ORIGINAL PAPER

Male *Enchenopa* treehoppers (Hemiptera: Membracidae) vary mate-searching behavior but not signaling behavior in response to spider silk

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Abstract Finding and attracting mates can impose costs on males in terms of increased encounters with, and attraction of, predators. To decrease the likelihood of predation, males may modify mate-acquisition efforts in two main ways: they may reduce mate-searching efforts or they may reduce mate-attraction efforts. The specific behavior that males change in the presence of predator cues should depend upon the nature of risk imposed by the type of predator present in the environment. For example, sit-and-wait predators impose greater costs to males moving in search of mates. Here, we test whether cues of the presence of a sit-and-wait predator lead to a reduction in mate-searching but not mate-acquisition behavior. We used a member of the *Enchenopa binotata* complex of treehoppers—a clade of vibrationally communicating insects in which males fly in search of mates and produce mate-attraction signals when they land on plant stems. We tested for changes in mate-searching and signaling behaviors when silk from a web-building spider was present or absent. We found that males delayed flight when spider silk was present but only if they were actively searching for mates. These results suggest that males have been selected to reduce predation risk by adjusting how they move about their environment according to the cues of sit-and-wait predators.

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Introduction

Mate-acquisition efforts are inherently risky. While searching for mates, males may experience higher encounter rates with predators due to increased movement and exposure (Moore 1987; Magnhagen 1991). Males that stay in one place and signal to attract mates may not incur such risks, but their signals may instead give their position away to predators in addition to also attracting them (Zuk and Kolluru 1998; Lima and Dill 1990). Thus, there is a tradeoff between mate-acquisition efforts and predator avoidance: an overall reduction in effort will reduce the likelihood of being preyed upon but at the cost of the number of mates a male can acquire. One way to resolve this tradeoff is for males to respond to cues indicative of specific types of predation risk by modifying specific components of their mate-acquisition efforts. The type of response should correspond to the nature of the threat of predation (Sih 1987). Thus, males should exhibit plasticity in either mate-attraction or mate-acquisition behavior according to the type of predator(s) present in the environment. For example, when cues of sit-and-wait predators are present, males should reduce mate-searching efforts but not mate-attraction efforts. Conversely, with cues of motile, eavesdropping predators, males should modify their signaling behavior but not mate-searching efforts. Furthermore, males that are inherently at greater risk of predation (e.g., because they are more conspicuous or more active) should exhibit greater plasticity (Hedrick 2000; Fowler-Finn and Hebets 2011). Thus, males that are more active in mate-acquisition efforts should show greater plasticity.

Here, we assess plasticity in the mate-searching and mate-attraction behaviors of a plant-feeding insect in response to

cues of a sit-and-wait predator. We used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Enchenopa* males use two different strategies to seek mates. When in conditions of low population density, males often engage in a behavior termed "call-fly," in which they fly from plant to plant, producing advertisement signals on each plant (Cocroft et al. 2008). As in many plant-feeding insects, these advertisement signals are substrate-borne vibrations that travel along the tissues of the plant as bending waves (Cocroft and Rodriguez 2005; see Fig. 1). If a receptive female is on the plant and she finds a male's signals attractive, she produces her own response signals, establishing a duet that facilitates pair formation (Rodríguez and Cocroft 2006; Cocroft et al. 2008; Rodríguez et al. 2012). If no female responds, the male flies off a short distance and signals again on another stem. Alternatively, males in high-density aggregations may not engage in call-fly, and females may not necessarily engage in duets with the males; instead, males engage in chorusing competitions with other males in the aggregation (Cocroft et al. 2010; 2008). In the *E. binotata* complex, the propensity for males to be in call-fly or chorusing "modes" varies among individuals and species (Cocroft et al. 2008), and in any given population, one can find males in either mode. This variation may influence the type of risk that males are exposed to, with males in call-fly mode being more vulnerable to predation through their increased movement (e.g., by sit-and-wait predators) and males in chorusing mode being more vulnerable to detection

of their signaling by eavesdropping motile predators. Consequently, the mode individual males are in should influence how they respond to predator cues, and we tested for greater levels of plasticity for males in call-fly mode. We tested males singly, and thus had no opportunity to observe chorusing behavior. We were, nevertheless, able to assess if males were in call-fly mode or not, as defined by a bimodal distribution of early vs. late onset of signaling during trials (see below).

We used the silk of spider webs as the cue of the presence of a sit-and-wait predator. Spiders are important generalist predators, and the presence of their silk has been shown to have wide-ranging effects on behavior across several insects and other arthropods (Schmitz et al. 2004; Schmidt-Entling and Siegenthaler 2009; Hawlena et al. 2012), including for those not considered typical prey item for the spider (Hlivko and Rypstra 2003; Fill et al. 2012). Arthropods often exhibit chemically mediated responses to the presence of silk that include changes in mating and signaling behavior, foraging behavior, and activity levels (Kortet and Hedrick 2004; Hlivko and Rypstra 2003; Persons et al. 2001; Rypstra and Buddle 2013). Spiders represent a potentially important predator of vibrationally communicating insects (Cocroft and Rodriguez 2005), and evidence suggests that they eavesdrop on male advertisement signals (Cocroft 2011; Virant-Doberlet et al. 2011). Spiders may pose a specific threat to *Enchenopa* males: we have often observed both wandering and web spiders on the branches of trees in which *Enchenopa* live, and we have also observed *Enchenopa* males to be entangled

Fig. 1 Illustration of the parameters we used to describe variation in *Enchenopa* male signals. **a** Spectrogram of an individual signal, with the dominant frequency of ~185 Hz indicated with a *bold arrow pointing to the y-axis*. **b** A waveform of the same signal illustrated in (**a**) with the whine length and pulses indicated by *brackets*. **c** Example of a signal bout from the same individual
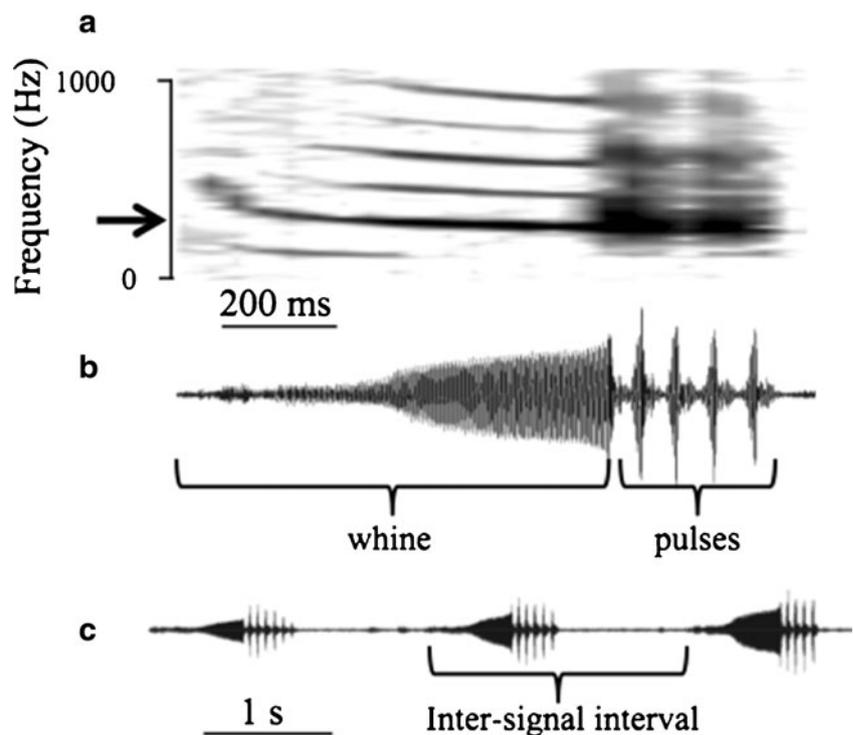
in the webs of spiders in the field during the mating season (Fowler-Finn and Rodríguez, personal observation; Cocroft, personal communication). These observations suggest that *Enchenopa* males are at risk of falling prey to various types of spiders, including through entanglement in webs.

We asked whether *Enchenopa* males modified their mate searching and/or signaling behavior in response to the presence of the silk of spider webs. In this experiment, one key prediction is that males should vary their mate-searching efforts but not their signaling behavior because spider silk indicates a risk of predation through aerial movement (see above). Specifically, we expected that with cues from sit-and-wait spiders, the treehoppers would be less motivated to fly off the stem (to avoid interception by the web), and that this would be manifest as a delay in flight. A second key prediction is that males more actively engaged in mate-acquisition efforts should show higher levels of plasticity. We thus analyzed patterns of plasticity for males in call-fly mode in separate analyses. We tested each male in treatments of silk present or absent. We then compared their signaling behavior and the time it took them to fly off the stem after signaling (taking into account whether they were in call-fly mode or not), with longer delays in departing indicating reduction of mate-searching effort.

Materials and methods

Study species

We used one of the members of the *E. binotata* complex that live on the host plant *Viburnum lentago* (Caprifoliaceae) in Wisconsin. There are two species that live on this host plant at our field sites, and we used the one that produces low-frequency signals (mean dominant frequency=~170 Hz). Formal description of many species in this complex is lacking, but male signal frequency is useful for distinguishing among species (Hamilton and Cocroft 2009; Rodríguez et al. 2004). After experimentation, we preserved voucher specimens in 95 % EtOH.

We collected insects as nymphs in the field May, 2010 at the University of Wisconsin-Milwaukee Field Station (Saukville, WI) and May 2013 at Tendick Nature Preserve (Saukville, WI). We reared the insects in groups on potted host plants at the University of Wisconsin-Milwaukee greenhouse. Upon maturation, we separated males from females. We tested males when they were approximately 6 weeks old.

Our experiment tested for responses to the general presence of spider silk. We used a spider that was abundant enough to provide silk from a full web from a different spider for each trial (see below). For both 2010 and 2013, we used *Frontinella communis* (Araneae: Lynyphiidae) collected from a patch of juniper bushes (*Juniperus communis*) adjacent to the *V. lentago*

patch at the University of Wisconsin-Milwaukee Field Station (Suakville, WI). We maintained spiders in the laboratory in 10×10×6 cm plastic containers lined with moist paper towel and covered in cling wrap. Spiders generally built a web on their first day of capture. In the spider silk-present trials, we used the first full web each spider constructed.

Experimental design

We tested each male on two consecutive days, once in the presence of spider silk and once without it, randomizing the sequence for each individual. We tested 18 males in 2010 and 14 males in 2013. In each trial, we placed a male at the midpoint of a freshly clipped host-plant stem placed in a vial of water. We used a fresh stem for each trial and standardized for length (approximately 25 cm long) and width across all trials. In the silk-present treatment, we had wrapped the stem in the silk from the full web of one spider individual (see above) by gently rolling the stem in the silk just prior to the trial. Webs were approximately 9×9 cm in size when stretched out, and consequently wrapped around the plant stem ~4 times. We wrapped the web so that it was centered on the midpoint of the stem. We used a different spider for each silk-present trial. Each trial lasted either 30 min or until the male flew off the stem (whichever came first).

We monitored and recorded signaling behavior by focusing the beam of a laser vibrometer (Polytech CLV 2534; Polytech Inc., Auburn, MA) on a small piece (ca. 2 mm) of reflective tape stuck to the stem. Signals from the laser were sent to an iMac computer and recorded as .wav files using the sound analysis program AUDACITY (version 1.2.5; http://audacity. sourceforge.net/). We recorded the time to the first call for each individual male in each trial and used this information to categorize males into call-fly mode or not (see "Statistical analyses"). We then measured male signal parameters in each trial from the .wav file recordings using the following criteria. Across the *E. binotata* complex, males produce groups of multiple signals per signaling bout (Fig. 1). Each signal within a bout consists of a frequency sweep, referred to as a whine, followed by one or more pulses (Fig. 1). Whine length and signal frequency (hertz) change throughout a bout and with progressive signaling bouts in a predictable manner (Rodríguez et al. 2006). To compensate for this variation introduced by the position of the signal within and across bouts, we analyzed signal parameters using a landmark signal: we selected the third signal from the second signal bout for each trial. Most males produced at least two signal bouts (64 %, N=53 trials) and three or more signals/bout (89 % N=53 trials). If a male only produced one signal bout, we used the first bout. Similarly, if males produced fewer than three signals in the selected bout, we used the second signal (or first if only one was available). We measured the following aspects

of the signals: number of bouts, bout rate (number of bouts divided by the time from the first signal to the end of the trial), number of signals/bout, inter-signal interval, whine length, number of pulses/signal, pulse length, and dominant frequency (Cocroft et al. 2008, 2010).

To determine if the silk treatment influenced mate-searching behavior, we determined the time to each male flying off the plant, as defined by the time elapsed during a trial before the male flew from the plant stem.

Statistical analyses

For all analyses, we excluded trials in which the male did not signal—there was no difference between treatments in the number of trials in which a male did not signal (four silk-absent trials and three silk-present trials). To categorize males according to the "mode" in which they came into a given trial (see above; $N=53$ trials), we examined the distribution of the time to onset of signaling. This distribution was bimodal and with a large gap between 10 and 15 min during which no males began to signal (Fig. 2). This allowed us to place males in categories of either call-fly mode (for those males that signaled before 10 min into the trial, henceforth referred to as early-onset signalers) or not (for those males that did not signal until 15 min of the trial had elapsed, henceforth referred to as late-onset signalers). The mode in which a male entered a trial could vary between trials, so we determined the mode for each male at the start of each trial. We tested if the proportion of males in each mode varied with spider silk treatments using a mixed-model logistic regression implemented in R v. 2.14.1 (R Development Core Team, Vienna, Austria 2011) using the *lme4* package, with silk treatment as a fixed effect. As each male contributed one to two data points to the model (note the

above seven excluded trials), we included male ID as a random effect. We also included year as a random effect.

For the above and following analyses, we originally included the order of treatment as independent variables, but it was not significant ($p>0.20$), and so we excluded this term from the final models.

Plasticity in mate-searching (flight) behavior

To analyze variation in flight behavior in response to silk treatment, we constructed a linear mixed model (using the EMS method in JMP v7.0, SAS Institute, North Carolina) with time to flight as the dependent variable and silk treatment as the independent variable. We included signaling mode as a covariate (which was independent of silk treatment, see "Results"), as well as the interaction between silk treatment and signaling mode. We included year as a random effect, and because each male was tested twice, we included male ID as a random effect. To test whether the bimodal distribution of flight times influenced the model, we ran a second model with flight times binned into five categories as follows: 1=flight time<1 min; 2=flight between 1 and 2 min; 3=flight between 2 and 10 min; 4=flight between 11 and 25 min; 5and=no flight during 30 min trial (Fig. 3). We then constructed a linear mixed model as above, except with a continuous response variable (with flight time category a proxy of an underlying continuous variable with a normal distribution).

We expected any patterns of plasticity to be strongest for males at higher risk (i.e., those in early-onset signaling mode), so we looked at variation in flight behavior in response to silk treatment for the subset of males in the early-onset signaling category ($N=45$ trials). This test asks whether, within early-onset signalers, plasticity in flight behavior corresponded to the onset of signaling. We constructed a linear mixed model (using the EMS method in JMP v7.0, SAS Institute, North Carolina) with time to flight as the dependent variable, and silk treatment, time to the onset of signaling, and the interaction between them as the independent variables. We also
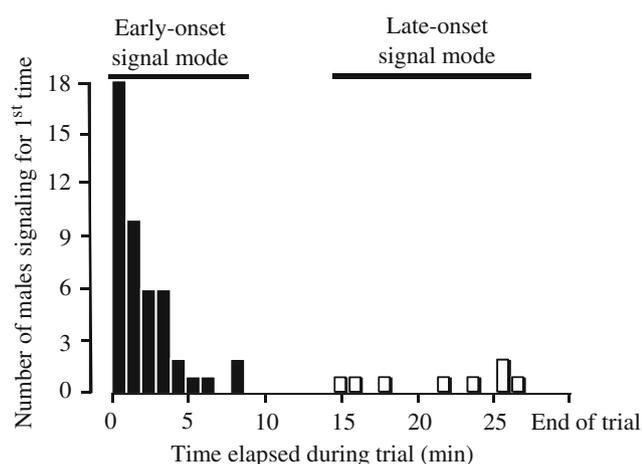

Fig. 2 Histogram of the time to the production of the first signal of *Enchenopa* males. Males producing their first signal before 10 min were classified as early-onset signalers (which we refer to as males in call-fly mode) and males producing their first signal after 15 min were classified as late signalers (males not in call-fly mode)
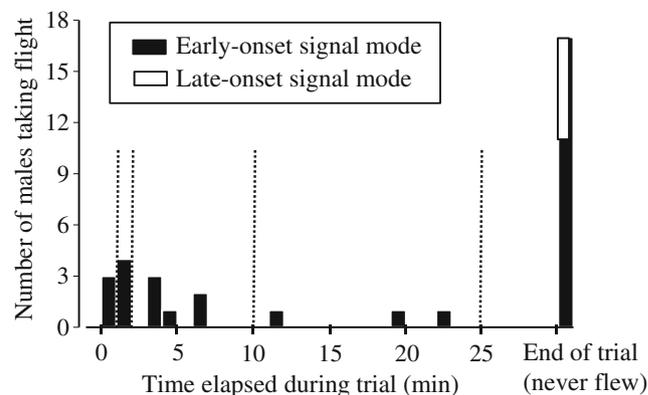

Fig. 3 Histogram of the time to flight of *Enchenopa* males with dotted lines indicating the categorical binning of flight times

included year and male ID nested within year as random effects. We were primarily concerned with the silk treatment term, which asks whether our silk treatments influence behavior. Also, the interaction term asks whether the effect of our silk treatment differs according to the propensity of males to signal earlier. If males that signal earlier show a greater response to silk cues, we expect a more negative correlation between flight time and signal onset in the silk-present versus silk-absent treatment.

Plasticity in signaling behavior

To analyze variation in signaling behavior corresponding to the silk treatment, we used a linear mixed model (REML method) in JMP with the signal trait (see above; Fig. 1) as a dependent variable, and silk absent/present and early/late-onset signaling as the independent variables. All variables were normally distributed except for the number of bouts

and bout rate, which we log-transformed before analysis. Year and male ID were included as random effects. Again, as we expected plasticity to be strongest for males at higher risk, we looked at whether, within early-onset males only, responses in signaling behavior to silk treatment varied with the onset of signaling. We used a linear mixed model (REML method) in JMP with the signal trait as a dependent variable, and silk absent/present and onset to signaling as independent variables, as well as their interaction. Year and male ID nested within year were included as random effects.

Having multiple tests with traits that are likely to be correlated increases the risk of spurious significance (Rice 1989), but corrections for this problem, such as the Bonferroni correction, decrease statistical power and should be avoided (Nakagawa 2004; Garcia 2004). We dealt with these problems in two ways. First, for continuous data (i.e., early-onset signalers), we examined the biological significance of the data for those traits on which silk treatment had a significant effect. We calculated effect sizes with the Pearson product–moment

Table 1 Variation in flight behavior of *Enchenopa binotata* males across spider silk treatments

	df	F ratio	p value
Time delay to flight[a]			
Whole model	32, 20	3.9	*0.0012*
Silk treatment	1	1.4	0.2569
Signal mode	1	21.1	*0.0002*
Silk × signal mode	1	6.8	*0.0172*
Year	1	0.2	0.6586
Male ID	28	2.9	*0.0081*
Categorical delay to flight[b]			
Whole model	32, 20	3.8	*0.0013*
Silk treatment	1	2.9	0.1041
Signal mode	1	26.1	*<0.0001*
Silk × signal mode	1	7.2	*0.0141*
Year	1	0.05	0.8196
Male ID	28	3.3	*0.0040*
Time delay to flight–early-onset signalers[c]			
Whole model	29, 15	3.2	*0.0104*
Silk treatment	1	10.2	*0.0061*
Time of signal onset	1	0.1	0.7725
Silk × signal onset	1	0.6	0.4493
Year	1	0.3	0.5867
Male ID	25	2.3	*0.0454*

Statistically significant values are italicized

[a] Variation in the delay to flight resulting from silk treatment and signaling mode (early- versus late-onset; see text)

[b] Variation in delay to flight as binned into five time delay categories (considered as a continuous variable) resulting from silk treatment and signaling mode

[c] Variation in the delay to flight resulting from silk treatment and the time to the onset of signaling within only early-onset signalers
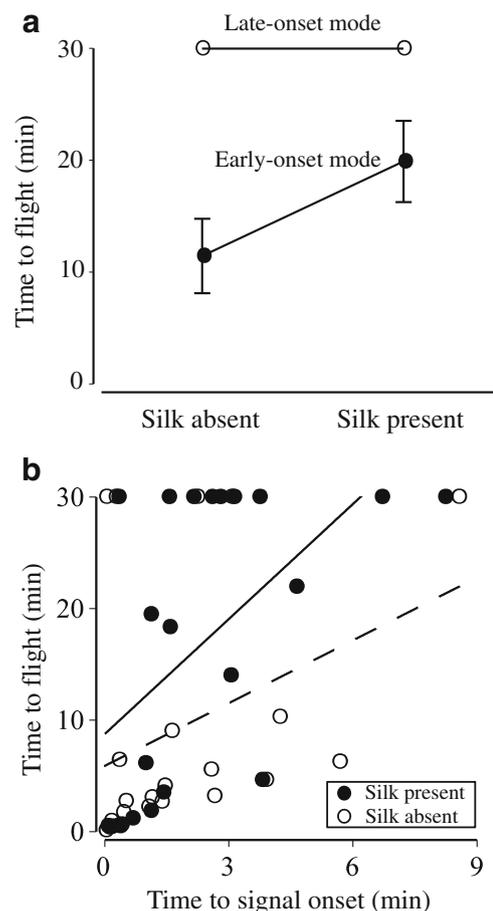

Fig. 4 Relationship between the onset of signaling and time to flight of *Enchenopa* males across silk treatments. **a** Variation in flight time for males in early- versus late-onset signaling modes in spider silk-absent and spider silk-present treatments. **b** Variation in flight time in relationship to the onset of signaling for early-onset signalers

correlation coefficients between the onset to signaling and the signal trait value for the two silk treatments independently. As this analysis is done for continuous data, this was performed for the early-onset signalers only. Second, we implemented the approach suggested by Moran (2003): the presence of multiple *p* values below but close to 0.05 likely indicates a significant pattern, but one *p* value below but close to 0.05 may be spurious.

Results

In the majority of trials, males signaled within the first 10 min of the trial, and thus were classified as being in early-onset mode (85 %, *N*=53 trials; Fig. 2). Whether a male began a trial in early- versus late-onset mode did not vary with silk treatment (proportion of males in early-onset mode in the silk-absent treatment=0.8 versus in the silk-present treatment= 0.76; test for treatment differences in the likelihood of being in early- versus late-onset mode: $z=1.1$, $p=0.28$).

Plasticity in mate searching (flight) behavior

The distribution of flight times was strongly bimodal, with several individuals never taking flight during the 30 min trial (Fig. 3). Early- and late-onset males responded differently to the treatments. Males in early-onset mode delayed flight significantly when silk was present whereas males in late-onset mode never flew, regardless of treatment (Table 1 (Time delay to flight); Figs. 3 and 4). This same pattern was confirmed using the categorical flight data (Table 1 (Categorical delay to flight)).

We then focused on the males in early-onset mode, and tested whether the time to flight varied with silk treatment and time to signal onset. We found an overall delay in flight when silk was present (Table 1 (Time delay to flight–early-onset signalers)). We also found a nonsignificant interaction between signal onset and silk treatment. This suggests that males that signaled earlier did not show an increased response to our silk treatments. Correlations between signal onset and time to flight corroborate this result: the correlation between the time to signal onset and time to flight in the silk-absent treatment

Table 2 Variation in *Enchenopa binotata* male signaling behavior across spider silk treatments

	Signaling mode	Silk treatment		Statistics			
		Silk absent (mean±SE)	Silk present (mean±SE)		df	F ratio	p value
# Bouts	Early onset	2.82±0.40	4.05±0.69	Silk treatment	1	0.4	0.55
	Late onset	1.00±0.00	1.17±0.17	Signaling Mode	**1**	*7.2*	*0.01*
				Silk×mode	1	0.07	0.80
Bout rate	Early onset	2.13±0.58	1.53±0.44	Silk yes/no	1	1.1	0.30
	Late onset	0.08±0.004	0.28±0.10	Signaling mode	**1**	*11.5*	*0.002*
				Silk×mode	**1**	*4.8*	*0.034*
Signals/bout	Early onset	4.95±0.64	4.59±0.41	Silk yes/no	1	0.01	0.92
	Late-onset	5.5±0.5	5.2±0.5	Signaling mode	1	0.03	0.87
				Silk×mode	1	0.04	0.85
Inter-signal interval	Early onset	2.7±0.1	2.8±0.1	Silk yes/no	1	0.0002	0.99
	Late onset	2.7±0.3	2.6±0.2	Signaling mode	1	0.04	0.84
				Silk×mode	1	0.46	0.50
Whine length (s)	Early onset	0.933±0.034	0.910±0.028	Silk yes/no	1	0.96	0.33
	Late onset	0.915±0.007	0.982±0.030	Signaling mode	1	3.0	0.10
				Silk×mode	1	0.49	0.49
# Pulses/signal	Early onset	4.00±0.15	4.05±0.17	Silk yes/no	1	1.1	0.31
	Late onset	4.50±0.50	3.83±0.17	Signaling mode	1	1.8	0.19
				Silk×mode	1	1.0	0.33
Pulse rate	Early onset	4.43±0.10	4.59±0.11	Silk yes/no	1	0.13	0.72
	Late onset	5.01±0.55	4.95±0.16	Signaling mode	**1**	*6.4*	*0.016*
				Silk×mode	1	1.1	0.29
Frequency (Hz)	Early onset	170.1±1.7	170.2±1.5	Silk yes/no	1	3.2	0.08
	Late onset	168.4±0.7	171.2±3.4	Signaling mode	1	2.2	0.15
				Silk×mode	1	3.0	0.09

Significant values are italicized

($r=0.35$, $p=0.104$; Fig. 4b) was similar to that in the silk present treatment ($r=0.56$, $p=0.006$; Fig. 4b).

Plasticity in signaling behavior

Overall, the variation we observed is within the normal range of within-species variation for those traits with previously published data (Cocroft et al. 2010).

Males in early-onset mode produced more signal bouts over the duration of the trial, had higher bout rates, and slower pulse rates (Table 2), which we interpret as consistent with the call-fly syndrome. We found a general lack of influence of silk treatment on the signaling behavior of males in either mode (Table 2). Early- and late-onset males seemed to respond differently to the silk treatments in terms of bout rates (significant treatment × signaling mode interaction term; Table 2): early-onset signalers did not modify their signaling rates ($p=0.14$; Table 2; Fig. 5a), but late-onset males seemed to show increased bout rates in the silk-present treatment ($p=0.03$; Table 2; Fig. 5a). However, this result is due to two males in late-onset mode producing a single signal bout early on in the trial. Given this, and the p value for the interaction term being close to 0.05, the criteria outlined above (see "Statistical analyses") lead us to consider that this difference is spurious.

Within just the males in early-onset mode, males that signaled later showed lower signaling efforts (lower bout rates, increased inter-signal intervals) and increased pulse rates (Table 3). We found a significant effect of silk treatment on bout rate (Table 3). However, this difference seems to be due to a single outlier male (Fig. 5b) and again we consider this result to be spurious.

Discussion

We tested for plasticity in mate-searching and signaling behavior in response to cues of a sit-and-wait predator. We asked whether the nature of the threat imposed by a spider web—which intercepts insects in flight (Harwood et al. 2003; Foelix 1996)—would correspond to greater plasticity in mate searching than in signaling. In particular, *Enchenopa* males in call-fly mode should be at greatest risk of interception in a web and should therefore show the greatest plasticity in mate-searching behavior as evidenced in longer delays in flying from the stem after signaling. In agreement with this prediction, we found that only males in call-fly mode altered their mate-searching behavior when spider silk was present and did so by delaying flight. This pattern of plasticity likely reflects a tradeoff between reducing the risk of predation—avoiding interception by the web by delaying flight—and minimizing missed opportunities for acquiring mates.

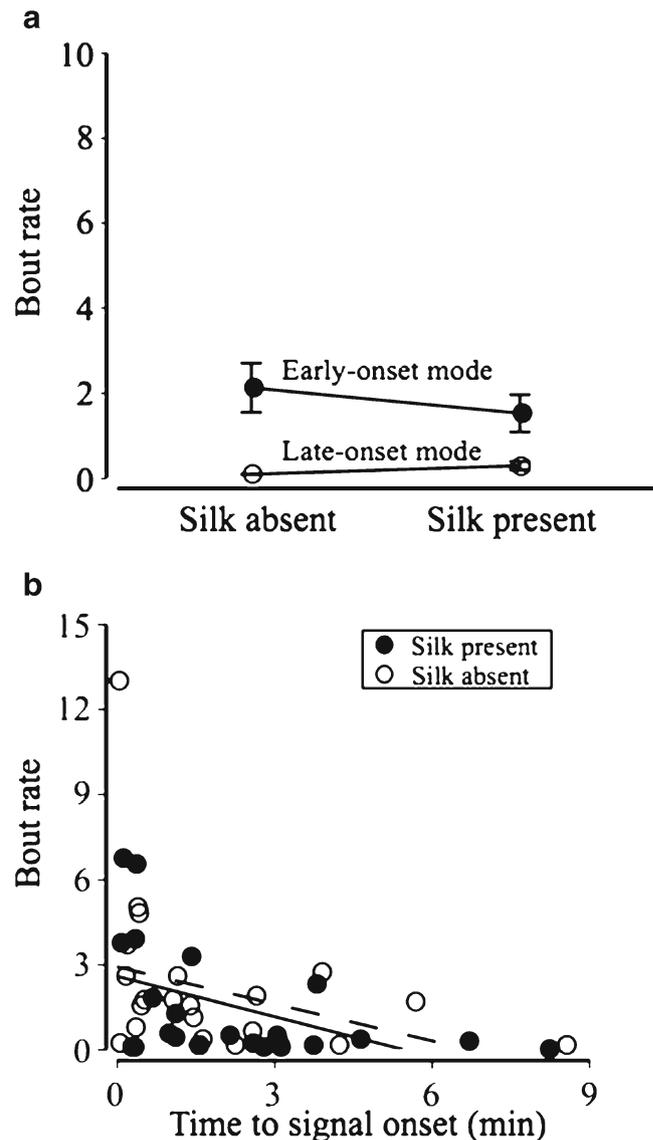Responses to predator cues should correspond with the magnitude of the risk of predation (Helfman 1989; Sih 1980;

Fig. 5 Variation in signal bout rate of *Enchenopa* males in response to spider silk treatments. **a** Relationship between the time to signal onset and signal bout rate in response to silk treatment for males in early- and late-onset signaling modes. **b** Relationship between signal onset and bout rates in response to silk treatment for only those males in early-onset signaling mode

Milinski and Heller 1978). The first component of this idea that we addressed was that the type of predation threat perceived by an individual should determine the pattern and form of plasticity exhibited. For example, anurans vary in their response to different predator cues according to the risk each predator imposes (Relyea 2001); water striders move toward the water's edge when predatory sunfish are nearby and avoid the water's edge when spiders sit on the banks (Krupa and Sih 1998); Guppies freeze or hide (depending on habitat cover) when aerial predators are overhead but will inspect aquatic predators (Templeton and Shriner 2004); vervet monkeys

Table 3 Variation in *Enchenopa binotata* male signaling behavior across spider silk treatments, and as functions of the time to the onset of signaling

	Statistics	df	F ratio	p value	Correlation (p)
# Bouts	Silk treatment	1, 25.5	1.9	0.1773	
	Time to signal	1, 39	0.4	0.5503	
	Silk×time to signal	1, 40.7	0.6	0.4265	
Bout rate	Silk treatment	1, 41	4.6	*0.0384*	
	Time to signal	1, 41	8.1	*0.0068*	−0.40, *0.0064*
	Silk×time to signal	1, 41	1.2	0.2853	
Signals/bout	Silk treatment	1, 23.1	0.2	0.6749	
	Time to signal	1, 38	0.3	0.5818	
	Silk×time to signal	1, 39.2	0.7	0.398	
Inter-signal interval	Silk treatment	1, 41	1	0.3267	
	Time to signal	1, 41	5.6	*0.0228*	0.33, *0.0267*
	Silk×time to signal	1, 41	0.01	0.9109	
Whine length (s)	Silk treatment	1, 16.6	0.09	0.7646	
	Time to signal	1, 32.7	0.22	0.6451	
	Silk×time to signal	1, 25.5	0.83	0.3704	
# Pulses/signal	Silk treatment	1, 12.4	0.01	0.9328	
	Time to signal	1, 26.5	3.0	0.0938	
	Silk×time to signal	1, 19.7	0.7	0.4078	
Pulse rate	Silk treatment	1, 15.4	2.6	0.1262	
	Time to signal	1, 30.4	10.7	*0.0026*	0.42, *0.0043*
	Silk×time to signal	1, 23.66	0.5	0.4974	
Frequency (Hz)	Silk treatment	1, 19.1	0.004	0.9533	
	Time to signal	1, 37.1	1.5	0.2325	
	Silk×time to signal	1, 30.3	0.05	0.8282	

Analyses are for only males in active mate-searching mode (i.e., early-onset signalers). Significant values are italicized

move up into the trees in response to terrestrial predators and down towards the ground in response to aerial predators (Makin et al. 2012). Even within a given type of predator, empirical and modeling approaches show that the plasticity in prey behavior should vary with the behavior of the predator (McGhee et al. 2013). Here, we support this idea from a different perspective. We show that males exhibit plasticity in the specific behavioral categories most likely to increase the risk of predation by specific predators (i.e., web-building spiders). While flight behavior associated with mate searching increases the risk of capture in a web, signaling males should not be at great risk of becoming entangled in a web while stationary on the plant stem and should show little plasticity in signaling behavior. Indeed, we find little change in signaling behavior (for males in either mode) in response to the silk treatments.

A complete test of the above prediction would include assessment of plasticity in mate-searching versus signaling behavior in the presence of cues from other types of predators. Males may modify mate-attraction behavior (e.g., their advertisement signals) in order to decrease the risk of attracting eavesdropping predators (Lima and Dill 1990; Simon 2007; Bertram et al. 2004; Taylor et al. 2005). For example, when acoustically orienting predators are present, we might predict greater plasticity in signaling behavior for *Enchenopa* males but weaker plasticity in flight behavior. Exploring these ideas further will increase our understanding of the complexity of factors that influence mate-acquisition behavior.

The second prediction that we tested was that males at inherently higher risk should show greater levels of plasticity in response to predator cues. For example, male spiders and crickets with more conspicuous courtship (and presumably higher risk of predation) also show higher plasticity in response to the threat of predation (Hedrick 2000; Fowler-Finn and Hebets 2011). Our first result was consistent with this prediction: males in call-fly mode (i.e., early-onset signalers) showed a significant response to silk treatment, whereas those males not in call-fly mode showed no response. However, we also expected that within males in call-fly mode, later

signalers should show weaker responses to the presence of predator cues. We found no support for this prediction: the correspondence between signaling onset and signaling behavior did not vary across treatments. This could be a result of a threshold response where males in call-fly mode are at higher risk, but variation in signal onset within call-fly mode does not significantly increase risk. A similar pattern has been found in wolf spiders where ornamented males show a greater response to predator cues than non-ornamented males, but within ornamented males, those with larger ornaments do not show an increased response (Fowler-Finn and Hebets 2011).

In general, plasticity is favored when no one behavioral phenotype achieves highest fitness across a variable environment (e.g., Gomulkiewicz and Kirkpatrick 1992; Via et al. 1995; Moran 1992; Ghalambor et al. 2007). Here, the tradeoff between mate acquisition and predator avoidance should vary across predator environments. When predator cues are present, delayed flight is likely to increase fitness because of a reduced risk of capture in spider webs; however, delaying flight reduces call-fly effort and the likelihood of encountering responsive females and will not be beneficial when predators are absent. Overall reduced movement or mate searching in the presence of predators can decrease encounter rates with predators and increase survival (Persons et al. 2001; Persons et al. 2002; Eiben and Persons 2007; Downes 2002). The tradeoff between these costs of reduced mating opportunities and costs of predation ultimately depends upon the nature of the risk imposed by the predator. Some predators may impose greater costs on males while mate searching and others while signaling. Ultimately, the levels and patterns of plasticity exhibited may vary with the type of predator and other factors in the environment and intrinsic to an individual that determine the potential loss of mating success incurred by reduction of mate-acquisition behavior.

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