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The relationship between a combinatorial processing rule and a continuous mate preference function in an insect

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Mate choice involves processing signals that can reach high levels of complexity and feature multiple components, even in small animals with tiny brains. This raises the question of whether and how such organisms deal with this complexity. One solution involves combinatorial processing, whereby different signal elements are processed as single units. Combinatorial processing has been described in several mammals and birds, and recently in a vibrationally signalling insect, *Enchenopa* treehoppers. Here, we ask about the relationship between combinatorial rules and mate preferences for continuously varying signal features. *Enchenopa* male advertisement signals are composed of two elements: a 'whine' followed by a set of pulses. The dominant frequency of the whine and element combination both matter to females. We presented synthetic signals varying in element order (natural [whine-pulses], reverse [pulses-whine]) and in frequency to *Enchenopa* females and recorded their responses. The reverse combination resulted in a decrease in attractiveness of the signals, and also slightly changed the shape of the preference for frequency. We found that females could be classified into three 'types': females with both a strong preference and a strong combinatorial rule, females with both a weak preference and weak rule, and females with a strong preference but a weak rule. Our results suggest that in *Enchenopa* signal processing, the mate preference for a continuous signal feature 'takes precedence' over, but also interacts with, the combinatorial rule. The relationship between the preference and the rule could evolve to take different forms according to selection on mate choice decisions. We suggest that exploring the relationship between such preferences and rules in species with more complex signals will bring insight into the evolution of the multi-component communication systems.

1. Introduction

To arrive at mate choice decisions, animals must often process complex signals that consist of multiple components in different modalities [1,2]. In many cases, signal complexity is extreme. Skylarks, for instance, have song repertoires containing an average of 340 different elements combined in varying sequences [3]. Even animals with small brains may have remarkably elaborate signals. In *Habronattus* jumping spiders, for example, courtship displays are a complex combination of visual and vibrational signals that include up to 20 different elements organized in motifs that vary during the display [4,5]. Simply describing these signals can be so challenging that they must be portrayed with the structure of movements within a symphony [4]. How do animal brains process such signal complexity?

One mechanism that may help animals to process complex, multi-component signals is combinatorial processing. With combinatorial processing, different signal elements are grouped and processed by receivers as single units, and groups of such composite units are then clustered into higher-level units
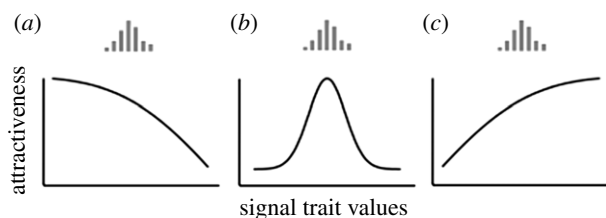
Figure 1. Signal trait distributions (top) and mate preference functions (bottom). Mate preference functions describe variation in the attractiveness of a signal as a function of variation in signal trait values [24,25]. Preference functions can be open-ended (favouring extreme trait values; *a,c*) or closed (favouring intermediate trait values; *b*). The relationship between the preferred signal trait value (i.e. 'peak preference', at the top of each function) and the trait distribution determines the form of selection due to mate choice: stabilizing when trait mean and peak preference match (e.g. as shown in *b*), directional when they do not match; how the curve drops as trait values deviate from the peak influences the strength of the resulting selection [24,26].

[6,7]. In human language, for example, phonemes are clustered into words, words into phrases, phrases into sentences, and so on. Those clusters (words, phrases, sentences) containing many lower level elements (phonemes, words) are more manageable for processing than all of the elements as a pile of individual items. Combinatorial processing involves rules that specify acceptable ways of combining signal elements [6–9]. English speakers, for instance, only accept some combinations of the same sounds (e.g. 'tire' and 'rite' as different but proper words while rejecting yet other combinations of the same sounds (airtie) as improper; [7]). Combinatorial processing has been described in several birds [10–16] and mammals [17–21]. The taxonomic range of species with combinatorial processing rules has recently been expanded, with the discovery of basic combinatorial processing (i.e. at the level of phonology or finite state grammar, as with 'tire' versus 'rite') in an insect [22], and there is evidence that suggests such rules or similar ones may be present in other insects as well [23].

Animal signals vary not only in the composition of their discrete elements, but also in the features of each element. A signal with two distinct elements, for instance, may vary among individuals and species in the length and dominant frequency of each element. In fact, most of the research on animal communication has focused on the relationship between continuous variation in such signal features and processing by receivers (e.g. mate preference functions in mate choice; figure 1). Some authors have used a univariate approach, analysing the relationship between variation in individual signal features and receiver responses (e.g. [24,27–30]). Others have used a multivariate approach, exploring more complex surfaces describing variation in attractiveness over signal trait space (e.g. [31–35]).

What is the relationship between the rules that govern combinatorial signal processing and the processing of continuous variation in individual signal features? Asking this question is an important step towards elucidating the cognitive architecture underlying mate choice decisions that involve processing complex signals (cf. [36]). The components of this architecture are in turn crucial for understanding the consequences of mate choice for sexual selection and speciation [25,37,38].

Here, we analyse the relationship between the combinatorial rule for a signal with two elements and a mate
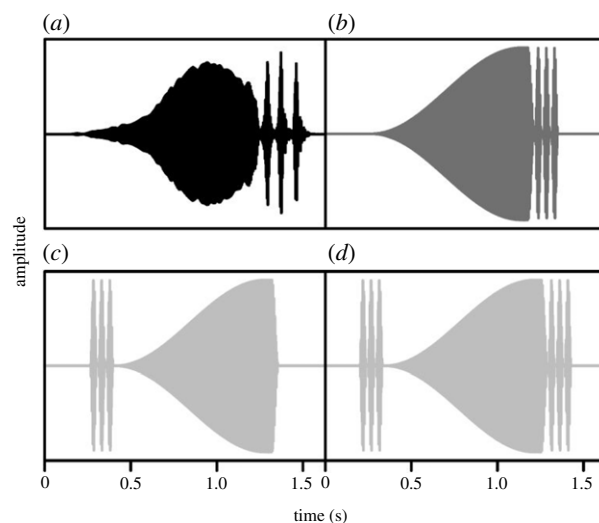

Figure 2. *Enchenopa binotata* male advertisement signals. (*a*) Recording of a signalling male, (*b*) synthetic male signal with the natural-element combination, (*c*) synthetic signal with reversed-element combination, (*d*) synthetic signal containing the natural-element combination but beginning with pulses, *Enchenopa* females prefer playback stimuli containing the natural signal-element combination (*b,d*) but discriminate against the reverse-combination stimuli (*c*), showing that their processing rule is combinatorial [22].

preference function for continuous variation in one of those signal elements. This preference function is closed or unimodal (as in figure 1*b*) in our study species (see below); however, the rationale we develop is also applicable for linear or open-ended functions.

Our study species is a member of the *Enchenopa binotata* species complex (Hemiptera: Membracidae). These treehoppers communicate with plant-borne vibrational signals [39]. Male advertisement signals are composed of two elements: a pure tone 'whine' that slightly sweeps down in frequency from beginning to end and a series of pulses that follow the whine (figure 2*a*; [39,40]). Females choose among males mainly on the basis of dominant signal frequency, and divergence in this preference seems to be the main cause of signal divergence in the complex [29,40–42]. Recent work revealed a combinatorial rule in *Enchenopa*, whereby females prefer playback stimuli containing the natural signal-element combination regardless of the beginning element but discriminate against reverse-combination stimuli [22].

We tested four competing hypotheses that analyse the relationship that may exist between a combinatorial rule and a preference function. We tested these hypotheses in terms of overall differences in preference functions that females expressed when presented with natural- and reverse-element combination playbacks. We were also interested in assessing patterns of individual variation, because within-population, individual differences in mate preference functions may have important consequences for the strength and form of selection stemming from mate choice [25,38]. We therefore also tested the hypotheses by assessing patterns of between-individual variation in: (i) the strength of the combinatorial rule and the preference function for signal frequency and (ii) the strength of the preference function for signal frequency when expressed with natural- and reverse-element combination stimuli.

The first hypothesis posits that the combinatorial rule takes precedence over the preference function, so that signals with the reverse-element combination (i.e. pulse-whine rather
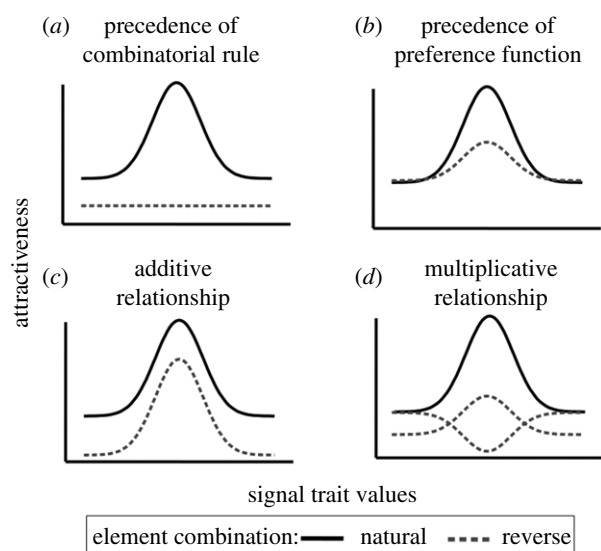
Figure 3. Relationships that may exist between a combinatorial rule and a continuous preference function. The solid black and grey dashed lines represent preference functions expressed with natural and reverse signal-element combinations, respectively. (*a*) The combinatorial rule takes precedence over the preference, so that signals with the reverse-element combination become completely unattractive regardless of the features of the elements; (*b*) the preference function takes precedence over the combinatorial rule, so that the combinatorial rule mostly reduces the attractiveness of stimuli with preferred frequencies (because stimuli with non-preferred frequencies have low attractiveness anyway); (*c*) the combinatorial rule and the preference relate additively, so that the shape of the two curves is similar, only the mean elevation varies; (*d*) the combinatorial rule and the preference relate in a multiplicative way, so that the shape of the preference varies markedly with the combination of signal elements. These graphs do not encompass the whole diversity of possible curves.

Figure 4. Relationships that may exist between the strengths of a combinatorial rule and of a continuous preference function. The numbered circles show the main possible 'types' of individual: (1) individuals with a strong preference and a weak combinatorial rule; (2) individuals with both a strong preference and a strong combinatorial rule; (3) individuals with both a weak preference and a weak combinatorial rule; and (4) individuals with a weak preference and a strong combinatorial rule.

than whine-pulse) become completely unacceptable regardless of the features of the elements. This hypothesis predicts that preference functions will flatline at low attractiveness for signals with the reverse-element combination (figure 3*a*). In terms of individual variation, we draw no prediction from this hypothesis about the strength of the preference function for signal frequency with natural- and reverse-element combination stimuli. However, this hypothesis predicts that individuals should have stronger combinatorial rules than preference functions, and that no individual should have a weak combinatorial rule but a strong preference function (i.e. no 'type 1' individuals as in figure 4). There is already some evidence against this hypothesis (the likelihood of female response did not drop to zero with reverse-element combination stimuli in the prior study [22]); here, we test it more fully.

The second hypothesis states that the preference function takes precedence over the combinatorial rule. This hypothesis predicts that the combinatorial rule will mostly influence the attractiveness of stimuli with preferred frequencies (because stimuli with non-preferred frequencies have low attractiveness anyway; figure 3*b*). In terms of individual variation, we draw no prediction from this hypothesis about the strength of the preference function for signal frequency with natural- and reverse-element combination stimuli. However, this hypothesis predicts that individuals should have a stronger preference function than combinatorial rule and that no individual should have a weak preference function
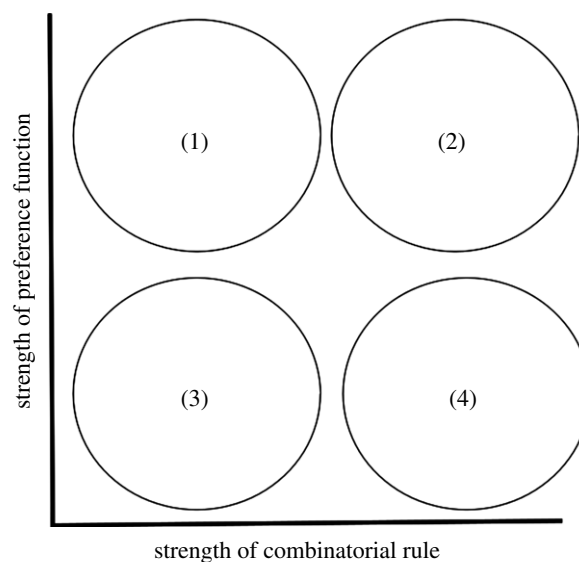
but a strong combinatorial rule (i.e. no 'type 4' individuals as in figure 4).

The third hypothesis posits that the combinatorial rule and the preference function relate in an additive way, so that attractiveness is evenly lowered for signals with the reverse-element combination. This hypothesis predicts that preference functions will have lower mean elevation for signals with the reverse-element combination (figure 3*c*). At the individual level, this hypothesis predicts that the shape of the preference for signal frequency will be similar for natural and reverse-element combinations (i.e. as in figure 3*c*). Consequently, there should be a correlation between the strength of the preference function for signal frequency with natural- and reverse-element combination stimuli (figure 5*a*). We draw no prediction from this hypothesis about the strengths of the combinatorial rule and the preference function for signal frequency.

The fourth hypothesis posits that the combinatorial rule and the preference function relate in a multiplicative way. This hypothesis predicts that preference functions will vary in shape (e.g. become steeper or shallower) according to signal-element combination (figure 3*b*,*d*). Note that the scenario depicted in figure 3*a* or *b* as supporting the second hypothesis above would constitute a form of interaction. However, this hypothesis allows for a wider variety of effects on the shape of the preference function (figure 3*d*). For example, an extreme version of the prediction would be for the valence of signal features to change sign with different signal-element combinations (cf. [43]; as with the inverse dotted line in figure 3*d*). At the individual level, this hypothesis predicts that the shape of the preference will be different with natural- and reverse-element combination stimuli (as in figure 3*d*). Consequently, there should be no correlation between the strength of the preference function for signal frequency with natural- and reverse-element combination stimuli (figure 5*b*). We draw no prediction from this
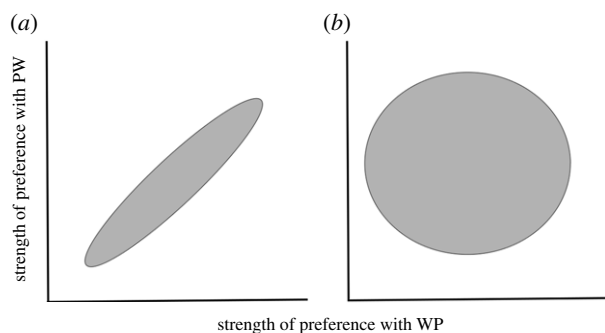
Figure 5. Predicted relationships between the strength of the preference function for signal frequency expressed with natural- (WP) and reverse-element (PW) combination stimuli. The grey area represents the predicted distribution of the points under different hypotheses. (a) If the combinatorial rule and the preference relate additively (as in figure 3c), the shape of preference will be similar with natural- and reverse-element combinations; consequently, the strength values of the preference expressed with natural- and reverse-element combinations will be correlated; (b) if the combinatorial rule and the preference interact (as in figure 3d), the shape of the preference will be different with natural- and reverse-element combination stimuli; consequently, the strength values expressed with natural- and reverse-element combinations will not be correlated.

hypothesis about the strengths of the combinatorial rule and the preference function for signal frequency.

The above scenarios do not deal with the position of the 'peak preference' (i.e. the preferred signal trait values; figure 1). We have no *a priori* rationale for expecting that signal-element combinations would change preferred values for continuous signal features. Nevertheless, to allow for this possibility, we also tested for correlations between peak preference and the strengths of the preference and the combinatorial rule, and for differences in peak preference across 'types' of females (figure 4). Exploring such relationships may help understand how the components of signal processing vary across individuals, and provide insight into how they generate selection on signals [25].

2. Material and methods

Most of the species in the *E. binotata* complex have not yet been described [44]. However, they can be readily recognized according to the colouration of the nymphs, the host plant species that they use, and the dominant frequency of the advertisement signals of adult males [39,40]. We used the species that lives on *Viburnuum lentago* host plants (Adoxaceae) in Wisconsin, USA, has grey nymph colouration, and male signals with a dominant frequency of about 165 Hz. We preserved all individuals used in our experiments in 70% ethanol.

We collected nymphs from two sites (Downer Woods on the University of Wisconsin-Milwaukee campus in Milwaukee, WI, USA, and the University of Wisconsin-Milwaukee Field Station in Saukville, WI, USA) at the beginning of June 2017. We reared the nymphs in aggregations of 30–40 individuals on potted host plants in the UWM Biological Sciences Greenhouse with natural photoperiod and a mean temperature of 24°C (s.d. = 4°C). As soon as the treehoppers moulted to the adult stage, we separated males and females on new potted plants to ensure females remained virgin and receptive to male signals.

(a) Vibrational playback trials
We tested females individually on a designated playback plant to avoid potential confounding factors of variation in plant signal transmission features [45,46]. 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, USA). We calibrated the playbacks to the average amplitude of a male signalling a few centimetres away from the female on the stem (0.15 mm s^{-1}) using an oscilloscope (HMO 1002 series, Rohde and Schwartz, Munich, Germany) and a laser vibrometer (see below).

We generated 10 synthetic playback stimuli using a custom-made script in MATLAB (MathWorks, Natick, MA, USA). The stimuli varied in element combination: natural (whine-pulse) and reversed (pulse-whine; figure 2b,c) and in frequency (140–220 Hz, in steps of 20 Hz centred around the population-level preferred frequency to allow for more resolution in the detection of variation in individual peak preference and preference strength). We set all other signal features to the population mean.

Our assay of female response to the playbacks was based on the natural communication system of the treehoppers. *Enchenopa* females express their mate preferences by means of selective duetting with males. The likelihood of female response is influenced by specific traits in the male signals, the most important of which is dominant frequency [29]. Thus, the duetting responses of *Enchenopa* females when interacting with playback stimuli offer a practical and realistic indication of their evaluation of signal attractiveness [29,41,47]. We noted whether females responded or not to the playbacks (scoring 0 if a female did not respond to a stimulus or 1 if she responded).

Before trials, we determined whether females were sexually receptive by playing back a recording of a male signal closely matching the population mean. We then presented receptive females with the 10 synthetic signals in random sequence, each separated by intervals of 20–25 s. Out of 46 receptive females tested, eight did not respond to any stimuli and one responded to all stimuli. Those nine females were thus excluded from subsequent analyses, yielding a sample of 37 females for our experiment.

We recorded the playbacks, as well as the response signals of 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 40–3000 Hz (Krohn-Hite 3202; Krohn-Hite Corp., Brockton, MA, 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.

(b) Describing mate preference functions
Mate preferences are function-valued traits—they are expressed as a function of the signals that females encounter [24,48]. We therefore used a function-valued approach to describe individual- and population-level female mate preference functions (see [24] for a discussion of preference functions and how to describe them). We used the program PFunc to fit cubic spline regressions to the female response data. This method does not assume any particular shape for the functions other than some level of smoothness that is determined empirically [24,49]. We generated two signal frequency preference functions for each individual female, one for each of the two signal-element combinations (natural and reverse). We then used these individual curves as the data in the analyses detailed below. We also used this method to generate population-level preference functions for each of the signal-element combinations.

(c) Statistical analysis
(i) Population-level preferences
We used a generalized linear mixed model in R (GLMM). The model included female response (0 for no response and 1 for a response) as the dependent variable. The error structure was a
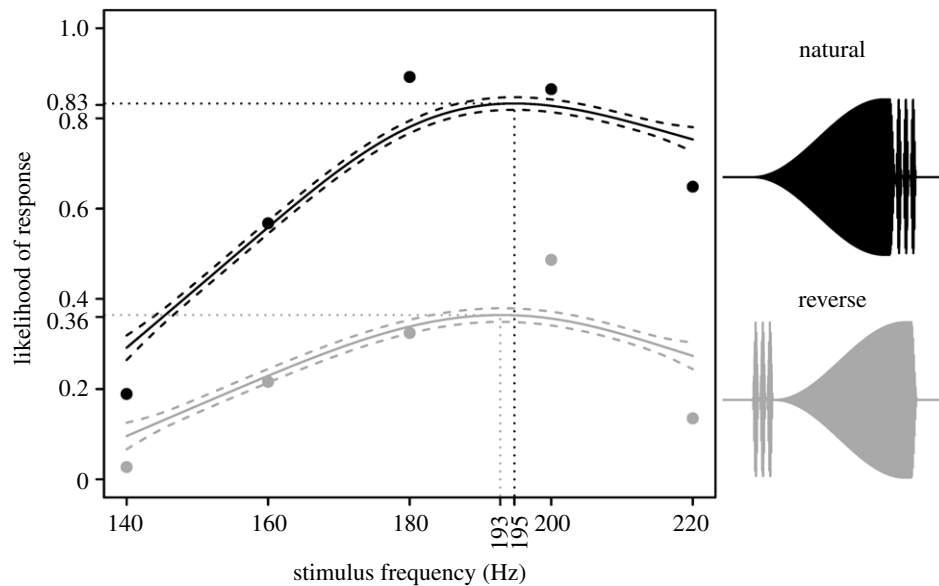
Figure 6. Likelihood of response of *Enchenopa* females to playback stimuli varying in signal-element combination and dominant frequency. Points are mean response likelihood for the 37 females. The curves are population-level preference functions generated with PFunc; dashed lines represent the 95% confidence interval for each preference function. Vertical and horizontal dotted lines indicate the peak preference and peak responsiveness for each function, respectively. Note the slight change in preference shape between stimuli with natural- versus reverse-element combinations, the latter being shallower.

Table 1. Variation in the likelihood of response of *Enchenopa* female treehoppers to playback stimuli varying in signal-element combination and dominant frequency. Significant terms indicated in italics. Random terms (individual female ID, and stimulus sequence) not shown.

term	estimate	z	d.f.	p-value
element combination	2.59	5.59	1, 375	*≪0.001*
frequency (linear)	0.82	2.69	1, 375	*0.007*
frequency (quadratic)	−1.35	−4.09	1, 375	*≪0.001*
element combination × frequency (linear)	0.21	0.59	1, 375	0.56
element combination × frequency (quadratic)	0.02	0.05	1, 375	0.96

binomial distribution. The explanatory variables included the following main terms: stimulus element combination, and linear and quadratic terms for stimulus frequency (the latter to account for curvilinearity in the *Enchenopa* preference function; i.e. as in figure 1*b*; [29]). We also included interaction terms between stimulus element combination and the linear and quadratic terms for stimulus frequency in order to test for differences in the shape of the preference function according to element combination. Because each female contributed two preference functions for signal frequency (one for each of the two signal-element combinations; 10 data points per female), we included female identity as a random factor in the model. A χ^2 test revealed no differences in responsiveness between females collected in the two sites ($\chi^2 = 0.0029$, d.f. = 1, $p = 0.96$). We therefore did not include site as a random term in the model. However, we found a marginally significant effect of stimulus sequence on female responsiveness ($\chi^2 = 15.27$, d.f. = 9, $p = 0.084$). We thus added stimulus sequence (from 1 for the first playback stimulus to 10 for the last one) as a random factor in our model. We fitted the model in R using the function glmer of the R-package lme4 (v. 1.1-21, [50]). We checked model stability by excluding data points one at a time from the data and found that all the estimates were stable except for the interaction term between element combination and quadratic frequency. We derived variance inflation factors using the function *vif* of the R-package car (v. 3.0-3, [51]) applied to a standard linear model excluding the random effects; this analysis did not indicate

collinearity between fixed effects to be an issue. We compared the full model with the null model (e.g. excluding all the predictors or the predictor tested) to test the significance of the model and predictors.

In addition to the interaction terms in the above model, we also conducted a complementary test for differences in preference shape according to signal-element combination. We estimated the correlation between the strengths of the two preference functions we obtained for each female (one with natural- and one with reverse-element combinations). If the shapes are similar (e.g. as in figure 3*c*), the strength values should be correlated (figure 5*a*); but if the shapes differ (e.g. as in figure 3*a,b,d*), the strength values should be uncorrelated (figure 5*b*).

To visualize population-level preference functions for each signal-element combination, we used the group-level feature in PFunc. This feature uses individual preference splines as new input data to fit a single group-level preference function [24].

(ii) Individual-level preferences
We used the PFunc-generated preference functions for each individual female as the trait for analysis. Some of the predictions we tested concern the relationship between the strengths of the preference function and of the combinatorial rule. Assessing that relationship requires measures of strength that would be comparable for the continuous preference and the discrete combinatorial rule. We therefore calculated the strength of the preference of each female as the difference between the highest and lowest
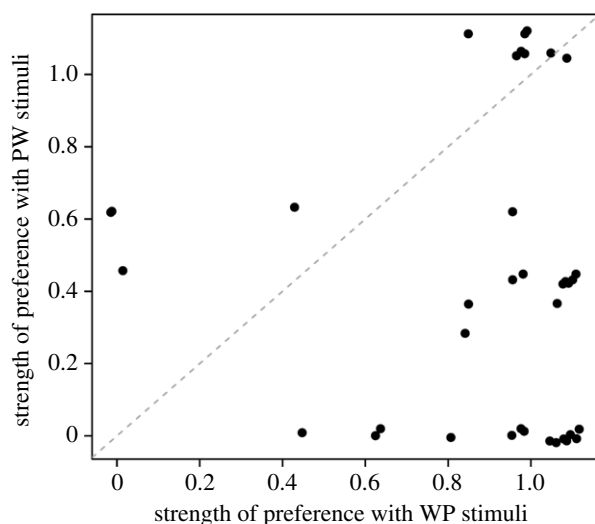
Figure 7. Individual variation in the strengths of the preference functions for frequency with natural (WP) and reverse (PW) signal-element combination in *Enchenopa* females. The dashed line indicates a one-to-one relationship. The correlation between strengths was of small effect size and non-significant ($\rho = -0.18$, $p = 0.29$). We jittered the points to handle overlapping.

response values from her preference function for signal frequency with natural-combination stimuli. And we calculated the strength of the combinatorial rule of each female as the difference between the two highest values from her preference functions for signal frequency with the natural- versus reverse-combination stimuli. As individual-level preference functions were constructed on the basis of a low number of stimuli (five for each element combination), we confirmed the reliability of our strength estimates with a simulation analysis (see electronic supplementary material for details).

We used a clustering approach to explore the relationship between individual variation in the strengths of the preference and the combinatorial rule. We computed the clusters with the *k*-means method with the *kmeans* function in R using the estimates for strength of the preference and combinatorial rule as input variables. To choose the number of clusters, we simulated the results obtained with 2–15 clusters and computed the within-cluster sum of squares for each number of clusters. When selecting the number of clusters, there is a trade-off between increasing the fit of each cluster and minimizing the number of clusters to get meaningful clusters. We thus selected a number of clusters corresponding to the start of the plateau for the sum of squares.

Finally, we compared the peak preference for signal frequency for functions with natural- and reverse-element combinations with a paired Wilcoxon rank test. We also tested for correlation between peak preference and the strengths of the preference function and of the combinatorial rule for each female. Because the data were not normally distributed, we used Spearman correlations instead of Pearson. We further tested for differences in peak preference between female 'types' (figure 4), with a Kruskal–Wallis rank-sum test, as the assumptions of normality of residuals were violated for the ANOVA. We then investigated the *post hoc* differences using pairwise Wilcoxon rank-sum tests. We did not correct for multiple testing as this added only three additional tests and multiple testing corrections tend to lower statistical power [52].

3. Results

(a) Population-level preferences
We found a significant effect of stimulus frequency (linear and quadratic terms) and of signal-element combination on the likelihood of female response (table 1 and figure 6). This confirms prior results of a closed or unimodal preference function for signal frequency [29,30] and the combinatorial rule [22] in *Enchenopa*.

The effect of reversing the signal elements was to reduce by 47% the likelihood of female response to the most preferred signal frequency value (i.e. at peak preference), from 83 to 36% likelihood of response (figure 6). The decrease was less pronounced away from the peak preference (figure 6). Nevertheless, the linear and quadratic terms remained significant and had similar estimate values if we retained only the reverse-element combination data (linear term: $z = 2.62$, $p = 0.009$; quadratic term: $z = -3.92$, $p \ll 0.0001$). Thus, even the preference function for signal frequency with the reverse-element combination was curvilinear and unimodal (figure 6).

None of the interaction terms between stimulus frequency and element combination were significant (table 1), suggesting that the shape of the preference did not change with element combination. However, visual inspection of the plots of the population-level preference functions for each signal-element combination (figure 6) suggested a subtle difference in the steepness of the curves, with the function with the reverse-element combination being shallower. We therefore conducted an additional test for changes in the shape of the functions: we tested for a correlation between the individual values for the strength of the preference for signal frequency with natural- and reverse-element combinations. This correlation was of small effect size and non-significant (figure 7), suggesting that the shape of the preference did vary with element combination.

(b) Individual-level preferences
When we explored individual variation in the strength of the preference function and the combinatorial rule, the within-cluster sum of squares revealed three clusters as the most appropriate solution (figure 8). This suggests the presence of three 'types' of females: individuals with both a strong preference function and a strong combinatorial rule (23 of 37 females; i.e. 62%); individuals with both a weak preference and a weak rule (5 females; i.e. 14%); and individuals with a strong preference but a weak combinatorial rule (9 females; i.e. 24%). Interestingly, there were no individuals with a weak preference but a strong rule (i.e. no 'type 4' females).

Individual peak preference had a mean of 187 Hz and did not differ between preference functions expressed with natural and the reversed-element combination stimuli (figure 9*a*). The three female 'types' (clusters) differed significantly in peak preference, and the *post hoc* test indicated that females with a high strength for both combinatorial rule and preference function had a higher preference peak than the other two female types (figure 9*b*). The preference peak was significantly and positively correlated with the strength of the combinatorial rule (weak effect size; figure 9*c*), as well as with the strength of the preference function with the natural-element combination (large effect size; figure 9*d*).

4. Discussion
We assessed the relationship between the rules that govern combinatorial signal processing and the processing of continuous variation in individual signal features. We worked with *Enchenopa* treehoppers, which have a basic
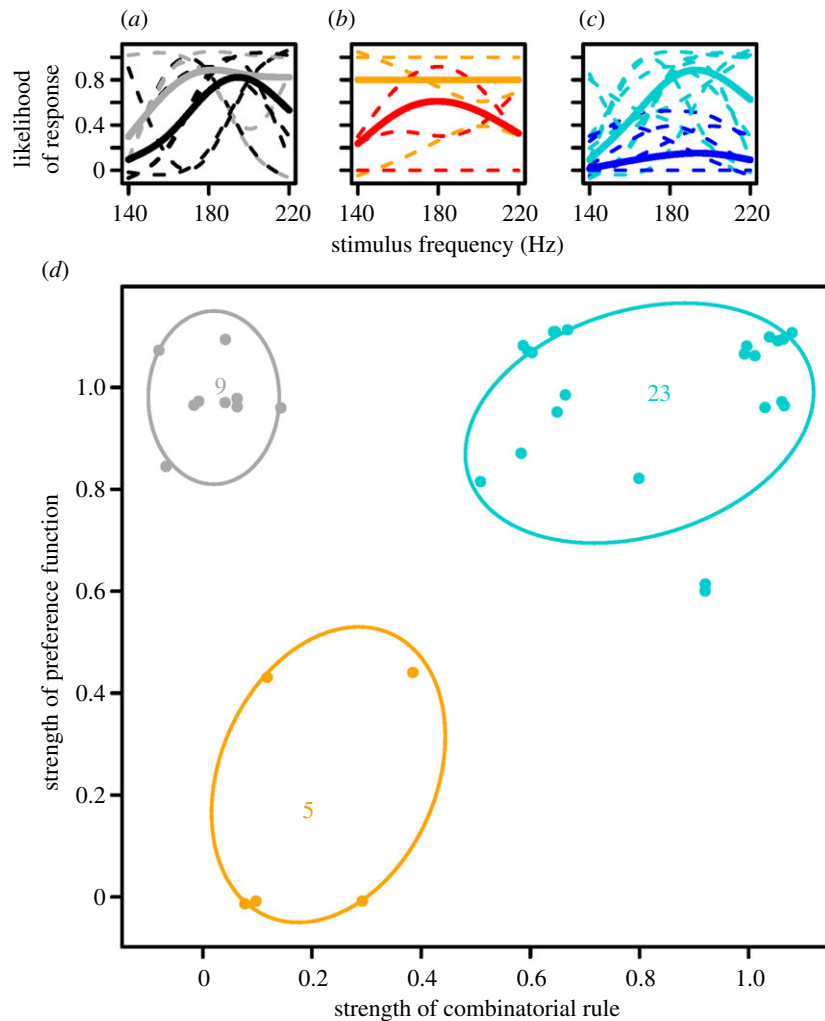
Figure 8. Individual variation in the strength of the preference function for frequency and the strength of the combinatorial processing rule in *Enchenopa* females. (*a*–*c*) Individual (dashed thin lines) and group-level (full thick lines) preference functions for each cluster (curves generated with PFunc). These preferences correspond to female type 1 (*a*), type 2 (*c*), and type 3 (*b*). Within each cluster, lighter colours (grey, orange, and light blue) correspond to the preference functions expressed with natural-element combinations, and dark colours (black, red, and blue) correspond to the preference function expressed with reverse-element combination. (*d*) There were three clusters, suggesting three 'types' of female. Each point represents one female. We jittered the points to handle overlapping. (Online version in colour.)

combinatorial rule for their two-component advertisement signal [22]. We contrasted this combinatorial rule against the treehoppers' strongest mate preference function, that for dominant signal frequency [29,30].

We first asked about the relationship between the combinatorial rule and the preference function in terms of potential changes in the shape of population-level preferences expressed with natural- versus reverse-element combination stimuli. We found that flipping the signal-element combination affected the elevation and the shape of the preference function without influencing preferred signal frequency values (i.e. without varying peak preference). Having the reverse-element combination lowered stimulus attractiveness by up to 51%. This decrease corresponds to a 30 Hz deviation in signal frequency away from the peak of the preference function (i.e. a deviation of *ca* 75% of the population range of variation, [53]). Thus, the strength of the combinatorial rule was comparable to the strongest mate preference in the *E. binotata* complex, which accounts for the largest species differences in signals in the complex [29,39,40].

In spite of the overall reduction in the likelihood of female response, flipping the signal-element combination did not zero out the function, opposing the combinatorial rule precedence hypothesis (figure 3*a*). The formal test for an effect of the combinatorial rule on the shape of the preference function (the interaction terms between element combination and frequency; table 1) did not detect a change in preference shape. However, the analysis using individual variation did suggest that the combinatorial rule affects the shape of the preference function: by mostly lowering response levels for preferred signal frequencies, the combinatorial rule not only makes the preference function lower overall, but also shallower. These results support both the preference function precedence hypothesis and a 'moderate' version of the interaction hypothesis (figure 3*b*,*d*), and oppose the additive hypothesis (figure 3*c*).

We also addressed the relationship between the combinatorial rule and the preference function in terms of patterns of between-individual variation. We found considerable individual variation in the strengths of the combinatorial rule and of the preference function. We detected three 'types' of female with distinct combinations of these traits. The majority of females (62% of 37) had both a strong combinatorial rule and a strong preference, and there were no females with a strong rule but a weak preference (i.e. no 'type 4' females; figure 4*b*), supporting the preference function precedence hypothesis.

All together, we consider that these results support the hypothesis that the preference function takes precedence over the combinatorial rule, which is a subcase of the
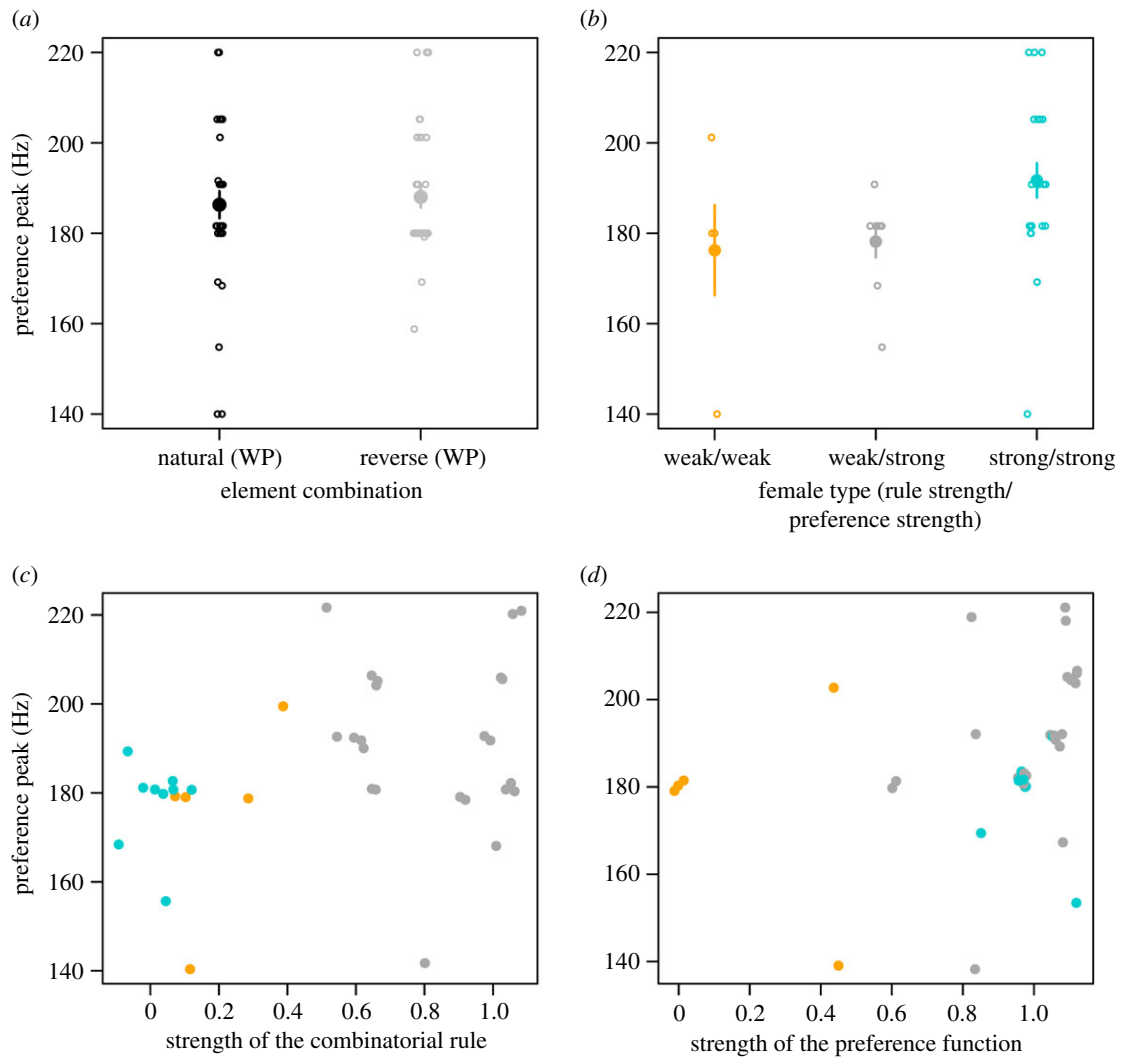
Figure 9. Patterns of individual variation in peak preference and the strength of the preference and the combinatorial rule in *Enchenopa* females. (*a*) Variation in peak preference between preference functions expressed with different element combination stimuli ($V = 268$, $p = 0.82$); (*b*) variation in peak preference across the three female 'types' identified by the cluster analysis ($\chi^2 = 7.48$, $p = 0.024$). (*c*) Relationship between peak preference and strength of the combinatorial rule ($\rho = 0.34$, $p = 0.039$). (*d*) relationship between peak preference and the strength of the preference function expressed with the natural-element combination ($\rho = 0.63$, $p \ll 0.0001$). Colours as in figure 8. We jittered the points to handle overlapping. (Online version in colour.)

multiplicative hypothesis. There was some evidence of a change in preference shape between stimuli having the natural- and reverse-element combination (which supports both hypotheses; figure 3*b,d*). However, the absence of females having the combination of a strong combinatorial rule and a weak preference tends to favour the former hypothesis.

Females with a strong preference and a strong rule ('type 2' females; figure 4) also had higher peak preferences for signal frequency. This result suggests that females may vary in their mate choice decisions and contribute differently to selection on male signals stemming from mate choice within the population (cf. [25,38]). We observed that females with the most stringent criteria (those with the strongest rule and preference) will tend to favour higher signal frequencies, whereas the less 'selective' females will favour lower frequencies. Although the latter females were the minority in our sample, they may help explain why the mean signal frequency tends to be below the mean peak preference in the population [53]. Moreover, individual variation suggests that the preference–rule relationship may be capable of responding to selection on mate choice decisions in different directions, given sufficient underlying genetic variation

(except perhaps in the direction of weakening the preference and strengthening the rule, as there were no individuals with this trait combination). Interestingly, although male signal frequency is the most divergent adult phenotype in the *E. binotata* complex (matching divergence in female peak preferences; [29,39,40]), signal-element structure is highly conserved and all species in the complex follow the whine-pulse pattern [39,40]. This suggests that the combinatorial rule may also be highly conserved in *E. binotata*.

The relatively simple communication system of *Enchenopa* treehoppers made it tractable to explore how a combinatorial rule and a preference function for a continuous signal feature are processed jointly. Our findings suggest that, as combinatorial rules help animals process complex signals (thereby allowing complex signals to evolve), they also influence the processing of the continuous features of the individual elements of those signals; e.g. increasing overall discrimination and contributing to within-population variation in likely mate choice decisions in this study. Even simpler processing rules may have important consequences for mate choice. Some frogs, for example, have a 'beginning rule', or temporal order effect, whereby novel changes to the

beginning portions of signals are never favoured, but the same changes at the end of signals can improve attractiveness [54]. This effect was strongest with shorter (less preferred) signals, highlighting the potential for such rules to influence selection on signals. It will be interesting to explore the form that such relationships take in species with much more complex signal structures. Do combinatorial rules take precedence in animals with very complex signals to facilitate processing? Or do combinatorial rules often function 'in parallel' with preferences for continuous signal traits in such cases? Addressing these questions will bring insight into the evolution of complex signals and of the cognitive architecture of mate choice decisions.

Ethics. *Enchenopa* treehoppers are insects which do not present any conservation concern. Our research was in compliance with national legislations.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.8cz8w9gn6 [55].

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