.

Wolf, J.B., Harris, W.E. & Royle, N.J. 2008. The capture of heritable variation for genetic quality through social competition. *Genetica* **134**: 89–97.

Wood, T.K. 1993. Speciation of the Enchenopa binotata complex (Insecta: Homoptera: Membracidae). In: *Evolutionary Patterns and Processes* (D.R. Lees & D. Edwards, eds), pp. 299–317. Academic Press, New York.

Wood, T.K. & Guttman, S.I. 1982. Ecological and behavioral basis for reproductive isolation in the sympatric *Enchenopa binotata* complex (Homoptera: Membracidae). *Evolution* **36**: 233–242.

Zuk, M., Bastiaans, E., Langkilde, T. & Swanger, E. 2014. The role of behaviour in the establishment of novel traits. *Anim. Behav.* **92**: 333–344.

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