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Social ontogeny in the communication system of an insect

Camille Desjonquères*, Jak Maliszewski, Emma Nicole Lewandowski, Bretta Speck, Rafael Lucas Rodríguez

Behavioral & Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, U.S.A.

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In humans and some other mammals and birds, the development of communication systems requires social feedback. How do such systems evolve from ancestral states featuring innate developmental mechanisms? We report evidence of a novel form of social ontogeny in the communication system of *Enchenopa* treehoppers that suggests an answer to this question. These insects use plant-borne vibrational signals throughout their lives. Signal repertoires of nymphs and adults differed and showed sexually dimorphic ontogenetic trajectories; individual differences projected into some of the features of adult signals and mate preferences. Signals and mate preferences differed between adults reared in isolation and adults reared in groups, but even individuals reared in isolation developed species-typical signals. In this type of social ontogeny, peer inputs cause variation in signals and preferences. Thus, even innate communication systems can be socially malleable. This may set the stage for the evolution of obligate social feedbacks in communication: the starting point is already socially plastic and does not require learning to arise de novo.

A key feature of human language — the need for feedback from social interactions early in life for proper development (Fitch, 2010; Pinker, 1994) — turns out to be surprisingly widespread among animals. To date, it has been documented in various other primates, whales and birds (Gultekin & Hage, 2017; Lipkind et al., 2013; Margoliash & Tchernichovski, 2015; Takahashi et al., 2015). Do these discoveries suggest potential ancestral states for human language? Perhaps they point to shared ancient adaptations that set the stage for further elaboration in our lineage (Pika, Wilkinson, Kendrick, & Vernes, 2018). Or are they independent derivations? And, how does the transition from innate to specialized social learning mechanisms occur? Innate communication systems and the ability to learn are both widespread in animals. But what are the early evolutionary stages of a system wherein the consequences of early social experience are expressed and selected on much later in life? Answering these questions will require broader phylogenetic exploration to establish the basis for comparative work.

Here we report on the social nature of the ontogeny of the communication system of an insect. Until recently, there seemed to be little call for research on such a process in invertebrates. The communication systems of the best-known case studies are expressed only in adults, and have been thought to be largely if not wholly innate (Gerhardt & Huber, 2002). However, three recent developments provide a strong rationale for analysing the ontogeny of insect communication. The first is the insight that innate behaviours need not be inflexible — that '"built-in" doesn't mean unmalleable; it means organized in advance of experience' (Marcus, 2004, p. 40). The second is the widespread occurrence of varied forms of socially mediated plasticity in the communication systems of insects and spiders (Bailey, Gray, & Zuk, 2010; Bailey & Zuk, 2008; Hebets & Sullivan-Beckers, 2010; Rodríguez, Rebar, & Fowler-Finn, 2013; Verzijden et al., 2012). The third is the discovery of unsuspected complexity in the social lives of many invertebrates based on research examining substrate-borne vibrational signalling — a widespread modality of communication (Cocroft, Gogala, Hill, & Wessel, 2014; Cocroft & Rodríguez, 2005; Hill, 2008). Many insects, for instance, use vibrational signals in various types of interaction throughout their lives, both as nymphs and adults; e.g. to coordinate cooperative foraging in nymphs; to solicit antipredator defence from parents or mutualists (Cocroft, 2005; Hamel & Cocroft, 2012; Morales, Barone, & Henry, 2008; Ramaswamy & Cocroft, 2009); as well as to compete for mates and select them (Cocroft & Rodríguez, 2005; Rodríguez & Desjonquères, in press). Such interactions could constitute a source of variation during the development of signalling behaviours and receiver responses of many species.

* Correspondence: C. Desjonquères, Behavioral & Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, 53201-0413, U.S.A.
E-mail address: desjonqu@uwm.edu (C. Desjonquères).

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We asked about social ontogeny in the communication system of a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). These plant-feeding insects communicate using substrate-borne vibrational signals and offer considerable evidence that social interactions are an important cause of variation in adult signals and preferences. Manipulating the experience of signal environments by young adults reveals social plasticity in male signal rate and length and in the breadth and strength of female mating preferences for signal frequency (but not in the peak of the preferences; Fowler-Finn & Rodríguez, 2012a,b). Manipulating the density and composition of the social groupings in which the treehoppers develop reveals stronger plasticity in signal frequency and in the peak preference for signal frequency (Fowler-Finn, Cruz, & Rodríguez, 2017; Rebar & Rodríguez, 2013, 2015). These observations suggest a parallel with the process of social ontogeny in the communication systems of humans and other vertebrates, in which early social experiences have greater importance for signal development (Fitch, 2010; Margoliash & Tchernichovski, 2015; Takahashi et al., 2015).

We first tested the hypothesis that there is an ontogeny to the *Enchenopa* communication system. This hypothesis makes the following predictions: (1) not only adults, but also nymphs, should engage in signalling interactions; (2) nymph signals should change throughout ontogeny; (3) as the signals of adult *Enchenopa* are sexually dimorphic (Cocroft, Rodríguez, & Hunt, 2008; Rodríguez & Cocroft, 2006; see below), the transition from nymph to adult signals should also feature sexually dimorphic trajectories; and (4) individual variation in nymph signals should be correlated with individual variation in adult signals (i.e. variation in ontogenetic trajectories will project onto the adult stage) and/or mate preferences. Prediction (1) already has support from prior work (Cocroft et al., 2008; Rodríguez, Rebar, & Bailey, 2018).

We then tested the hypothesis that the ontogeny of the *Enchenopa* communication system is social. This hypothesis predicts that (5) signals and/or mate preferences should vary between individuals that develop in social groupings or in isolation. An effect of developing in aggregations of varying size has already been demonstrated (Fowler-Finn et al., 2017), but the effect of full isolation still needs to be documented.

Finally, we tested the hypothesis that the ontogeny of the *Enchenopa* communication system is obligately social. This hypothesis predicts that individuals that develop in isolation should either (6) have signals and/or preferences that are not species typical or (7) produce no signals and/or exhibit no mate preferences at all.

METHODS

Enchenopa treehoppers have a communal social system (Costa, 2006). Females aggregate to lay eggs on their host plants, and nymphs develop in peer groupings wherein they interact with each other using vibrational signals (Cocroft et al., 2008; Rodríguez et al., 2018). In the adults, male–female signal exchanges guide pair formation (Cocroft et al., 2008). Thus, although the *Enchenopa* life cycle features limited opportunity for nymphs to be exposed to adult signals, there are considerable signalling interactions with peers all throughout ontogeny.

Most of the species of the *E. binotata* species complex remain to be described (Hamilton & Cocroft, 2009). However, they can be readily identified by the host plant species they use, the coloration of the nymphs and the signals and preferences of the adults (Cocroft et al., 2008). We worked with one of the two *E. binotata* species that live on *Viburnum lentago* host plants in Wisconsin, U.S.A.; this species has nymphs of a uniform grey body coloration and male signals with a dominant frequency of ca. 165 Hz (Rodríguez et al., 2018). We preserved all individuals used in our experiments in 70% ethanol.

We ran the experiments over the spring and summer of 2017. We first used treehoppers hatched from eggs laid on potted *V. lentago* host plants by mated females collected in August of 2016 at Downer Woods, on the University of Wisconsin-Milwaukee (UWM) campus. We induced the nymphs to hatch in March by bringing the plants out of winter pause at the UWM Biological Sciences Greenhouse. In May, we collected a second group of naturally hatched nymphs at Downer Woods.

Rearing Experiments

To test predictions (1)–(4), we reared treehoppers in isolation, each nymph on its own potted host plant. This allowed us to track the ontogeny of each individual's signals and preferences, starting from the first-instar stage, without influence of any interactions with other individuals. We sexed the treehoppers after the adult moult. This allowed us to test for sex differences in the individual ontogenetic trajectories.

To test predictions (5)–(7), we manipulated the social experience of the treehoppers during development. We reared nymphs either in isolation (this treatment consisted of the above nymphs) or in groups of 30–40 individuals per potted host plant, approximating aggregations in the wild. When the treehoppers in the group treatment reached the adult stage, we separated them by sex; at this point the groups consisted of 10–20 males or females per potted host plant. We then compared the signals and preferences of the adults that developed in these treatments. Although plant quality (and thus nutritional resources) may have varied between the isolation and group treatments, we discuss below why we think the results likely represent the effects of social experience rather than plant quality during development.

Sample sizes for all experiments are given in the Appendix, Table A1.

Signal Recording and Vibrational Playbacks

We recorded *Enchenopa* nymph and adult signals with laser vibrometry. We used a portable laser Doppler vibrometer (Polytec PLV-100; Polytec Inc. Auburn, MA, U.S.A.). 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; 109 Krohn-Hite Corp., Brockton, MA, U.S.A.) 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. Treehoppers were introduced in a standardized way on the recording plant, which led them to settle within 5 cm of the reflective tape. The health of plants was maintained with a regular watering schedule limiting discrepancies in transmission properties. We noted the air temperature near the signalling treehoppers with a thermometer and used it as a control covariate in all statistical analyses (see below).

We recorded each of the singly reared nymphs on its potted host plant for 30 min once per week throughout development and past the final moult into the adult stage. The nymphs moulted as adults 5–6 weeks after they hatched. We also weighed them weekly with a microbalance (XP26; Mettler Toledo, Columbus, OH, U.S.A.). Once the treehoppers likely had reached sexual maturity (at approximately 2 weeks after the adult moult for males and approximately 4 weeks after the adult moult for females; Fowler-Finn et al., 2017), we attempted to obtain a final recording of their adult signals. Of the 104 adult male 30 min recordings made in the 2 weeks before

sexual maturity, only 30 contained male signals. Similarly, 16 out of the 139 adult female recordings made in the 4 weeks before sexual maturity contained female signals.

To record mature males, we placed each individual singly on a designated recording plant and allowed them 10 min to signal. If they did not signal within that interval we returned them to their rearing plant and tried again 2 days later, and so on until they signalled. Mean (±SD) age at recording for males was 25 ± 6 days after the adult moult.

To record mature females, we used vibrational playback experiments to induce them to signal. These playbacks also served to describe mate preferences for male signal frequency (the most distinctive signal feature among the species in the complex and the signal feature for which females have the strongest preference; Cocroft, Rodríguez, & Hunt, 2010; Rodríguez, Ramaswamy, & Cocroft, 2006; Rodríguez et al., 2013). To test each female, we placed her on a designated playback plant. We presented vibrational playback stimuli through a piezoelectric stack coupled to the stem of the plant with soft wax, driven by a piezoelectric controller (Thorlabs, Newton, NJ, U.S.A.) and recorded female signals with the laser vibrometer as described above.

Before trials, we determined whether females were sexually receptive by playing back a recording of a male signal closely matching the population mean. We used the response signal to this playback for the analysis of female signal features (see below). We then proceeded to the playback experiment to describe mate preferences. If the female was not responsive, we returned her to the treatment plant and tried again 2 days later, and so on until the female became receptive. Mean (±SD) age at testing for females was 46 ± 6 days after the adult moult.

We calibrated the playbacks to an amplitude approximating the mean of a male signalling a few centimetres away from the female on the stem (0.15 mm/s) using an oscilloscope (HMO 1002 series, Rohde and Schwartz, Munich, Germany).

To describe preferences for signal frequency, we used 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 four signals/bout, so each of our stimuli had four signals, all with the same frequency; further details in Fowler-Finn et al., 2017). We presented each female with a random sequence of 17 playback stimuli (with frequencies from 130 to 230 Hz). This range of stimuli 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).

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. *Enchenopa* females express their mate preferences through selective duetting with males, and the number of response signals produced by females when interacting with playback stimuli offers a practical and realistic indication of their evaluation of signal attractiveness (Rodríguez et al., 2004, 2006, 2012).

Description of Mate Preference Functions

Mate preference functions describe variation in signal attractiveness over a range of signal trait values (Jennions & Petrie, 1997; Ritchie, 1996; Wagner, 1998). Preference functions are expressed as a function of the signals that 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.

Recording Analysis

Enchenopa nymphs and adults produced diverse signal repertoires. We examined variation in their frequency and temporal features (Fig. 1). All acoustic measurements were conducted using the program Audacity and R (v.3.3.2, R Foundation for Statistical Computing, Vienna, Austria). To estimate the signalling rate and signal features, we visually and aurally inspected each nymph recording in Audacity and labelled each signalling event. The signalling rates and signal features were quantified by processing the labels in R.

Statistical Analysis

We conducted all analyses in R using the functions 'lmer' or 'glmer' of the R package lme4 (v.1.1–12; Bates, Maechler, Bolker, & Walker, 2014). Our data included three signal types for nymphs, one signal type for males and one signal type for females as well as female preference. For each of these signal types, several signal features were measured. This introduces the risk of spurious significance in our analyses (Rice, 1989) because we ran many 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 only included traits that were not highly correlated with each other ($r < 0.5$). 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 and it was of special interest to assess the effect of social ontogeny on them (Rodríguez et al., 2006). Second, we followed a tablewide 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 represent spurious significance (Moran, 2003).

Ontogeny

To test for ontogenetic trajectories (predictions 1–3), we used generalized linear mixed models. We ran a separate test for each dependent variable; these were nymph mass (log transformed), signalling rate for each signal type (log transformed) and the independent signal features defined in Fig. 1a–c. In each test, the explanatory variables were sex, recording week (linear and quadratic terms, to test for linear and curvilinear relationships), the sex * week interaction (to test for a potential sex dimorphism of the

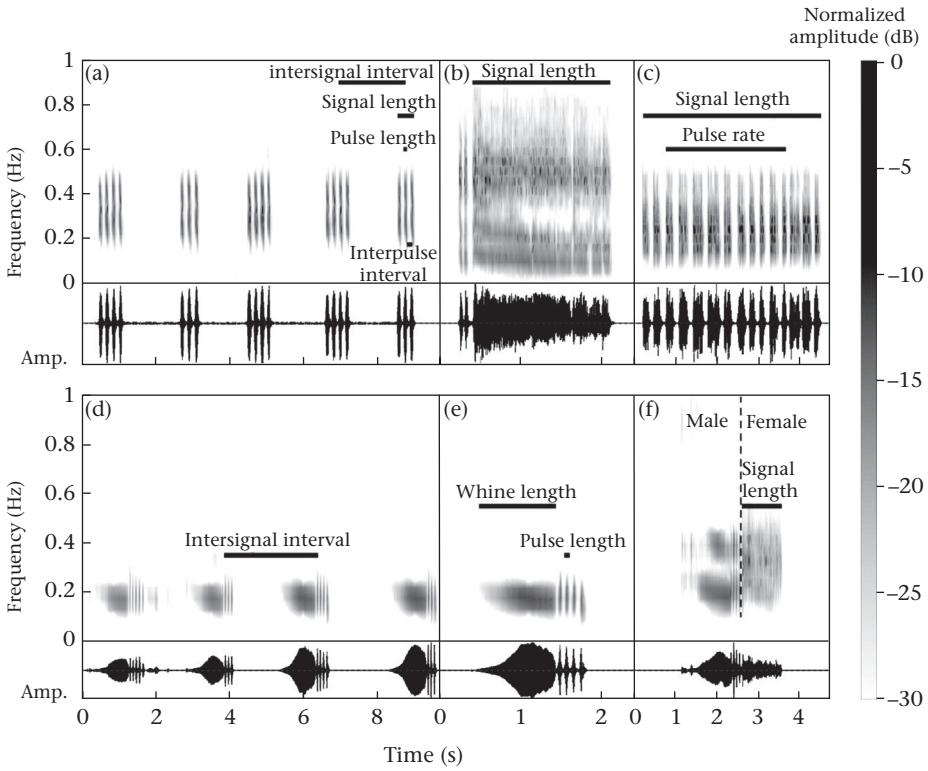

Figure 1. *Enchenopa* signal repertoire. Inventory of the signal types produced by nymphs and adults monitored in this study. For each signal type, we define the traits that we measured. (a–c) Signals produced by nymphs. (a) Short signals: groups of one to five pulses usually produced in bouts of two or more signals. A pulse was defined as a sound lasting less than 0.2 s. We measured the number of signals per bout, the duration of the fifth signal (or the signal closest to fifth) in seconds (signal length), the interval between the fourth and fifth signals in the bout in seconds (intersignal interval), the duration between the second and third pulses in the fifth signal of the bout (interpulse interval), the duration of the third pulse in seconds (pulse length) and the dominant frequency of the fifth signal. (b) Long signals: produced singly, sometimes preceded by one to five pulses. We measured the length, the dominant frequency and the number of pulses before the signal. (c) Modulated signals: a continuous succession of pulses. We measured signal length, dominant frequency and the pulse rate, measured as the duration of 10 pulses. (d–f) Signals produced by adults. (d) Male signalling bouts, each consisting of several signals. We measured the number of signals per bout (signal number) and the interval between the end of the second and third signals (intersignal interval); (e) Male signals, which consisted of two elements: a whine and pulses. We measured the duration of the whine of the third signal (or the whine closest to third signal) in seconds (whine length), the duration of the second pulse (pulse length), the number of pulses in the third signal (or closest to third signal) and the dominant frequency. (f) Female duet signal in response to the standard male primer. Female calls consisted of a broadband sound lasting 0.3–3 s. We measured signal length, fundamental frequency and the frequency modulation between the beginning and the end of the signal. Amp. = amplitude.

ontogenetic trajectories) and recording temperature as a control. As we recorded individuals repeatedly, nymph identity was a random factor in all models. The error structure was Gaussian for all but two models with count values (number of short signals per bout and number of introductory pulses for the long signal) 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' (v.2.1–4; Fox & Weisberg, 2011) applied to a standard linear model excluding the random effects, 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.

To test prediction (4), we used linear models to assess the relationship between variation in nymph and adult signal rates, signal features and preferences. Given the number of potential comparisons and our sample size limitations (Table A1), we restricted the tests to features that we considered comparable; that is, by comparing length with length, frequency with frequency or signal number with signal number. We included one exception by comparing the length of the nymph signal and the whine length in male signals as whine length is correlated with male signal frequency. The only adult feature that we deemed comparable to signalling rate was the number of signals per bout in males. We therefore used a linear model with the number of signals in adults and the signalling rates for the three signals of nymphs during their last week before moulting. We also included mass at the last nymph stage and recording temperature as covariates. We then compared signal features in nymphs and adults. We used four features of the short signal in nymphs as a covariate for male signal features models because sample sizes were too limited (fewer than 5 individuals) for the other signal types and features. Similarly, for female signal features, we used two features of the short signal in nymphs. Finally, we looked at the relationship between preference traits and two short-signal features. The assumptions and model significance were tested as above. The error structure was Gaussian for all models except number of pulses and number of signals, for which it was a Poisson distribution.

Social ontogeny

To test for an effect of social experience treatments on adult signal features and preferences (predictions 5–7), we used generalized linear models. We ran a separate test for each dependent variable; these were male and female signal features (defined in Fig. 1) and female preference traits (defined above). In each test, the explanatory variables were social experience (group

Table 1
Ontogeny of signalling rate in *Enchenopa* nymphs

Term	Nymph mass			Short signal rate			Long signal rate			Modulated signal rate		
	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*
Sex	31.2	3, 190	**<0.0001**	3.52	3, 190	0.26	1.15	3, 190	0.76	0.20	3, 190	0.98
Week	185.6	2, 190	**<0.0001**	2.47	2, 190	0.29	0.46	2, 190	0.79	0.27	2,190	0.87
Week^2	45.5	2, 190	**<0.0001**	6.12	2, 190	**0.047**	1.94	2, 190	0.38	0.39	2, 190	0.82
Sex∗week	8.10	1, 190	**0.0044**	1.96	1, 190	0.16	0.22	1, 190	0.64	0.0002	1, 190	0.99
Sex∗week^2	0.0041	1, 190	0.95	2.58	1, 190	0.11	0.03	1, 190	0.86	0.0083	1, 190	0.93

Results of generalized linear mixed models testing for a relationship between signalling rate or mass and nymph sex, recording week, and their interaction. All dependent variables were log-transformed for the analysis (see Methods). Significant and marginally significant *P* values are shown in bold. The week (linear and quadratic) test for an ontogenetic trajectory and the interaction between sex and week tests for a sexually dimorphic ontogenetic trajectory. The criterion of tablewide significance (Moran, 2003) suggests that the detected change over ontogeny in the signal rate may be spurious.

or isolation), age (in days after adult moult) and recording temperature (as a control). We included age as a control variable mainly for female preferences and signals as age has been shown to have no effect on signal features in males (Sattman & Cocroft, 2003). We tested the assumptions and model significance as above. 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.

RESULTS

There Is an Ontogeny to Enchenopa *Signals*

Prior work (Rodríguez et al., 2018) showed that *Enchenopa*, as do many other vibrational insects (Rodríguez & Desjonquères, in press), produce signals not only as adults but also as juveniles, using different signal types as nymphs and adults. Here we provide greater detail on the signals and how they change over development.

We use the increase in body mass over ontogeny as a standard for comparison. The growth trajectories of *Enchenopa* nymphs were sexually dimorphic. Females were larger than males since the earliest instars and grew more quickly (significant main and interaction terms in Table 1, Fig. 2a).

Nymphs produced three different signal types (short, long and modulated signals; Fig. 1a–c). This repertoire did not change throughout nymph ontogeny, nor did it differ between developing male and female nymphs. As expected from prior work (Cocroft et al., 2008), adult signals were sexually dimorphic and different from nymph signals (Fig. 1d–f). While males and females each produced one signal type in our study (Fig. 1d–f), males have other signal types in their repertoire (Sullivan-Beckers, 2008).

In the nymph recordings, 47% of the files contained short signals, 15% contained long signals and 14% contained modulated signals. There was evidence that signalling rates changed over ontogeny for one of the three nymph signal types, but this may be a spurious result (short signals; Figs 1 and 2b, Table 1). By contrast ontogenetic changes in the features of nymph signals were prevalent (Table 2, Fig. 3). These ontogenetic trajectories were sexually dimorphic for 6 out of 12 features in the three signal types (Table 2, Fig. 3).

We found a few relationships between nymph and adult male signals: between the rate of one of the three nymph signals types (short signals) and the number of signals per bout of adult males; between the frequency and the interpulse interval of nymph short

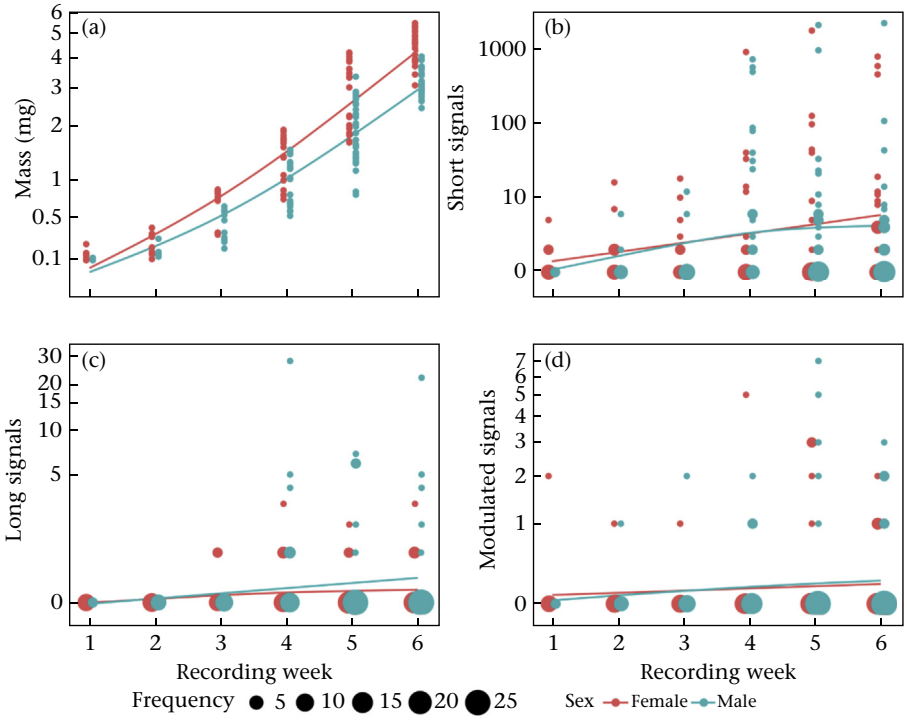

Figure 2. Ontogeny of mass and signalling rate in *Enchenopa* nymphs. Lines represent splines fitted to the means for each week.

Table 2
Ontogeny of signal features in *Enchenopa* nymphs

Signal type	Sex			Week			Week^2			Sex∗week			Sex∗week^2		
	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*
Short															
Signal number	8.20	3, 111	**0.042**	0.48	2, 111	0.79	14.51	2, 111	**0.0007**	0.16	1, 111	0.69	6.06	1, 111	**0.014**
Signal interval	7.17	3, 112	**0.067**	6.96	3, 112	**0.031**	1.05	2, 112	0.59	4.50	1, 112	**0.034**	0.12	1, 112	0.73
Pulse length	18.23	3, 268	**0.0004**	13.20	2, 268	**0.0014**	24.98	2, 268	**<0.001**	13.20	1, 268	**0.0003**	4.98	1, 268	**0.026**
Frequency	2.83	3, 268	0.42	15.88	2, 268	**0.00034**	2.49	2, 268	0.29	0.61	1, 268	0.43	2.45	1, 268	0.12
Signal length	13.51	3, 140	**0.0037**	0.22	2, 140	0.89	14.00	2, 140	**0.0009**	0.22	1, 140	0.64	12.62	1, 140	**0.0004**
Interpulse interval	2.06	3, 139	0.56	2.05	2, 139	0.36	6.34	2, 139	**0.042**	0.05	1, 139	0.82	0.93	1, 139	0.33
Long															
Frequency	1.97	3, 58	0.58	15.70	2, 58	**0.0004**	0.47	2, 58	0.79	6.85	1, 58	**0.0089**	0.12	1, 58	0.73
Signal length	4.15	3, 60	0.25	3.06	2, 60	0.22	4.36	2, 60	0.11	0.07	1, 60	0.79	2.05	1, 60	0.15
Introductory pulses	1.57	3, 60	0.67	9.06	2, 60	**0.011**	2.78	2, 60	0.25	0.008	1, 60	0.93	1.16	1, 60	0.28
Modulated															
Frequency	0.28	3, 35	0.28	5.28	2, 35	**0.071**	3.21	2, 35	0.20	0.0001	1, 35	0.99	2.53	1, 35	0.11
Signal length	1.05	3, 35	0.79	0.98	2, 35	0.61	0.87	2, 35	0.65	0.98	1, 35	0.32	0.68	1, 35	0.41
Pulse rate	23.95	3, 35	**<0.001**	1.02	2, 35	0.60	20.87	2, 35	**<0.001**	0.50	1, 35	0.48	15.94	1, 35	**<0.001**

Results of generalized mixed linear models testing for a relationship between signal features and nymph sex, recording week, and their interaction. Significant and marginally significant *P* values are shown in bold. The week (linear and quadratic) test for an ontogenetic trajectory and the interaction between sex and week tests for a sexually dimorphic ontogenetic trajectory. The criterion of tablewide significance (Moran, 2003) suggests that the relationships supported here are likely real and not spurious (see Methods).

signals and the frequency of adult male signals; and between the frequency of nymph short signals and the whine length of adult male signals (Fig. 4, Table 3). The other relationships were weak and nonsignificant (Table 3). For females, we found no relationship between signal features in nymphs and adults (Table 4). However, we did find a relationship between the frequency of the short signals of females as nymphs and preference strength in adult females (Table 4).

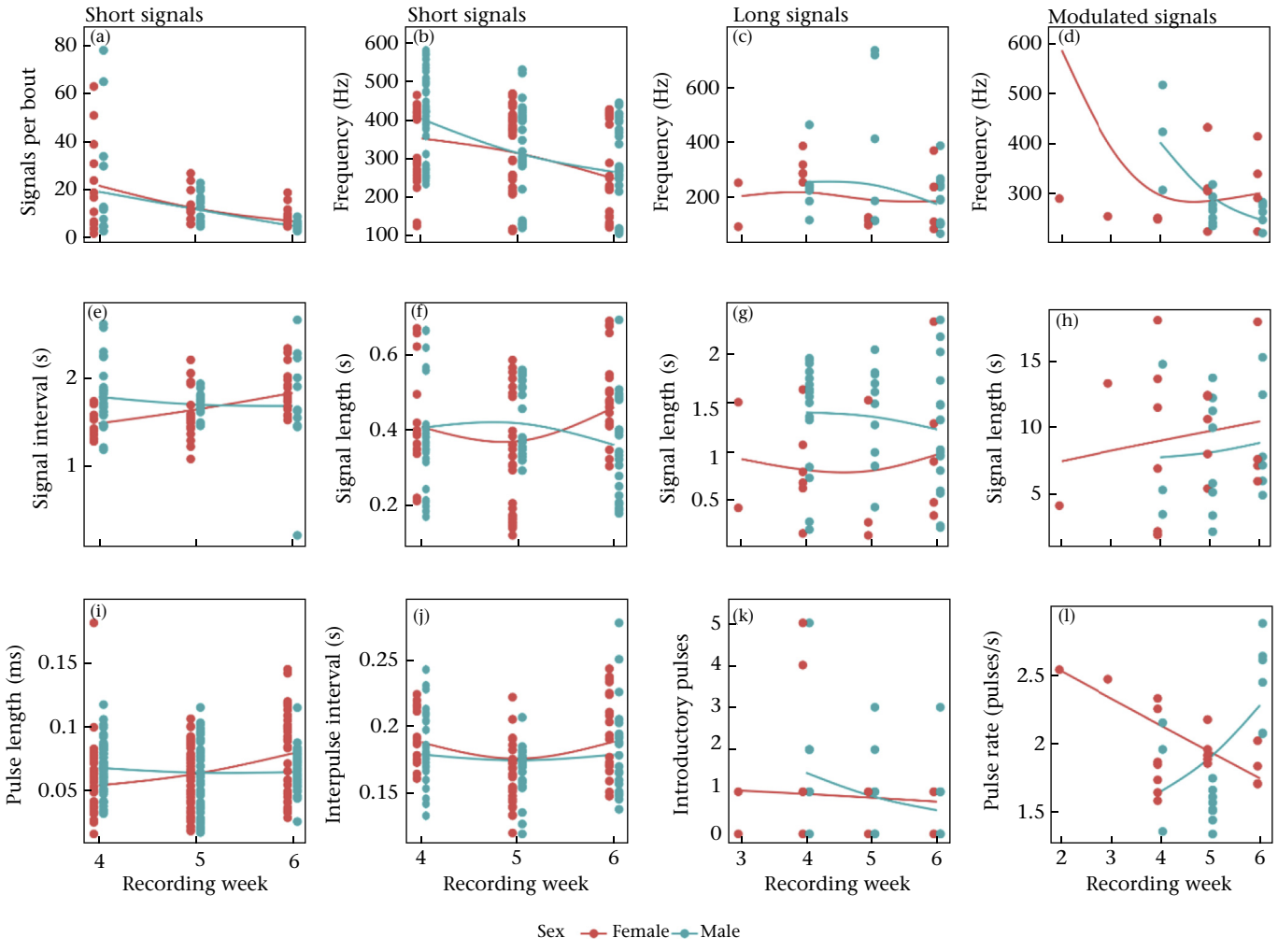

Figure 3. Ontogeny of signal features in *Enchenopa* nymphs. Lines represent splines fitted to the means for each week.

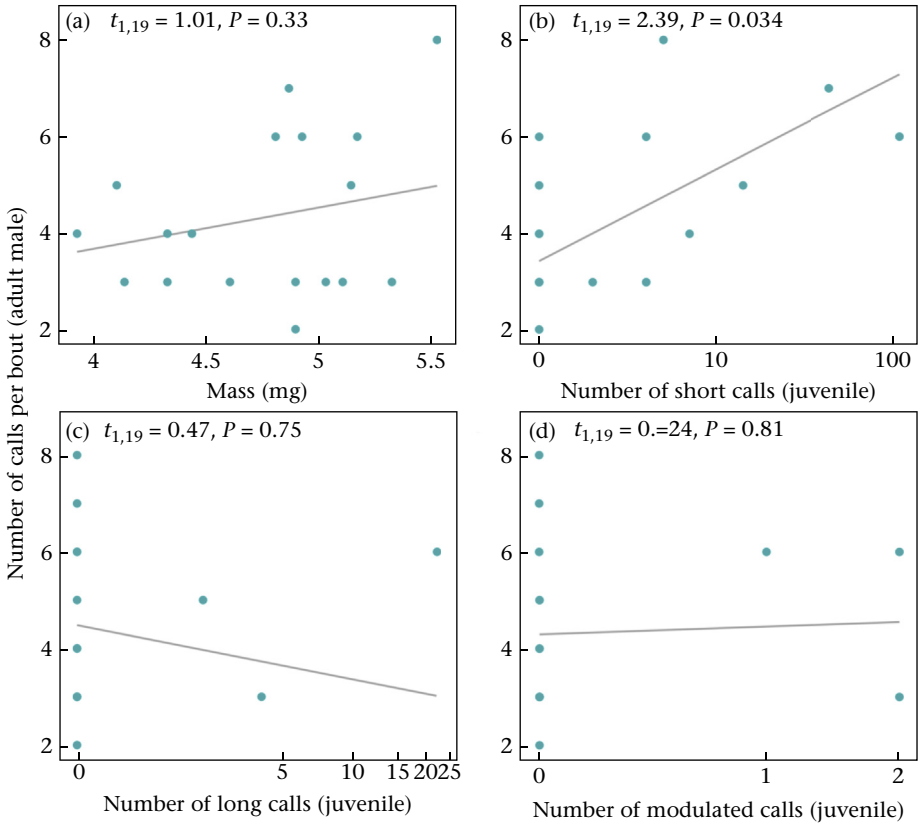

Figure 4. Relationships between the number of calls per bout in adult males and (a) body mass, (b) number of short calls as nymphs, (c) number of long calls as nymphs and (d) number of modulated calls as nymphs. Each point represents an individual male. Solid lines represent the values fitted by the linear model testing for a relationship between nymph mass and signal type rate and number of signals per bout of male signalling. Statistics, degrees of freedom and *P* values for the fitted models are shown.

The Ontogeny of Enchenopa *Signals and Preferences Is Social*

Social experience affected two signal features in males: males reared in isolation had significantly lower pulse lengths and marginally significantly higher intersignal intervals than males reared in groups (Fig. 5). The difference in signal features reached 29% for pulse duration. Social experience did not affect any of the female signal features (Fig. 5).

Social experience affected two features of female mate preference: females reared in isolation had significantly lower peak preference and significantly higher peak responsiveness than females reared in groups (Fig. 6). The change in preference features reached 24% for peak responsiveness.

The Ontogeny of Enchenopa *Signals and Preferences Is Not Obligately Social*

Males and females in both social experience treatments produced species-typical signals (Fig. 1d–f). Similarly, females in both treatments had species-typical preferences.

DISCUSSION

Our results indicate that there is an ontogeny to *Enchenopa* communication systems: nymphs and adults signalled; nymph signals differed from adult signals and changed gradually in a sexually dimorphic way throughout development; and some variation in nymph signals was correlated with variation in the signals and mate preferences of adults (predictions 1–4). Some of these changes could be explained by an increase in body size during development; e.g. as nymphs grow larger, their signals would likely decrease in frequency (as for short signals). The overall data reject this simple interpretation, however. Besides the signal rate of one out of three signal types, 10 out of 12 signal features changed along the nymphs' ontogeny, and some patterns directly opposed the above expectation (e.g., juvenile females did not have significantly lower-frequency signals for any signal type even though they were larger than males throughout development). Thus, additional factors besides a simple increase in body size are likely to explain the ontogeny of signals in *Enchenopa*.

Our results also suggest that there is a social component to the ontogeny of *Enchenopa* communication: the presence or absence of social experience influenced some of the features of male signals and female preferences (prediction 5). We interpret the effects of social experience on male signal traits with caution because they could represent spurious significance according to our tablewide criterion (Moran, 2003). However, the effect of social experience on mating preference was robust and consistent with prior experiments (Fowler-Finn et al., 2017). In the present study, the quality of plants used (and thus the resources available) may have differed during development for isolated versus grouped individuals, potentially confounding our interpretation of the results. Teasing these variables apart would require manipulation of plant quality independent of the number of nymphs per plant. Nevertheless, we consider that the results likely reflect the effect of social experience. All the plants appeared to have similar vigour and health. Additionally, in a separate experiment, we manipulated the social experience of nymphs with playbacks during development of isolated individuals, and this treatment resulted in adult phenotypes that were similar or intermediate between this study's grouped and

Table 3
Individual variation in signal features in *Enchenopa* treehoppers

Response variable (adult signal features)	Term (nymph short signal features)	*r*	*t*	*df*	*P*
Male					
Frequency	Pulse length	0.061	0.15	1, 7	0.89
	Frequency	0.729	2.61	1, 7	**0.080**
	Signal length	0.666	2.19	1, 7	0.12
	Interpulse interval	0.814	3.44	1, 7	**0.041**
Whine length	Pulse length	−0.010	−0.03	1, 7	0.98
	Frequency	−0.762	−2.88	1, 7	**0.063**
	Signal length	−0.164	−0.41	1, 7	0.71
	Interpulse interval	0.277	0.71	1, 7	0.53
Pulse length	Pulse length	0.026	0.06	1, 7	0.95
	Frequency	0.245	0.62	1, 7	0.58
	Signal length	0.345	0.90	1, 7	0.43
	Interpulse interval	0.440	1.20	1, 7	0.32
Intersignal interval	Pulse length	0.134	0.33	1, 7	0.76
	Frequency	0.278	0.71	1, 7	0.53
	Signal length	−0.196	−0.49	1, 7	0.66
	Interpulse interval	0.475	1.32	1, 7	0.28
Number of signals	Pulse length	−0.262	−0.66 (*z* value)	1, 7	0.51
	Frequency	−0.375	−0.99 (*z* value)	1, 7	0.32
	Signal length	0.186	0.47 (*z* value)	1, 7	0.64
	Interpulse interval	−0.214	−0.54 (*z* value)	1, 7	0.59
Number of pulses	Pulse length	0.008	0.02 (*z* value)	1, 7	0.98
	Frequency	0.040	0.10 (*z* value)	1, 7	0.92
	Signal length	0.059	0.14 (*z* value)	1, 7	0.89
	Interpulse interval	0.042	0.10 (*z* value)	1, 7	0.92
Female					
Signal length	Pulse length	0.028	0.06	1, 6	0.95
	Frequency	−0.365	−0.88	1, 6	0.45
Fundamental frequency	Pulse length	0.006	0.01	1, 6	0.99
	Frequency	0.287	0.67	1, 6	0.55
Frequency modulation	Pulse length	−0.350	−1.11	1, 6	0.35
	Frequency	−0.445	−0.84	1, 6	0.47

Results of generalized linear mixed models testing for a relationship between nymph short signal features and male or female signal features. Significant and marginally significant *P* values are shown in bold. The criterion of tablewide significance (Moran, 2003) suggests that some of the relationships observed here may be spurious.

isolated conditions (Desjonquères, Speck, & Rodríguez, n.d.). These results suggest that acoustic interactions during development are at least partly responsible for the observed differences between the treatments. Thus, we conclude that social interactions may play a strong role in the ontogeny of *Enchenopa* signals.

Finally, the ontogeny of the *Enchenopa* communication system is not obligately social: adults reared in isolation produced species-typical signals and had species-typical mate preferences, rejecting predictions (6)–(7).

Socially mediated plasticity has a surprisingly strong role in generating signal and mate preference variance in animals like *Enchenopa* (Fowler-Finn et al., 2017; Fowler-Finn & Rodríguez, 2012a,b; Rebar & Rodríguez, 2013, 2015). Our results suggest why. In these insects, an individual's behaviour may influence (and be influenced by) the behaviour of other individuals: the signals of each individual in an aggregation could act as inputs into the expression of the signals and preferences of the others. In other words, the consequences of their lifelong social dynamics are best understood within the 'interacting phenotypes' framework, wherein the causes of variation and selection coevolve with the traits under selection (Rodríguez et al., in press; West-Eberhard, 1983, 2014). Consider the most divergent aspect of the phenotype of adults in the *E. binotata* complex: male signal frequency and the corresponding female mate preference (Cocroft et al., 2010; Rodríguez et al., 2006). In the species we studied here, both male signals and female preferences covaried with some of the features of nymph signals, and female peak preference varied between social treatments. Consequently, the form and strength of sexual selection on male signals may vary among aggregations (if the aggregations are composed of individuals with different ontogenetic trajectories), as well as according to social experience. Specifically, females in this population prefer higher-than-average male signal frequencies (Fowler-Finn et al., 2017), and the preference became more strongly directional in our grouped social treatment relative to treehoppers reared in isolation (Fig. 6). This result is in agreement with a prior study that found more strongly directional preferences for signal frequency when the treehoppers were reared in groups of higher density (Fowler-Finn et al., 2017).

Our results identify a novel type of social ontogeny in animal communication systems. Most known instances involve learning or imprinting by juveniles from adult tutors (Akçay, Campbell, & Beecher, 2017; Hebets & Sullivan-Beckers, 2010; Ljubičić, Hyland Bruno, & Tchernichovski, 2016; Verzijden et al., 2012). By contrast, in this species, the most important social inputs seem to originate from peers of similar age. This may not be an unusual scenario. Plant-feeding insects are highly diverse: they often

Table 4
Link between mating preference and nymph signal features in *Enchenopa* treehoppers

Response variable	Term	*r*	*t*	*df*	*P*
Preference peak	Pulse length	−0.322	−0.90	1, 8	0.41
	Frequency	−0.204	−0.55	1, 8	0.60
Peak responsiveness	Pulse length	−0.584	−1.90	1, 8	0.12
	Frequency	−0.120	−0.32	1, 8	0.76
Preference strength	Pulse length	0.723	2.77	1, 8	**0.039**
	Frequency	0.803	3.56	1, 8	**0.016**

Results of generalized linear mixed models testing for a relationship between nymph short signal features and female mating preference traits. Significant *P* values are shown in bold. The criterion of tablewide significance (Moran, 2003) suggests that the relationships observed here are real and not spurious.

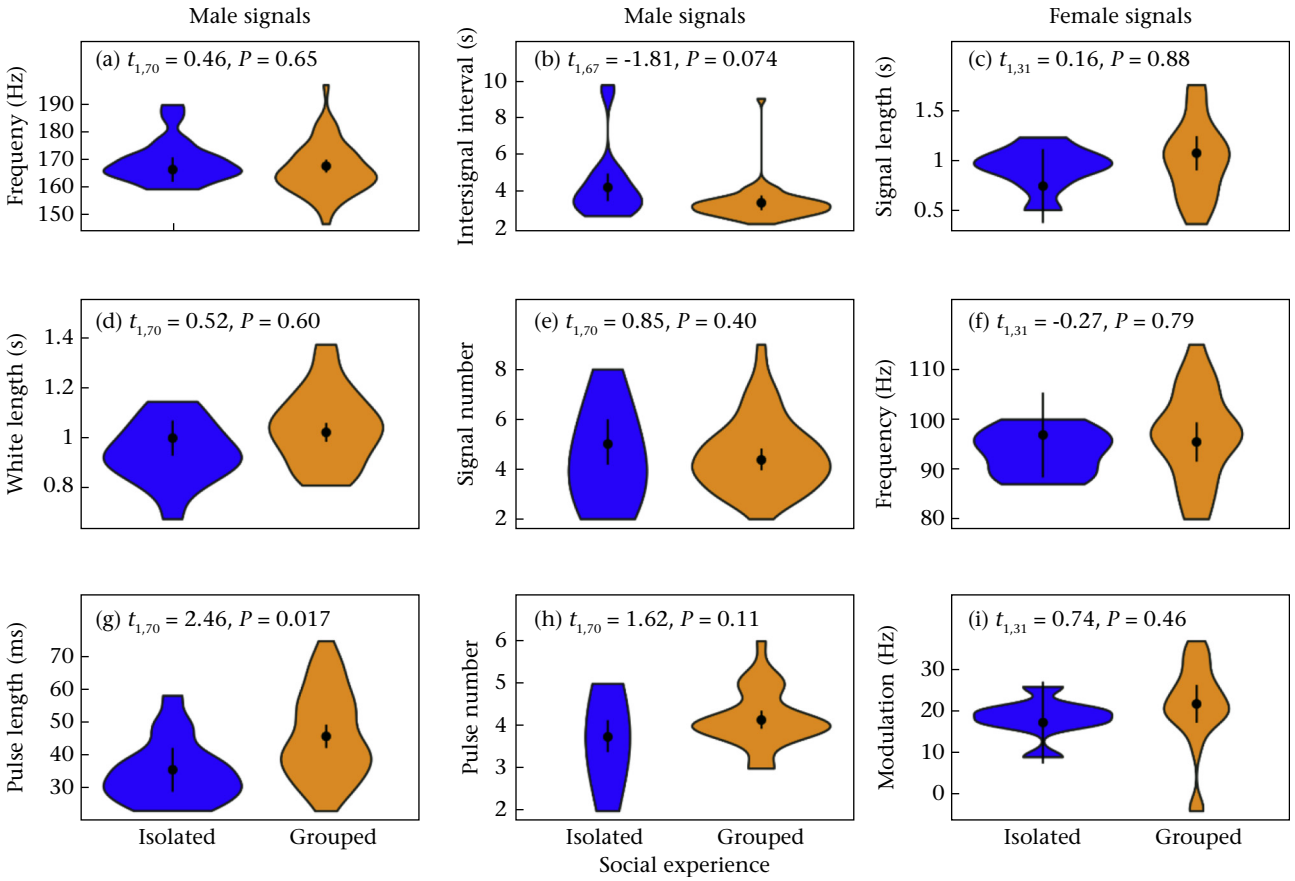

Figure 5. Effect of social treatments in *Enchenopa* adult signal features. Distribution of the signal features of adults are represented in violin plots. Each violin plot represents the kernel density plot for a social experience (isolated or grouped). Points and error bars show fitted values and the 95% confidence interval for the values fitted by the linear models testing for an effect of social experience on adult signal features and controlling for temperature. Statistics, degrees of freedom and *P* values for the fitted models are shown. The criterion of tablewide significance (Moran, 2003) suggests that some of the relationships observed here may be spurious.

communicate with vibrational signals as juveniles and adults, and they frequently have communal or subsocial social systems (Cocroft & Rodríguez, 2005; Costa, 2006; Rodríguez & Desjonquères, in press). The phylogenetic distribution of such lifelong social interactions, whether through substrate-borne vibrational signals or other modalities, may be much broader still.

The implication of our findings is that even innate signalling systems that do not require social experience or learning for proper

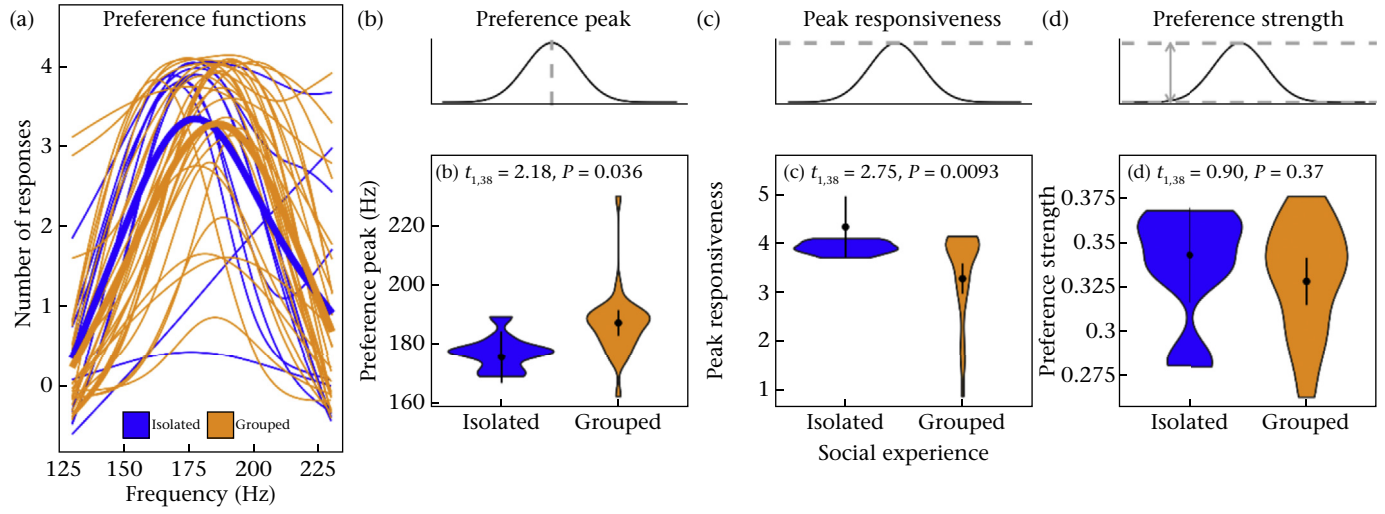

Figure 6. Effect of social treatments in *Enchenopa* female preference. (a) Preference functions for the two social experiences. (b-d) Top row: definition of each preference trait. Bottom row: distribution of the female preference traits represented in violin plots. Each violin plot represents the kernel density plot for a social experience (isolated or grouped). Points and error bars show fitted values and the 95% confidence interval for the values fitted by the linear models testing for effect of social experience on female preferences and controlling for temperature. Statistics, degrees of freedom and *P* values for the fitted models are shown.

development may nevertheless be socially malleable. They are expressed and used in social interactions throughout the lives of the animal and vary according to the particularities of individuals of different sexes, including their particular social experiences. All of these are causes of variation in communication systems on which selection may act.

This conclusion contributes to the ongoing revision of the traditional view of language as a monolithic. Recent multidisciplinary work has brought the twin insights that human language is a complex faculty consisting of a suite of components that evolved sophisticated coordination in our lineage; and that each of these components occurs in various forms and combinations in other species (Fitch, 2010). Social ontogenies such as we describe here for *Enchenopa* treehoppers may represent one of these components: a basic 'building block' that underlies the ancestral state from which more complex communications systems may arise (Pika et al., 2018). Similarity between arthropod and vertebrate communication probably represents convergence, as the last common ancestor of these groups likely had a very simple neural system (Feinberg & Mallatt, 2016). However, if we posit that the complex communication systems of humans and some other mammals and birds (Fitch, 2010; Gultekin & Hage, 2017; Lipkind et al., 2013; Margoliash & Tchernichovski, 2015; Pinker, 1994; Takahashi et al., 2015) evolved from simpler, innate systems, then our results suggest that even those ancestral innate systems may have featured ontogenies with some degree of social plasticity. It may therefore be that the transition from innate to specialized and socially learned mechanisms involves selection acting on already-present variation in lifelong patterns of social plasticity, rather than the de novo advent of learning in communication systems. In this context, it is interesting to note that social interactions earlier in life seem to have stronger effects on mate preferences (influencing the peak and overall shape of mate preferences) whereas interactions taking place later in life (e.g. social experience of young adult females) only influence the overall shape but not the peak of mate preferences (Fowler-Finn et al., 2017; Fowler-Finn & Rodríguez, 2012a,b; Rebar & Rodríguez, 2013). Thus, the main variance generated by social plasticity arises when it is most likely to result in divergence and assortative mating — i.e. predispersal (Verzijden et al., 2012). We hope that our understanding of the evolution of the components that interact to generate human language will continue to grow synergistically with progress in our understanding of the structure of the communication systems of other animals (Soha & Peters, 2015).

Declaration of Interest

We declare that we have no competing interests.

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References

Akçay, Ç., Campbell, S. E., & Beecher, M. D. (2017). Good tutors are not dear enemies in song sparrows. *Animal Behaviour, 129*, 223–228.

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

Bailey, N. W., & Zuk, M. (2008). Acoustic experience shapes female mate choice in field crickets. *Proceedings of the Royal Society B: Biological Sciences, 275*, 2645–2650.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). lme4: Linear mixed-effects models using 'Eigen' and S4 *(R package* version 1.1-7. https://CRAN.R-project.org/package=lme4.

Cocroft, R. B. (2005). Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. *Proceedings of the Royal Society B: Biological Sciences, 272*, 1023–1029.

Cocroft, R. B., Gogala, M., Hill, P. S. M., & Wessel, A. (Eds.). (2014). *Studying vibrational communication*. Berlin, Germany: Springer.

Cocroft, R. B., & Rodríguez, R. L. (2005). The behavioural ecology of insect vibrational communication. *BioScience, 55*, 323–334.

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 K. J. Tilmon (Ed.), *Species, speciation, and radiation: The evolutionary biology of herbivorous insects* (pp. 88–100). Berkeley, CA: University of California Press.

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. *Biological Journal of the Linnean Society, 99*, 60–72.

Costa, J. T. (2006). *The other insect societies*. Cambridge, MA: Harvard University Press.

Desjonquères, C., Speck, B., & Rodríguez, R. L. (n.d.). Causes of social ontogeny in the signal and preference of *Enchenopa* treehoppers (Membracidae, Hemiptera). Manuscript in preparation.

Feinberg, T. E., & Mallatt, J. M. (2016). *The ancient origins of consciousness: How the brain created experience*. Cambridge, MA: MIT Press.

Field, A. (2009). *Discovering statistics using SPSS*. Thousand Oaks, CA: Sage.

Fitch, W. T. (2010). *The evolution of language*. Cambridge, U.K.: Cambridge University Press.

Fowler-Finn, K. D., Cruz, D. C., & Rodríguez, R. L. (2017). Local population density and group composition influence the signal-preference relationship in *Enchenopa* treehoppers (Hemiptera: Membracidae). *Journal of Evolutionary Biology, 30*, 13–25.

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. *Journal of Evolutionary Biology, 25*, 1855–1863.

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Thousand Oaks, CA: Sage.

Gerhardt, H. C., & Huber, F. (2002). *Acoustic communication in insects and anurans: Common problems and diverse solutions*. Chicago, IL: University of Chicago Press.

Gultekin, Y. B., & Hage, S. R. (2017). Limiting parental feedback disrupts vocal development in marmoset monkeys. *Nature Communications, 8*, 14046.

Hamel, J. A., & Cocroft, R. B. (2012). Negative feedback from maternal signals reduces false alarms by collectively signalling offspring. *Proceedings of the Royal Society B: Biological Sciences, 279*, 3820–3826.

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). *Entomological News, 120*, 554–565.

Hebets, E., & Sullivan-Beckers, L. (2010). Mate choice and learning. In M. Breed, & J. Moore (Eds.), *Encyclopedia of animal behavior* (pp. 389–393). London, U.K.: Academic Press.

Hill, P. S. (2008). *Vibrational communication in animals*. Cambridge, MA: Harvard University Press.

Jennions, M. D., & Petrie, M. (1997). Variation in mate choice and mating preferences: A review of causes and consequences. *Biological Reviews, 72*, 283–327.

Kilmer, J. T., Fowler-Finn, K. D., Gray, D. A., Höbel, G., Rebar, D., Reichert, M. S., et al. (2017). Describing mate preference functions and other function-valued traits. *Journal of Evolutionary Biology, 30*, 1658–1673.

Lipkind, D., Marcus, G. F., Bemis, D. K., Sasahara, K., Jacoby, N., Takahasi, M., et al. (2013). Stepwise acquisition of vocal combinatorial capacity in songbirds and human infants. *Nature, 498*, 104–108.

Ljubičić, I., Hyland Bruno, J., & Tchernichovski, O. (2016). Social influences on song learning. *Current Opinion in Behavioral Sciences, 7*, 101–107.

Marcus, G. (2004). *The birth of the mind*. New York, NY: Basic Books.

Margoliash, D., & Tchernichovski, O. (2015). Marmoset kids actually listen. *Science, 349*(6249), 688–689.

Morales, M. A., Barone, J. L., & Henry, C. S. (2008). Acoustic alarm signalling facilitates predator protection of treehoppers by mutualist ant bodyguards. *Proceedings of the Royal Society B: Biological Sciences, 275*, 1935–1941.

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. *Behavioral Ecology, 15*, 1044–1045.
Pika, S., Wilkinson, R., Kendrick, K. H., & Vernes, S. C. (2018). Taking turns: Bridging the gap between human and animal communication. *Proceedings of the Royal Society B: Biological Sciences, 285*, 20180598.
Pinker, S. (1994). *The language instinct: How the mind creates language*. New York, NY: William Morrow.
Ramaswamy, K., & Cocroft, R. B. (2009). Collective signals in treehopper broods provide predator localization cues to the defending mother. *Animal Behaviour, 78*, 697–704.
Rebar, D., & Rodríguez, R. L. (2013). Genetic variation in social influence on mate preferences. *Proceedings of the Royal Society B: Biological Sciences, 280*, 20130803–20130803.
Rebar, D., & Rodríguez, R. L. (2015). Insect mating signal and mate preference phenotypes covary among host plant genotypes. *Evolution, 69*, 602–610.
Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution, 43*, 223–225.
Ritchie, M. G. (1996). The shape of female mating preferences. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 14628–14631.
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., & Desjonquères, C. (2018). Vibrational signals: Sounds transmitted through solids. In B. D. Roitberg (Ed.), *Reference module in life sciences* (pp. 1–10). Amsterdam, The Netherlands: Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.90702-7.
Rodríguez, R. L., Haen, C., Cocroft, R. B., & Fowler-Finn, K. D. (2012). Males adjust signalling effort based on female mate-preference cues. *Behavioral Ecology, 23*, 1218–1225.
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. *Proceedings of the Royal Society B: Biological Sciences, 273*, 2585–2593.
Rodríguez, R. L., Rebar, D., & Bailey, N. W. (2018). Indirect genetic effects. In B. D. Roitberg (Ed.), *Reference module in life sciences* (pp. 1–6). Amsterdam, The Netherlands: Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.90048-7.
Rodríguez, R. L., Rebar, D., & Fowler-Finn, K. D. (2013). The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behaviour, 85*, 1041–1047.
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., Wojcinski, J. E., & Maliszewski, J. (2018). Between-group variation in *Enchenopa* treehopper juvenile signalling (Hemiptera Membracidae). *Ethology Ecology & Evolution, 30*, 245–255.
Sattman, D. A., & Cocroft, R. B. (2003). Phenotypic plasticity and repeatability in the mating signals of *Enchenopa* treehoppers, with implications for reduced gene flow among host-shifted populations. *Ethology, 109*, 981–994.
Schluter, D. (1988). Estimating the form of natural selection on a quantitative trait. *Evolution*, 849–861.
Soha, J. A., & Peters, S. (2015). Vocal learning in songbirds and humans: A retrospective in honor of Peter Marler. *Ethology, 121*, 933–945.
Stinchcombe, J. R., Function-valued Traits Working Group, & Kirkpatrick, M. (2012). Genetics and evolution of function-valued traits: Understanding environmentally responsive phenotypes. *Trends in Ecology & Evolution, 27*, 637–647.
Sullivan-Beckers, L. (2008). *The ecology of mate choice: Identifying the agents of sexual selection on mating signals in* Enchenopa *treehoppers* (Ph.D. thesis). Columbia, MO: University of Missouri.
Takahashi, D. Y., Fenley, A. R., Teramoto, Y., Narayanan, D. Z., Borjon, J. I., Holmes, P., et al. (2015). The developmental dynamics of marmoset monkey vocal production. *Science, 349*, 734–738.
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 in Ecology & Evolution, 27*, 511–519.
Wagner, W. L. (1998). Measuring female mating preferences. *Animal Behaviour, 55*, 1029–1042.
West-Eberhard, M. J. (1983). Sexual selection, social competition, and speciation. *Quarterly Review of Biology, 58*, 155–183.
West-Eberhard, M. J. (2014). Darwin's forgotten idea: The social essence of sexual selection. *Neuroscience & Biobehavioral Reviews, 46*, 501–508.

Appendix

Table A1
Sample sizes for the experimental tests of social ontogeny in *Enchenopa* treehoppers

Experimental test	Sample sizes		
Ontogeny	**Males**	**Females**	**Total**
Signalling rate	32	21	53 (190 recordings)
Short signal	12	13	25 (31 recordings)
Long signal	12	12	24 (60 recordings)
Modulated signal	10	9	19 (35 recordings)
Signalling rate adult–juvenile	19	0	19
Signal features adult–juvenile	7	6	13
Mating preference and signal feature	0	8	8
Social experience	**Isolated**	**Normal**	**Total**
Male signals	19	52	71
Female signals	8	24	32
Female preferences	10	29	39