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Signalling interactions during ontogeny are a cause of social plasticity in *Enchenopa* treehoppers (Hemiptera: Membracidae)

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ABSTRACT

We recently discovered that there is a social ontogeny of signals and preferences in *Enchenopa* treehoppers. Nymphs signalled throughout their development; some signal features changed gradually and in sexually dimorphic ways throughout ontogeny; and some adult male signal features and female mate preferences differed between individuals reared in isolation or groups. In this paper, we investigate whether signalling interactions during ontogeny are a cause of plasticity in mating signals and preferences. We subjected *Enchenopa* nymphs to treatments of either: rearing in aggregations (the natural condition), in isolation, or in isolation with playbacks of nymph signals. We then described variation in the signals and mating preferences of individuals that developed in those conditions. The playback treatments partially "rescued" the signal and preference phenotypes, resulting in phenotypes either similar to those that result from rearing in aggregations, or intermediate between those that result from rearing in isolation or in aggregations. These results pin-point signalling interactions during ontogeny as an important cause of plasticity in signals and mate preferences.

1. Introduction

Interacting phenotypes are traits whose expression in an individual is at least in part a function of interactions with other individuals (Moore et al., 1997). How an animal acts, for instance, depends in no small measure on how others around it behave and react to it, whether in foraging, aggression or courtship. Interacting phenotypes have a special role in evolution because they generate feedbacks at two levels: they are causes of plasticity that are themselves plastic, and they are causes of selection that are themselves targets of selection. These feedbacks influence trait variation, the form and strength of selection, and the ability of traits to respond to selection (West-Eberhard, 1983, 2014; Moore et al., 1997; Wolf et al., 1998; Bailey et al., 2018; Rodríguez et al., 2018a).

The potential evolutionary consequences of interacting phenotypes are of special interest in the case of mating signals and mate preferences. Signal-preference feedbacks may speed divergence and promote speciation, even in the absence of direct genetic covariance between signals and preferences (Bailey and Moore, 2012; Rebar and Rodríguez, 2015). The mechanisms underlying interacting phenotype dynamics are diverse, ranging from the physiological effects of physical contact, and simple forms of experience-mediated plasticity, to complex systems of learning involving social feedback during ontogeny (Rodríguez et al., 2013; Stamps, 2016; Svensson et al., 2010; Verzijden et al., 2012). For example in many oscine birds, humans, and some other mammals, young individuals must practice and learn their signals with tutors (Akçay et al., 2017; Fitch, 2010; Mennill et al., 2018; Prat et al., 2015; Takahashi et al., 2015). In the absence of such feedback, individuals do not develop species typical songs or develop them slower.

The general importance of ontogenetic causes of signal-preference variation depends on the phylogenetic distribution of social ontogeny, and on the timing of the induction of variants relative to dispersal and mating (Verzijden et al., 2012). It might seem that early-life social-induction of signal-preference plasticity is restricted to a few vertebrate groups. However, there is evidence that prior experience influences communication systems of some insects and spiders (Grüter and Czaczkes, 2019; Hebets and Sullivan-Beckers, 2010; Rodríguez et al., 2013).

We recently reported on a process of social ontogeny as a cause of variation in the communication system of an insect, a member of the *Enchenopa binotata* species complex of treehoppers (Desjonquères et al., 2019). We found that *Enchenopa* nymphs interacted with each other throughout their development with signals that showed sexually dimorphic ontogenetic trajectories; further mate preferences and some signal features differed between adults reared in isolation or in groups,

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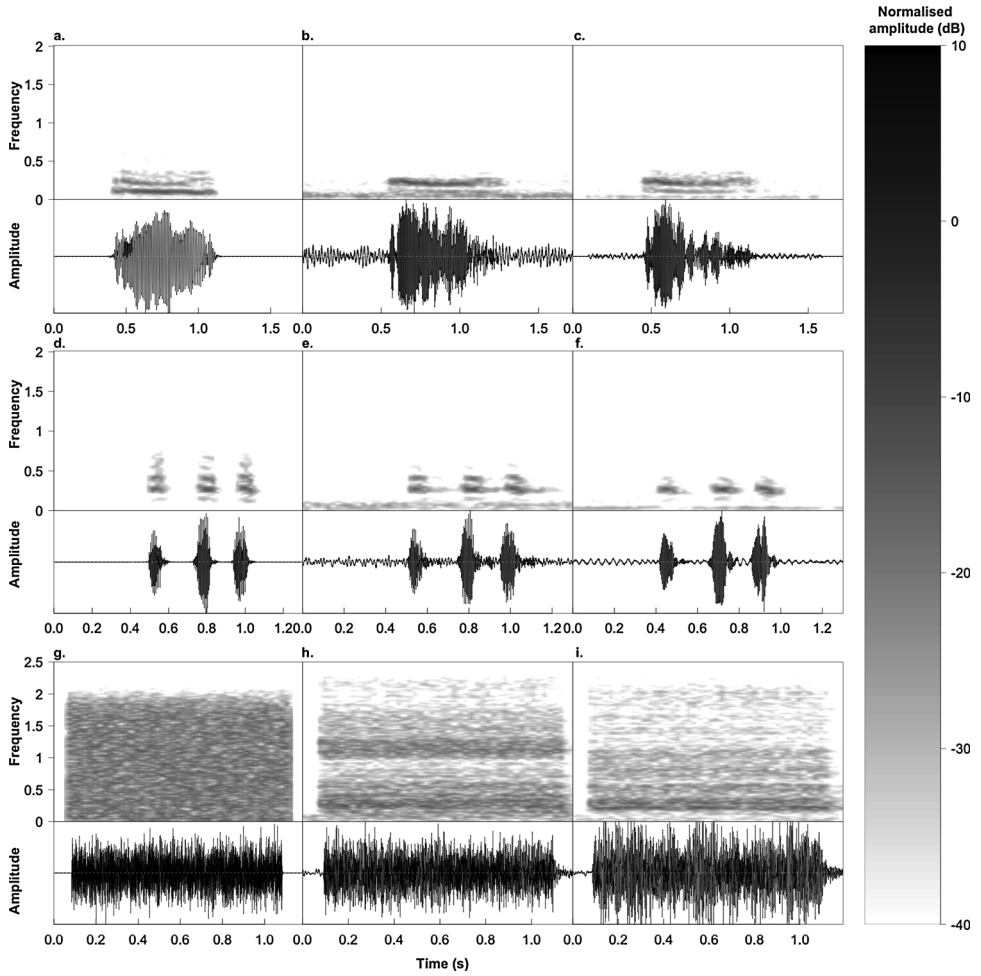

Fig. 1. Playback stimuli used in the experiment. (a) Example of one of the playback recordings for long signal. (b–c) Recording of the long signal example emitted by the speaker and transmitted through the plant. (d) Example of one of the playback recordings for short signal. (e–f) Recording of the short signal example emitted by the speaker and transmitted through the plant. (g) Example of the playback recording for noise. (h–i) Recording of the noise emitted by the speaker and transmitted through the plant. Fourier window length: 2048 samples, frame overlap: 50%, window type: Hanning.

although even individuals in isolation developed species-typical signals and preferences. We interpret this as an innate communication system in which the developing environment is nevertheless an important cause of variation which can influence the strength and direction of sexual selection.

The finding of a social ontogeny to the communication system of an insect suggests that early-life induction of interacting phenotype dynamics may be more widespread than currently anticipated, occurring even in species without sophisticated learning mechanisms (Desjonquères et al., 2019). This suggestion is in agreement with other observations that variation in the groupings in which *Enchenopa* treehoppers develop influences nymph signalling rates (Rodríguez et al., 2018b) and adult mating signals and mate preferences (Fowler-Finn et al., 2017; Rebar and Rodríguez, 2015, 2013), as well as sexual receptivity and signalling effort in another insect species (Kasumovic et al., 2012). Social experience can even have effect on non signal traits such as web building in spiders (DiRienzo et al., 2019) or grouping behaviour (Schausberger et al., 2017). These effects may be due to a number of factors, however; e.g., the key variable may be experience of signalling environments, actual between-individual signalling interactions, physical contact, and so on.

Here we report on a vibrational playback experiment that we used to test the hypothesis that signalling interactions during development are the main cause of plasticity in the social ontogeny of *Enchenopa* signals and preferences. In this experiment, the hypothesis predicts that: (i) only traits affected by group-isolated rearing treatments (Desjonquères et al., 2019) should be affected by playbacks of nymph signals to individuals reared in isolation; and (ii) playbacks to isolated nymphs throughout ontogeny should rescue adult signals and preferences — i.e. the playback treatment should have the same effects on adult signals and preferences as rearing nymphs in groups. Alternatively, signalling interactions may be only one of many causes of plasticity during ontogeny. If so, playing back conspecific signals to isolated nymphs should result in intermediate signal and preference phenotypes between socially isolated and grouped individuals. Finally, the null hypothesis that signalling interactions are not the cause of social plasticity predicts that playing back conspecific signals to isolated nymphs should result in the same phenotypes as fully isolated individuals.

2. Material and methods

Most of the species in the *E. binotata* complex remain to be described (Hamilton and Cocroft, 2009) but can be distinguished based on the coloration of the nymphs, the host plant they use, and the frequency of adult male signals (Cocroft et al., 2010, 2008). We used the species present on nannyberry plants (*Viburnum lentago*, Adoxaceae), that has grey nymph coloration, and adult male signals with a dominant frequency of about 165 Hz. As our species of interest has not yet been formally described, we preserved all individuals used in our

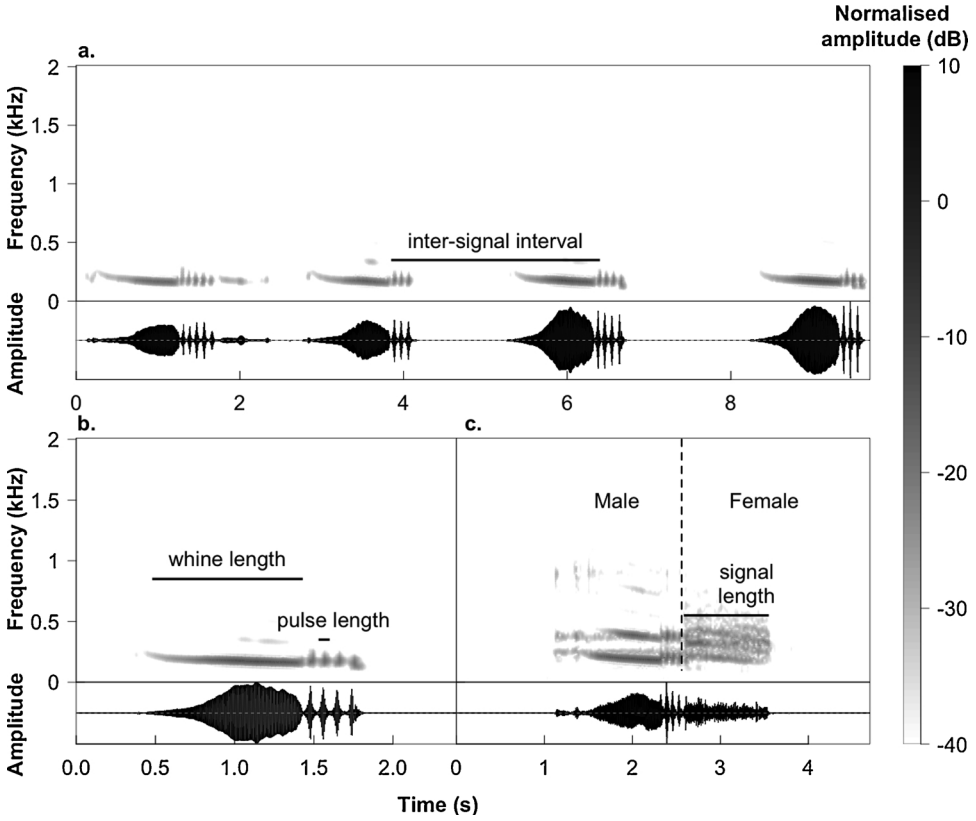

Fig. 2. *Enchenopa* male and female signal measurements. (a) Male signalling bout. Males produced bouts of several signals. We measured the number of signals per bout (signal number); and the interval between the end of the 2nd and 3rd signals (intersignal interval); (b) Male signal. Male signals consisted of two elements, a whine and pulses. We measured the duration of the whine of the 3rd (or closest to 3rd) signal in seconds (whine length); the duration of the 2nd pulse (pulse length); the number of pulses in the 3rd (or closest to 3rd) signal; and the dominant frequency. (c) Female duet signal in response to the standard male primer. Female signal consisted in a broad band sound lasting between 0.3–3 seconds. We measured signal length; fundamental frequency; and the frequency modulation between the beginning and the end of the signal. Fourier window length: 2048 samples, frame overlap: 50%, window type: Hanning.

experiments in 70% ethanol. This species is common over North-America and there are no specific regulations on its use for laboratory experiments.

We acquired the data for the present paper in the course of a prior study (Desjonquères et al., 2019) in which we reared treehoppers on potted host plants either in isolation (1 nymph/host plant) or in groups (30–40 nymphs/plant). We ran the experiment over the Spring and Summer of 2017 with treehoppers from the population in Downer Woods on the UWM campus.

For the current study, we randomly assigned the above singly-raised treehoppers to treatments of weekly playbacks (playback treatment) or no playback (silent treatment). We have already reported on the ontogenetic signal trajectories of singly-raised treehoppers in the silent treatment and how their adult signals and preferences differed from those of group-raised treehoppers (Desjonquères et al., 2019). Here we compare those signal and preference phenotypes with those of singly-raised treehoppers in the playback treatment, in order to test the role of signalling interactions and experience *per se* in the social ontogeny of *Enchenopa* adult signals and preferences.

Enchenopa nymphs and adults emit vibrational signals throughout their life (Cocroft et al., 2008; Desjonquères et al., 2019; Rodríguez et al., 2018b). In membracids, the main mechanism of signal production is contraction of muscles in the thorax and abdomen, which causes periodic movements of the abdomen that impart vibrations onto the substrate (Miles et al., 2017). *Enchenopa* adult signals function in pair formation and mate choice (Cocroft et al., 2008; Rodríguez et al., 2006, 2004). The functions of *Enchenopa* nymph signals are still unknown, but in other membracid nymph, signals are used in cooperative feeding or alarm systems (Cocroft, 2005; Hamel and Cocroft, 2012; Morales et al., 2008; Ramaswamy and Cocroft, 2009).

Once a week until adult moult, we presented each nymph in the playback treatment with three types of stimulus delivered in random sequence: recordings of two nymph signal types (short and long call; Rodríguez et al., 2018b; Desjonquères et al., 2019) and a white noise playback (Fig. 1). We obtained the playback stimuli from a reference library of nymph recordings. We selected 10 low-noise recordings for each signal type, and cycled them randomly for use as playbacks. The noise playback was a synthetic white noise generated in R (v. 3.5.2; R Core Team, 2015) with the package seewave (v. 2.1.0; Sueur et al., 2018).

The weekly sequence of stimulus playback lasted for 15 min. It consisted of three five minute intervals containing one type of stimulus played for 30 s followed by 4 min and 30 s of silence. After the random sequence of playbacks, we also brushed each nymph three times with a paintbrush, to mimic a predator attack (following Ramaswamy and Cocroft, 2009; Rodríguez et al., 2018b); this final treatment was another potential way to induce nymphs to signal (e.g. if they produce alarm signals). On average, each nymph received 3 ± 1 (mean ± sd) weeks of playback sessions until they moulted to the adult stage.

As we had a large number of nymphs on individual potted plants to treat, we delivered the playbacks with loudspeakers (Logitech Z130, Silicon Valley, California). This results in the airborne sounds being imparted onto the plant as substrate vibrations (Rebar et al., 2012). This method is likely to alter the features of the stimuli, due to filtering as the playbacks are imparted onto the plants and transmitted along the plant tissues (Cocroft et al., 2006; Virant-Doberlet and Cokl, 2004). Nevertheless, the temporal and spectral features of the nymph signal stimuli were largely unaffected, although the noise playback lost some frequency bands (Fig. 1). We calibrated stimulus amplitude to correspond to the typical amplitude of the nymph signals (see below for recording method).

2.1. Recording and measurement of adult signals

We recorded the above playbacks, as well as the signals of adult males and females with a portable laser Doppler vibrometer (Polytec PLV-100; Polytec Inc. Auburn, MA, USA). We focused the laser beam on a piece of adhesive reflective tape (ca. 5 mm^2) secured on the stem of the recording plant. We sent the laser signal through a band pass filter set to 40–4000 Hz (Krohn-Hite 3202; Krohn-Hite Corp., Brockton, MA,

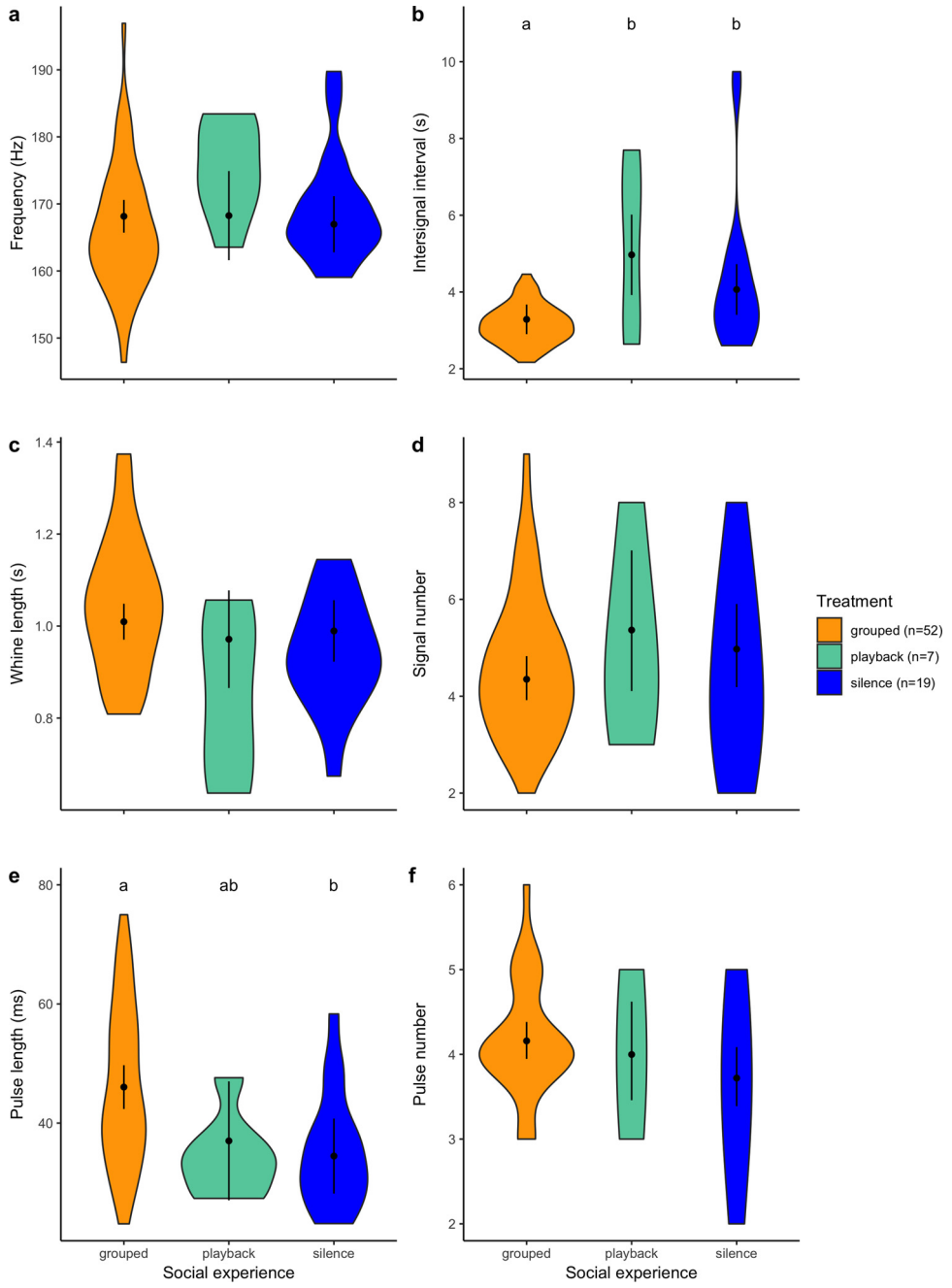

Fig. 3. Effect of signalling experience during ontogeny on *Enchenopa* male signal features. Distribution of the signal features of males are represented in violin plots. Each violin plot represents the kernel density plot for a social experience. Point and error bar showing fitted values and 95% confidence interval for the values fitted by the linear models testing for an effect of social experience on male signal features; and controlling for temperature and age (measured as days after adult moult).

USA) and then to an iMac computer through a USB audio interface (Edirol UA-25; Roland, Corp. Hamamatsu, Japan). We recorded the signals with the program AUDACITY (v. 2.1.2; http://audacity.sourceforge.net/) at a sampling rate of 44.1 Hz.

Sexually active *Enchenopa* males (starting approximately two weeks after the adult moult) signal spontaneously when placed on a stem of their host plant. We took advantage of this behaviour to record them. We placed a male on a potted plant and if he did not signal after the first three minutes, we primed him with a playback of a recorded male (see below for vibrational playback method).

Sexually receptive *Enchenopa* females duet with the signals of males that they find attractive (Cocroft et al., 2008; Rodríguez et al., 2012, 2006, Rodríguez et al., 2004). We used this behaviour to record female signals (see below for vibrational playback method).

Males and females that did not signal at the time of the trial were placed back on their plant and tested again two days later. Male signals were recorded 24 ± 6 days (mean ± sd) after adult moult and female signals 44 ± 7 days after adult moult.

We analysed the male and female signals using AUDACITY and R. For males, we measured the number of signals per bouts and pulses per signals, the bout, signal, whine and pulse length, the intersignal interval, the pulse rate, and the dominant frequency of the call (Fig. 2a-b). We later excluded measures that were highly correlated with the other (we excluded the following: signals/bout, signal length, and pulse rate; see below). For female signals, we measured signal length, fundamental frequency and frequency modulation (Fig. 2c).

Table 1
Effect of signalling experience during ontogeny on *Enchenopa* male signals. Results of linear models testing for effect of signalling experience and time after moult on male signals, and controlling for the effect of temperature.

Response variable	Term	Statistics (F or z)	Degrees of freedom	P-value
Signal dominant frequency	Signalling experience	0.08	2, 73	0.925
	Time after moult	0.11	1, 73	0.737
Intersignal interval	Signalling experience	13.08	2, 73	**< < 0.001**
	Time after moult	0.41	1, 73	0.522
Whine length	Signalling experience	0.87	2, 73	0.423
	Time after moult	0.71	1, 73	0.402
Signal number	Signalling experience	−1.12	2, 73	0.571
	Time after moult	1.92	1, 73	**0.055**
Pulse length	Signalling experience	3.79	2, 73	**0.027**
	Time after moult	1.28	1, 73	0.263
Pulse number	Signalling experience	0.42	2, 73	0.810
	Time after moult	0.19	1, 73	0.850

2.2. Recording and description of female mate preferences

To describe female preferences for signal frequency, we used vibrational playbacks of synthetic stimuli varying in frequency, with all other features set to the population mean (e.g., males in the population produce bouts with a mean of 4 signals/bout, so each of our stimuli had 4 signals per bout; further details in Fowler-Finn et al., 2017). We presented each female with a random sequence of 17 playback stimuli with frequencies varying from 130 to 230 Hz. This range of stimuli slightly exceeds the range of signal frequency values in the population, which is the recommended practice to capture the full shape of the preference functions (Kilmer et al., 2017). The increments of frequency were smaller near the likely peak of the preferences (steps of 2–10 Hz) to allow us better to capture variation in peak preference (see below).

Enchenopa females express their mate preferences through selective duetting with males, and their behaviour when interacting with playback stimuli offers a practical and realistic indication of their evaluation of signal attractiveness (Rodríguez et al., 2012, 2006, Rodríguez et al., 2004). Our assay of female preference was the number of responses (between 0 if she did not respond and 4 if she responded to all the signals in the synthetic bout) that each female produced in response to each of the 17 stimuli.

Mate preference functions describe variation in signal attractiveness over a range of signal trait values (Jennions and Petrie, 1997; Ritchie, 1996; Wagner, 1998). They are expressed as a function of the signals females encounter: they are function-valued traits (Kilmer et al., 2017; Stinchcombe et al., 2012). We therefore used a function-valued approach to describe individual female mate preference functions. We used the program PFunc (v. 1.0.0; https://github.com/Joccalor/PFunc) to fit cubic spline regressions to the response data for each female and generate individual preference function curves; this is an approach that does not assume any particular shape for the functions other than some level of smoothness that is determined empirically (Kilmer et al., 2017; Schluter, 1988).

We then analysed variation in the individual preference functions using three metrics out of the five inbuilt in PFunc (see below; Kilmer et al., 2017): (1) preference peak: most preferred display trait value, measured as the frequency corresponding to the highest point on the preference function; (2) peak responsiveness: response value at the preference peak, measured as the highest point on the preference function; (3) preference strength: degree to which attractiveness falls away from peak preference as display values change, measured as the standard deviation of y values normalized by the mean.

2.3. Statistical analysis

We conducted all analyses in R using the functions lm or glm of the package lme4 (v. 1.1–12; Bates et al., 2014). Our data included six signals features for males, and three for females as well as three preference metrics for female mating preference. This may introduce two sources of risk of spurious significance for our analyses (Rice, 1989): we ran a high number of tests, and some of the traits in the data sets were correlated with each other. However, corrections for multiple testing compromise statistical power (Moran, 2003; Nakagawa, 2004). We dealt with this problem in two ways. First, we excluded from the analyses traits that were highly correlated ($r > 0.5$) with other traits already included (see above). We allowed one exception to this rule: the frequency and whine length of adult male signals were correlated, but we retained them in the analyses because they are associated with the strongest mate preferences in the complex (Rodríguez et al., 2006). Second, we followed a table-wide criterion for analysing significance tests: whenever a test or statistical table contained five or more tests, we asked whether significant terms were widespread and diverse across the tables or whether only a single or a few terms were significant; in the latter case, we deemed it likely to indicate spurious significance (Moran, 2003).

To test for an effect of social experience treatments on adult signal features and preferences, we used generalized linear models. We ran a separate test for each dependent variable; these were: male and female signal features defined in Fig. 2 and female preference traits defined above. In each test, the explanatory variables were: social experience (group, playback, or isolation), age (in days after adult moult) and recording temperature (as a control). The error structure was Gaussian for all models except number of pulses and number of signals for which the error structure was a Poisson distribution. We checked the assumptions of normality and homogeneity of the residuals by visually inspecting a quantile-quantile plot and the residuals against the fitted values, both indicating no deviation from these assumptions. We inspected model stability by excluding data points one at a time from the data. We derived variance inflation factors (Field, 2009) using the function vif of the R-package car (version 2.1–4; Fox and Weisberg, 2011) and they did not indicate collinearity between fixed effects to be an issue. We compared the full model with the null model (excluding the predictor tested) for significance testing.

3. Results

The rearing and playback treatments had little effect on adult male signals. Only two out of the six signal traits we measured varied significantly between treatments (this study, Fig. 3; Table 1; Desjonquères et al., 2019), and the patterns varied for the two traits. Intersignal interval was similar in the playback and silent treatments and different from the group-rearing treatment (Fig. 3b; Table 1), whereas pulse length was intermediate in the playback treatment (Fig. 3e; Table 1).

None of the three adult female signal traits that we measured varied significantly between treatments (this study, Fig. 4; Table 2; Desjonquères et al., 2019).

By contrast, two of the three female mate preference function traits

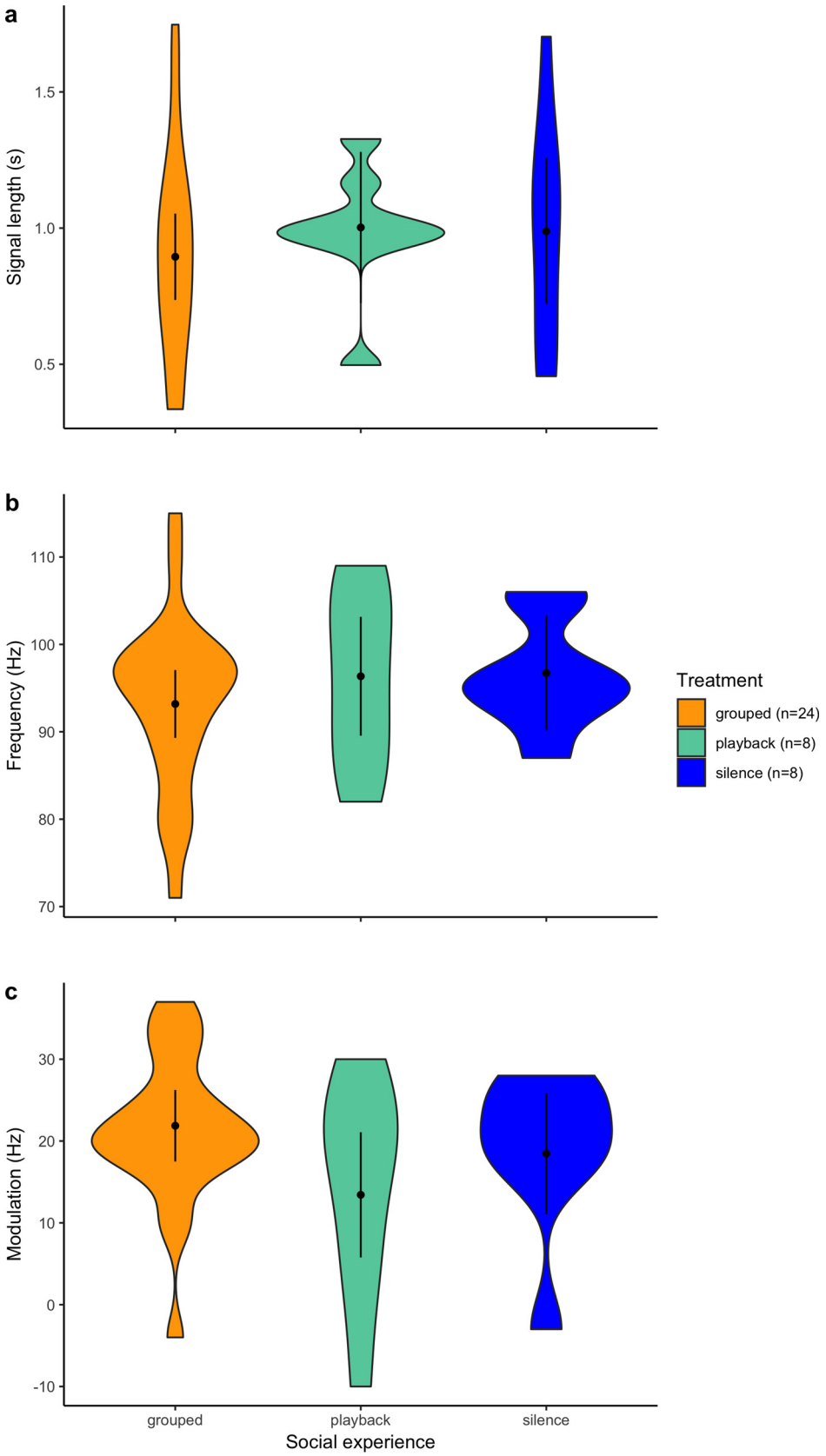

Fig. 4. Effect of signalling experience during ontogeny on *Enchenopa* female signal features. Distribution of the signal features of females are represented in violin plots. Each violin plot represents the kernel density plot for a social experience. Point and error bar showing fitted values and 95% confidence interval for the values fitted by the linear models testing for an effect of social experience on female signal features; and controlling for temperature and age (measured as days after adult moult).

Table 2
Effect of signalling experience during ontogeny on *Enchenopa* female signals. Results of linear models testing for effect of signalling experience and time after moult on female signals, and controlling for the effect of temperature.

Response variable	Term	Statistics (F)	Degrees of freedom	P-value
Signal length	Developmental experience	0.39	2, 35	0.680
	Time after moult	0.22	1, 35	0.641
Signal fundamental frequency	Developmental experience	0.95	2, 35	0.396
	Time after moult	2.56	1, 35	0.119
Signal frequency modulation	Developmental experience	1.62	2, 35	0.213
	Time after moult	0.41	1, 35	0.527

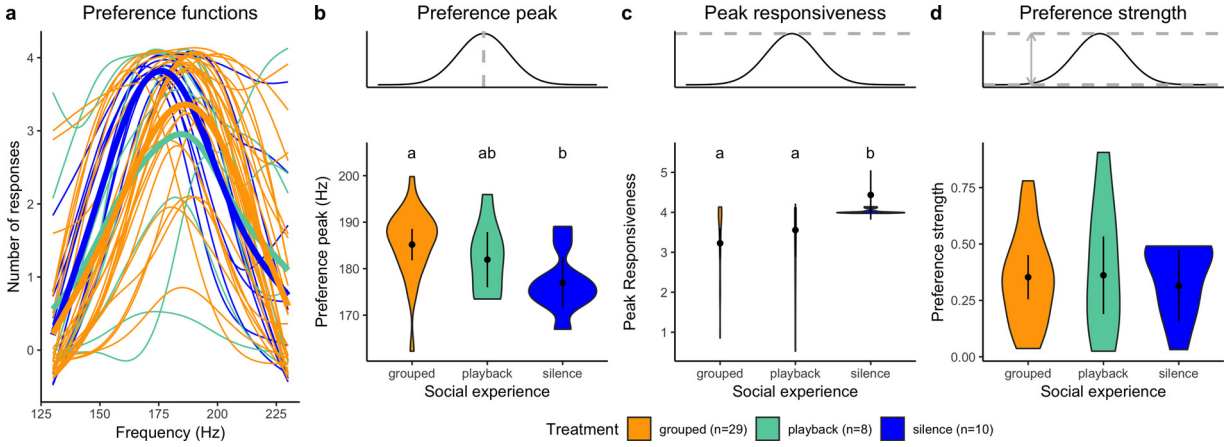

Fig. 5. Effect of signalling experience during ontogeny on *Enchenopa* female preference. (a) Preference functions for the three social experience treatments. (b–d) Top: definition of each preference trait. Bottom: distribution of the female preferences are represented in violin plots. Each violin plot represents the kernel density plot for a social experience. Point and error bar showing fitted values and 95% confidence interval for the values fitted by the linear models testing for effect of social experience on female preferences; and controlling for temperature and age (measured as days after adult moult).

Table 3
Effect of signalling experience during ontogeny on *Enchenopa* female preference. Results of linear models testing for effect of signalling experience and time after moult on female preferences, and controlling for the effect of temperature.

Response variable	Term	Statistics (F)	Degrees of freedom	P-value
Preference peak	Developmental experience	4.35	2, 41	**0.019**
	Time after moult	0.002	1, 41	0.969
Peak responsiveness	Developmental experience	3.04	2, 41	**0.059**
	Time after moult	4.89	1, 41	**0.032**
Preference strength	Developmental experience	0.20	2, 41	0.820
	Time after moult	0.46	1, 41	0.501

that we measured varied between treatments (this study, Fig. 5; Table 3; Desjonquères et al., 2019). Peak preference was intermediate in the playback treatment (Fig. 5b; Table 3), and peak responsiveness was similar in the playback and group-rearing treatment and different from the silent treatment (Fig. 5c; Table 3). Preference strength did not vary significantly among the treatments (this study, Fig. 5d; Table 3; Desjonquères et al., 2019).

4. Discussion

We asked about the role of signalling interactions in the social ontogeny of *Enchenopa* mating signals and mate preferences. We found that only the two signal traits and two preference traits that were influenced by group- vs isolated-rearing treatments (Desjonquères et al., 2019) were also influenced by our playbacks to isolated nymphs. Further, one out of the two signal traits and two out of two mating preference traits were at least partially rescued by the playback of conspecific signals during signal ontogeny.

In the *E. binotata* complex, the strongest female mate preferences are for male signal frequency (Rodríguez et al., 2006), rather than for the signal traits that were affected by our experiment (intersignal interval and pulse length). This, added to the fact that we only detected effects in two out of six male signal traits (and three female signal traits) would suggest that this result may be spurious (Moran, 2003). However, the two male signal traits affected by the playback are the same as were affected by rearing in isolation-groups (this study; Desjonquères et al., 2019), which indicated that the effect is likely real and due to signalling interactions. Further, even weak preferences may influence male signal attractiveness and mating success. By contrast, two of the three mate preference function traits that we measured were affected by our experiment, and these were the same as the traits affected by rearing in isolation-groups (this study; Desjonquères et al., 2019), a result unlikely to be spurious. Thus the effect of signalling interactions on signals and preferences may have important consequences for selection on signals.

In this experiment, we did not include a control with only noise playbacks during the development. However, in an another study, nymphs interacted significantly more with conspecific signals (the short signal stimulus) than with the noise playback (Desjonquères et al. in prep). This suggests that the effects we detect are due to the experience of signalling and interacting with playbacks as conspecific signals rather than noise.

Our results also suggest that signalling interactions are one of the

mechanisms shaping the development of mating signals and preferences. These long term developmental effect on signals and preferences, termed social ontogeny, are also observed in other species such as many species of birds and some mammals (Abramson et al., 2018; Margoliash and Tchernichovski, 2015; Mennill et al., 2018; Verzijden et al., 2012). Recent findings indicate that the above social development effects can occur even in animals with innate communication systems (this study; Desjonquères et al., 2019; Takahashi et al., 2017).

Different signal and preference traits were influenced by signalling interactions in different ways. Some traits such as frequency, whine length or pulse rate for signal features as well as preference strength were not affected at all by social experience or signalling interactions. By contrast, other traits were influenced only by social experience but not signalling interactions, such as male intersignal interval, and some others were influenced both by social experience and signalling interactions, such as pulse duration in males as well as preference peak and peak responsiveness in females (Desjonquères et al., 2019; Fowler-Finn et al., 2017). Variation in ontogenetic trajectories of different trait has also been shown in other groups; e.g., male but not female birds imprint on beak colour in zebra finches (Vos, 1995). Different traits were also affected at different timing. For example in *Enchenopa* treehoppers, acoustic experience during the early adult phase, increased signal rate and signal length (Rebar and Rodríguez, 2016) but not pulse duration or intersignal interval. Thus different features of mating signals and preferences may vary and evolve partly independently. The variations in the timing and causes of plasticity for different traits may therefore have important evolutionary consequences (Verzijden et al., 2012).

Our treatment re-created only the acoustic (vibrational) component to social interactions. It is interesting to observe that in three out of four traits, the phenotype was either partially or not influenced by acoustic experience. One potential explanation for this result is that the rate of our playback treatment was too low. Although nymphs have highly variable rates of signalling, they tend to signal every day at a few signals per minute (Desjonquères et al., 2019; Rodríguez et al., 2018b). Our playbacks, with a few signals over 15 min, only once a week are likely quite low levels of interactions compared to what nymphs typically encounter. Another potential explanation for the partial influence of the playbacks is that other factors may also influence the ontogeny of signals. There could be effects of social interactions through direct contacts. Indeed in the desert locust, *Schistocerca gregaria*, the switch between two extreme behavioural syndromes, solitary and gregarious, can be initiated by a simple tactile stimulation of the hind femora for only four hours (Rogers et al., 2014). Behavioural plasticity can also be mediated by surface molecules called cuticular hydrocarbon (e.g., Griffith and Ejima, 2009). Cuticular hydrocarbons can also be expressed plastically leading to potential interactions between the plastic response to contacts and the plastic expression of those surface molecules (Pascoal et al., 2016). Thus, contacts during social interactions may be a source of plasticity explaining the observed remaining variance. Another factor that could explain some of the residual variance is plant identity. Although in our experiments, plants were replicated for all groups and randomly assigned, plant genotype is known to influence courtship signals and mating preferences in *Enchenopa* treehoppers (Rebar and Rodríguez, 2015, 2014). Additionally, denser aggregations of treehopers on a plant could influence the nutritional value available at a particular location on a plant. Plants with denser aggregation could also have stronger defence responses. Indeed, some plants increase their emission of defence chemicals after vibrational playbacks (Appel and Cocroft, 2014). The increase was only observed with herbivorous chewing sounds and not leafhopper sounds but it would be interesting to test nymph treehopper sounds for which calling rates increase with increasing nymph density on the plant (Rodríguez et al., 2018b). There are therefore remaining causes of plasticity that could potentially be explained by social interactions or other mechanisms linked to the social environment. Although the effect of signalling interactions is in some cases partial, our results clearly demonstrate a significant influence of the playbacks.

In conclusion, our results demonstrate the role of signalling interactions on the development of mating signals and preferences in an insect. This finding broadens the scope for the dynamics of interacting phenotypes and social plasticity to influence the patterns of variation and the evolution of mating signals and mate preferences.

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