JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.13122

Describing mate preference functions and other function-valued traits

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Keywords:

cognitive phenotype;
developmental trajectories;
mate choice;
plasticity;
reaction norm;
sexual selection.

Abstract

Mate preferences are important causes of sexual selection. They shape the evolution of sexual ornaments and displays, sometimes maintaining genetic diversity and sometimes promoting speciation. Mate preferences can be challenging to study because they are expressed in animal brains and because they are a function of the features of potential mates that are encountered. Describing them requires taking this into account. We present a method for describing and analysing mate preference functions, and introduce a freely available computer program that implements the method. We give an overview of how the program works, and we discuss how it can be used to visualize and quantitatively analyse preference functions. In addition, we provide an informal review of different methods of testing mate preferences, with recommendations for how best to set up experiments on mate preferences. Although the program was written with mate preferences in mind, it can be used to study any function-valued trait, and we hope researchers will take advantage of it across a broad range of traits.

Introduction

Darwin (1871) identified a broadly fascinating topic of modern biology when he pointed out the special nature (and consequences) of mate preferences. Mate preferences are mental phenomena – representations of the attractiveness of potential mates, expressed in animal brains as the product of sensory and neural processing. They are a kind of cognitive phenotype, and studying them is at the forefront of the objective study of how mental phenomena influence behaviour and decision-making (Mendelson *et al.*, 2016). Because mate preferences influence mate choice decisions, they can generate strong sexual selection (Darwin, 1871; West-Eberhard, 1983, 2014, 2014; Andersson, 1994; Ritchie, 1996; Jennions & Petrie, 1997; Wagner, 1998; Widemo & Sæther, 1999; Andersson & Simmons, 2006; Cotton *et al.*, 2006; Prum, 2012). This places the study of mate preferences at the intersection of behavioural ecology, neuroscience and evolutionary biology.

Mate preferences are expressed as a function of the features of potential mates that are encountered. In other words, they are function-valued traits (Meyer & Kirkpatrick, 2005; Stinchcombe *et al.*, 2012). The function-valued nature of mate preferences means that describing them requires assessment across a range of display trait values. When an individual encounters a potential mate, only a fraction of its mate preference is expressed – the part that corresponds to the potential mate's trait values. Confronted with another potential mate with different features, that same individual with that same mate preference may respond differently.

Describing mate preferences brings insight into the causes of selection and speciation, and into how organisms perceive and process some of the most salient aspects of their environments (Ritchie, 1996; Jennions &

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Petrie, 1997; Wagner, 1998; Widemo & Sæther, 1999; Andersson & Simmons, 2006; Chenoweth & Blows, 2006; Cotton *et al.*, 2006; McPeek & Gavrilets, 2006; Rodríguez *et al.*, 2006, 2013a; Sullivan-Beckers & Cocroft, 2010). When used to characterize populations or species, descriptions of mate preferences offer a straightforward framework to test hypotheses about the action of sexual selection due to mate choice (Fig. 1). Meanwhile, studying mate preferences at the level of individuals can shed light on the developmental and genetic underpinnings of preferences, which is key for assessing the generality of theoretical models of sexual selection and speciation (Jennions & Petrie, 1997; Mead & Arnold, 2004; Kuijper *et al.*, 2012; Roff & Fairbairn, 2014; Sharma *et al.*, 2016).

Here, we present a simple method for describing and analysing mate preference functions, and a computer program that implements it. The method takes a function-valued approach, viewing the entire preference function as the trait of interest for an individual (or population). It then extracts some measurements (preference function traits) from the curve that describe the preferred sexual display values and the shape of the curve around those preferred values (Fig. 1). These traits are key for testing hypotheses about mate preferences as causes of sexual selection (Rodríguez *et al.*, 2006, 2013a). For example, *peak preference* indicates the display value that should have peak fitness under sexual selection due to mate choice; other traits indicate the expected strength of selection (Fig. 1) (see below). The traits are also useful for describing components of variation in mate preferences such as repeatability, heritability and plasticity (Fowler-Finn & Rodríguez, 2012a,b, 2013; Rebar & Rodríguez, 2013, 2014; Rodríguez *et al.*, 2013b).

Describing and analysing mate preference functions

Consider an experiment that we might use to describe mate preferences for one of our study animals, a species of *Enchenopa* treehopper (Hemiptera: Membracidae) (Fig. 2). *Enchenopa* females respond to male advertisement signals that they find attractive with signals of their own, establishing a duet that continues while the male searches for the female, until copulation begins (Fig. 2a) (Cocroft *et al.*, 2008). By playing back a sample of male signals to a female and seeing how she responds to each one, we describe her preference across a range of male signal trait values. Such playback experiments produce data sets with two basic components: an independent variable encompassing variation in a signal trait (in this example, dominant frequency, or pitch) and a dependent variable reflecting the female's ranking of the attractiveness of the stimuli (measured here as the number of responses she emitted when presented with each male signal) (Fig. 2b).

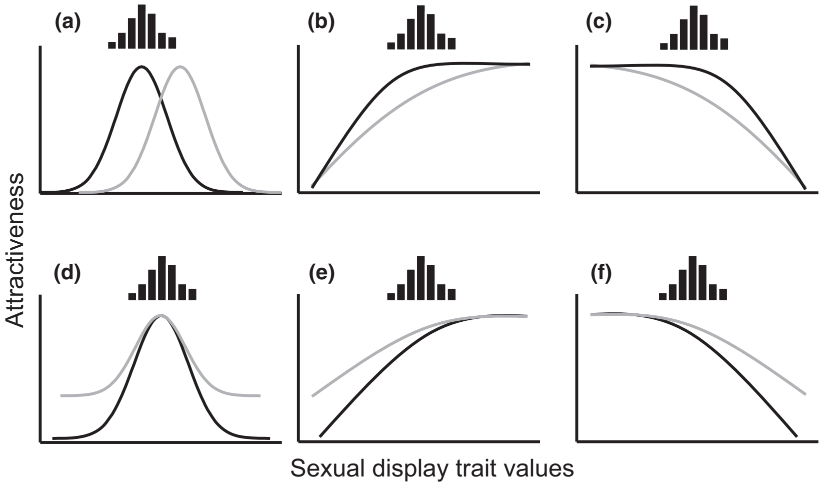


Fig. 1 Mate preferences represent variation in the attractiveness of sexual displays and can take various forms. In each panel, curves depict preferences, and the histograms correspond to the distribution of sexual display traits. (a) Mate preferences may have closed shapes, meaning that peak display attractiveness is reached at some intermediate display trait value. There can be variation in peak preference (black vs. grey curves). (b, c) Mate preferences may also have open-ended shapes. Here too, peak preference may vary; for example, the grey curves would have peaks at the extreme value of the test range, whereas the black curves might have a peak part-way across their plateaus. Mate preferences may also vary in the extent of the decrease in attractiveness as displays deviate from the peak preference (d, e, f: black vs. grey curves). When contrasted against the distribution of sexual display traits (histograms), mate preferences constitute hypotheses about the form of sexual selection. For example, in (a), the preference in black represents stabilizing selection, whereas the preference in grey represents directional selection. Similarly, in (b) and (c), the preferences in black and grey represent varying levels of directional selection.

Data such as the above can be used to generate mate preference functions. A variety of approaches have been used for this purpose, but they all share the goal of producing a function that describes variation in the attractiveness of a sexual trait. One method is to use sets of comparisons between paired stimuli, drawing a line linking the attractiveness of each stimulus pair to assemble an overall shape that is inspected visually (e.g. Jang & Greenfield, 1996; Gerhardt *et al.*, 2000; Murphy & Gerhardt, 2000; Shaw, 2000; Shaw & Herlihy, 2000; Höbel & Gerhardt, 2003; Gerhardt, 2005a, b). Another method is to assume given function shapes, such as linear, quadratic, logistic or sigmoidal, and fit such functions to the data (e.g. Endler & Houde, 1995; Brooks & Endler, 2001; Burke & Murphy, 2007). Yet another method is to use nonparametric analyses that do not assume any given shape (e.g. Ritchie, 1996; Jang & Greenfield, 1998; Brooks & Endler, 2001; Ritchie *et al.*, 2001; Simmons *et al.*, 2001; Reinhold *et al.*, 2002; Brooks *et al.*, 2005; Rodríguez *et al.*, 2006). We value this variety of approaches, because it allows researchers to adjust to the biology and behaviour of their study organism (Reinhold & Schielzeth, 2015). Indeed, researchers often combine some of the above approaches within a single study. For example, linear and quadratic terms are often used for significance testing in combination with nonparametric regression for visualization (see below).

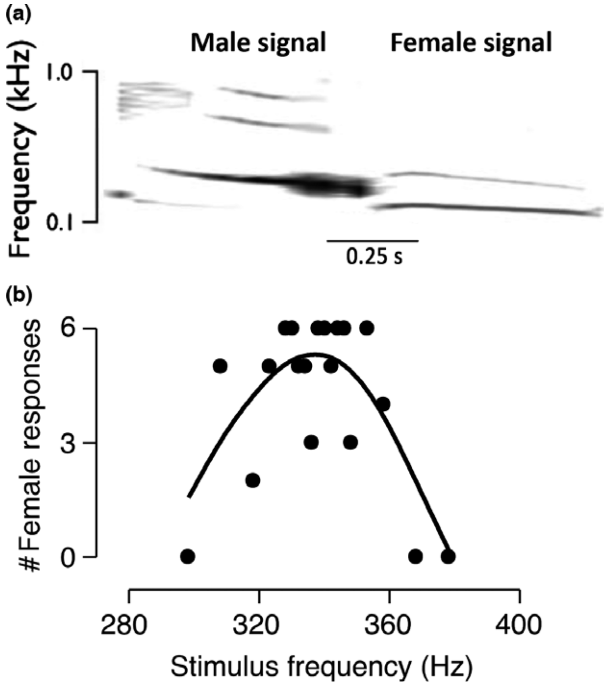


Fig. 2 Example of a mate preference function, from a study with *Enchenopa* treehoppers (Hemiptera: Membracidae), which are plant-feeding insects that communicate with plant-borne vibrational signals (Cocroft & Rodríguez, 2005). (a) Spectrogram of male and female *Enchenopa* duetting signals, including 0.25-s bar for scale. Males travel in search of females, signalling when they reach a new plant. A female that finds a male's signals attractive will engage in a duet with him until he locates her (Cocroft *et al.*, 2008). (b) Behaviour of an *Enchenopa* female interacting with playback stimuli resembling male advertisement signals. The data points indicate the duetting signals that the female produced when presented with each stimulus, and the curve shows the cubic spline generated from those data points (see text), which depicts the female's mate preference function. The preference function peaks at a narrow range of stimulus frequencies and drops sharply as stimuli deviate in either direction away from those preferred frequencies. Note that, in any one encounter with a given male, only a portion of this female's preference function would be expressed. Nevertheless, the entire function is the trait of interest.

Our method adopts the philosophy of making as few assumptions as possible about the shape of mate preferences, following Schluter (1988) and Brodie *et al.* (1995). We use generalized additive models (GAMs) to fit nonparametric curves – termed cubic splines – to the raw response data (Wood, 2006). This allows the shape of the mate preference to be determined primarily by the pattern of responses across a range of stimuli (Fig. 2b), rather than by an *a priori* expectation. As a result, there are no real restrictions to the data that can be analysed as long as it broadly follows the stimulus-response structure that we outline here. We favour this approach because we are interested in exploring variation in mate preferences, and thus, fewer shape assumptions afford greater potential for discovery. Note that although our method avoids prespecifying linear or quadratic shapes, it can be used in conjunction with analyses that do use those terms (see below).

With cubic splines, the shape of mate preferences depends in part on a smoothing parameter (Schluter, 1988; Wood, 2006). Less smoothing produces wobblier functions that can detect more local peaks and troughs, whereas more smoothing produces less wobbly functions that highlight only the major peaks and troughs (Fig. 3). Current statistical software automatically chooses a smoothing parameter that optimizes the predictive power of the function for a given data set (Schluter, 1988; Wood, 2006, 2011). The default in our method is to smooth out functions that are very wobbly – to extract the underlying function from the (sometimes noisy) raw behavioural data – but we allow users to adjust smoothing values when necessary (see below). With this default, we follow the rationale from studies of the form of selection that the true function is more likely to be smoother than very wobbly (Schluter, 1988). We consider that this rationale should fit mate preferences well: being based on neural activity, mate preference functions are likely to be relatively smooth, continuous functions, rather than very wobbly or jagged. Even preference functions with sharp transitions (e.g. step functions with thresholds) are not likely to have the many tiny peaks of a wobbly spline

(Fig. 3a), and their analysis can benefit from visualizing them as somewhat smooth functions.

Mate preference function traits

Once a function has been generated, our method uses several metrics to describe a few key aspects of variation in mate preferences (Fig. 4).

- *Peak preference*: the most preferred display trait value (the point on the *x*-axis where the function reaches maximum value) (Fig. 4a).
- *Peak height*: the maximum elevation of the function (on the *y*-axis) at the preferred stimulus (Fig. 4b). This trait is a measure of the magnitude of the response to the preferred value. We have not used it in prior analyses of variation in mate preferences, but offer it here for researchers that may be interested in it.
- *Tolerance*: the width of the preference function at a given height (in other words, the range of stimulus values over which the function remains relatively high) (Fig. 4c). The height at which we have measured tolerance in prior work is relative to the height of the preference function (e.g. the width of the curve one-third of the way down from the peak), but it can also be measured at a set absolute height on the *y*-axis (see Table 2).
- *Preference strength*: the degree to which attractiveness falls away from peak preference as display values change (in other words, how much the preference 'punishes' deviation from the peak) (Fig. 4d). Our main measure of strength takes into account the overall height of the preference function, because any given amount of drop-off from the peak is relatively greater for preferences that are lower overall (i.e. those with lower responsiveness; defined below). We do, however, offer a height-independent option for users who want a measure of strength that is independent of responsiveness (Fig. 5). In the mate preference literature, measures akin to our preference strength have been termed 'tolerance', 'discrimination' or 'choosiness' (Gray & Cade, 1999; McPeek & Gavrilets, 2006; Bailey, 2008; Edward, 2015; Reinhold & Schielzeth, 2015). We prefer 'strength' for several reasons. 'Discrimination' refers intuitively to the acuity of variation in sexual response, rather than to its magnitude; it is thus better suited to questions about the acuity of perception, as with the framework of just-noticeable and just-meaningful differences (Kirkpatrick *et al.*, 2006). And 'choosiness' has convincingly been applied to the effort an individual invests in mate assessment, which may vary independently from the shape of mate preferences (Jennions & Petrie, 1997). (A related sense of the term 'choosiness' could be the effort invested in obtaining a preferred mate type.) Other authors suggest measuring the strength of mate preferences with the slope of the preference function (e.g. the slope of linear or quadratic functions) (Brooks & Endler, 2001; Edward, 2015). We favour our measures of strength because they do not rely on assumed shapes for the preference function and because they provide a direct link between the mate preference function as a hypothesis about the form of sexual selection due to mate choice and the standard measure of the strength of selection (Schluter, 1988).
- *Responsiveness*: the overall elevation of the preference function (the mean of the *y*-axis values along the function curve) (Fig. 4e). In the mate choice literature, the term 'responsiveness' is often used to denote the motivation to mate, or overall sexual receptivity (see review by Edward, 2015). In this sense, responsiveness refers to a variable that is broader than an aspect of mate preferences (Jennions & Petrie, 1997; Cotton *et al.*, 2006).

These preference traits are useful for analysing variation in mate preferences between individuals, experimental treatments, populations, species, and so on (e.g. Fowler-Finn & Rodríguez, 2012a,b; Rodríguez *et al.*, 2013b). Characterized for different populations or species and contrasted with the distribution of male traits, the preference traits directly relate to hypotheses about the action of sexual selection due to mate choice (e.g.

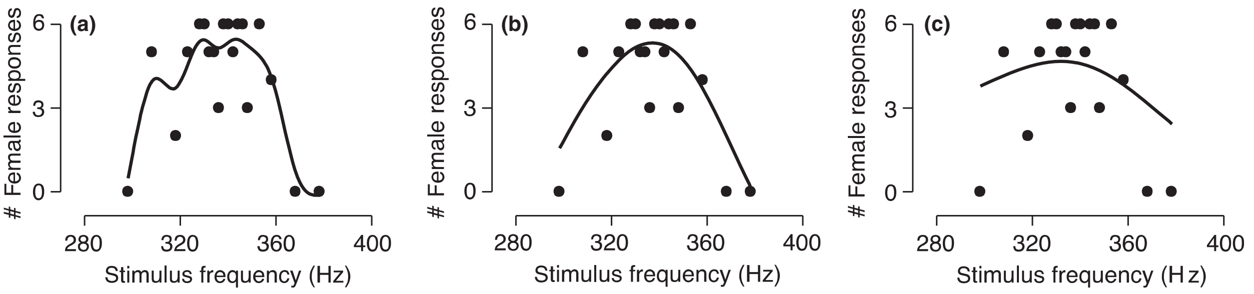
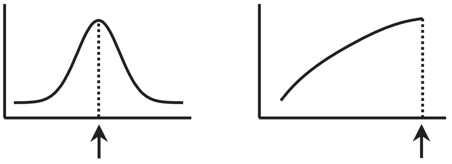
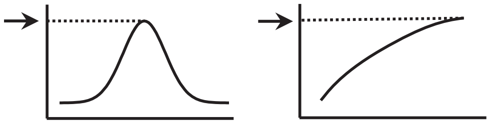


Fig. 3 Example of how the smoothing parameter influences the shape of mate preference functions generated with cubic splines. Panels (a–c) go from weaker to stronger smoothing, yielding wobblier to stiffer functions. The function in panel (b) is generated by the default settings in PFunc and is the same as in Fig. 2b.

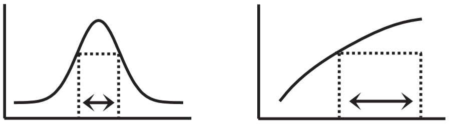
(a) Peak preference



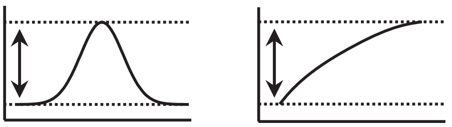
(b) Peak height



(c) Tolerance



(d) Strength



(e) Responsiveness

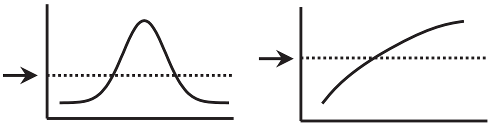


Fig. 4 Mate preference function traits. (a) Peak preference: the preferred display trait value. (b) Peak height: the maximum elevation of the function (on the *y*-axis) at the preferred stimulus. (c) Tolerance: the width of the preference function at a given elevation, either relative to the height of the peak or at a set value. (d) Preference strength: the degree to which attractiveness falls away from peak preference (see Fig. 5). (e) Responsiveness: overall elevation of the preference function.

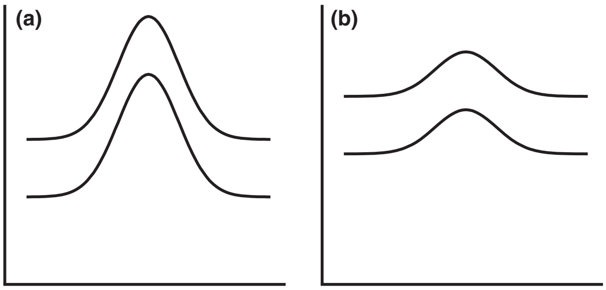


Fig. 5 Examples of differences in preference function strength. Strength is a unitless measure of the vertical range of a spline. We offer two different measures of strength: one that is height dependent {calculated as: [SD(response values)/mean(response values)]^2} and one that is height independent {calculated as: [SD (response values)/(max response – min response)]}. In panel (a), both curves have higher strength (by both measures) than the curves in panel (b). Within each panel, both curves have the same height-independent strength, but different height-dependent strength (higher curves have lower height-dependent strength). We recommend using height-dependent strength unless there is specific reason to use the height-independent measure.

Rodríguez *et al.*, 2006, 2013a). Peak preference (Fig. 4a) predicts the display trait value that ought to occupy the peak of fitness functions if selection was mainly due to mate choice. Preference strength (Fig. 4b) predicts the degree to which mate preferences may contribute to overall selection (relative to other potential sources of selection) (Rodríguez *et al.*, 2006, 2013a). (Note that the strength of sexual selection will also be influenced by mating system and mate searching strategies.) Similarly, tolerance and responsiveness (Fig. 4c,e) likely reflect aspects of the predicted strength of selection.

In our work, we have often found that strength, tolerance and responsiveness are correlated with each other and independent of peak – broad preferences (i.e. those with high tolerance) tend to be shallower than narrow preferences, regardless of the position of the peak (e.g. Fowler-Finn & Rodríguez, 2012a,b, 2013; Rodríguez *et al.*, 2013b). However, the relationship between preference traits may vary among species and experiments, and we allow estimating the preference traits separately so that researchers may explore how they relate to each other. For group-level functions, there may be some additional considerations for analysis. For instance, if data are combined from multiple individuals, standardization of response scores by researchers may be necessary (say, standardizing the maximum response of each individual to a common value). This of course would take meaning away from the responsiveness and peak-height measurements.

The program: PFunc

We wrote a computer program called PFunc to describe and analyse mate preference functions. It was developed and coded by JTK with input from all authors. PFunc allows users to instantly visualize preference functions and several key metrics of the curves. Users can fine-tune individual functions when necessary and can output their results into csv files that can be used for further analysis and graphing in other programs.

The program is built from two main components. The first is code written in R (R Core Team, 2017) that handles most of the analysis. It fits curves through data using the gam function in the *mgcv* package (Wood, 2006), and it outputs measurements of those curves to the user. The second component is a graphical user interface (GUI) (Fig. 6) that is written in Python and uses the rpy2 package to interface with R (see Appendix S1 for set-up and usage instructions; see

Appendix S2 for the program files. The most recent version can be downloaded from https://github.com/joccalor/pfunc/releases/latest).

Here, we focus on using PFunc with the GUI to make it accessible to a broad audience. However, for those comfortable working in the R environment, it is also possible to run PFunc directly from the R command line (see Appendix S1).

Getting started with PFunc

Consider an experiment in which females are presented with a set of playback stimuli – the experiment that yielded the data in Fig. 2, in which females were exposed to 19 different stimuli resembling male advertisement signals (in random order) that varied in pitch. A female's response was measured as the number of times she responded positively to these signals over multiple exposures (with the maximum number being the number of times a given stimulus was played).

To analyse these data in PFunc, the user first organizes them in a spreadsheet, following one of the two layout options described in the data layout section below, and saves it as a csv file. Once the data are in this format, the user can open them in PFunc and begin analysis immediately.

PFunc plots the data points and fits cubic splines to them. The program displays up to nine graphs per page at a time, and the user can navigate between pages with the controls at the bottom of the screen to access all the graphs. Clicking on a graph brings up its information in the control panel on the right-hand side of the window (Fig. 6), including the smoothing parameter and the trait-by-trait breakdown of the curve. Double-clicking on a graph enlarges it for better visibility, and double-clicking on an enlarged graph returns the user to the previous view. Users can also view PFunc's running message log, which displays certain messages and warnings from the current session (Advanced > Show Message Log) (Fig. 6d).

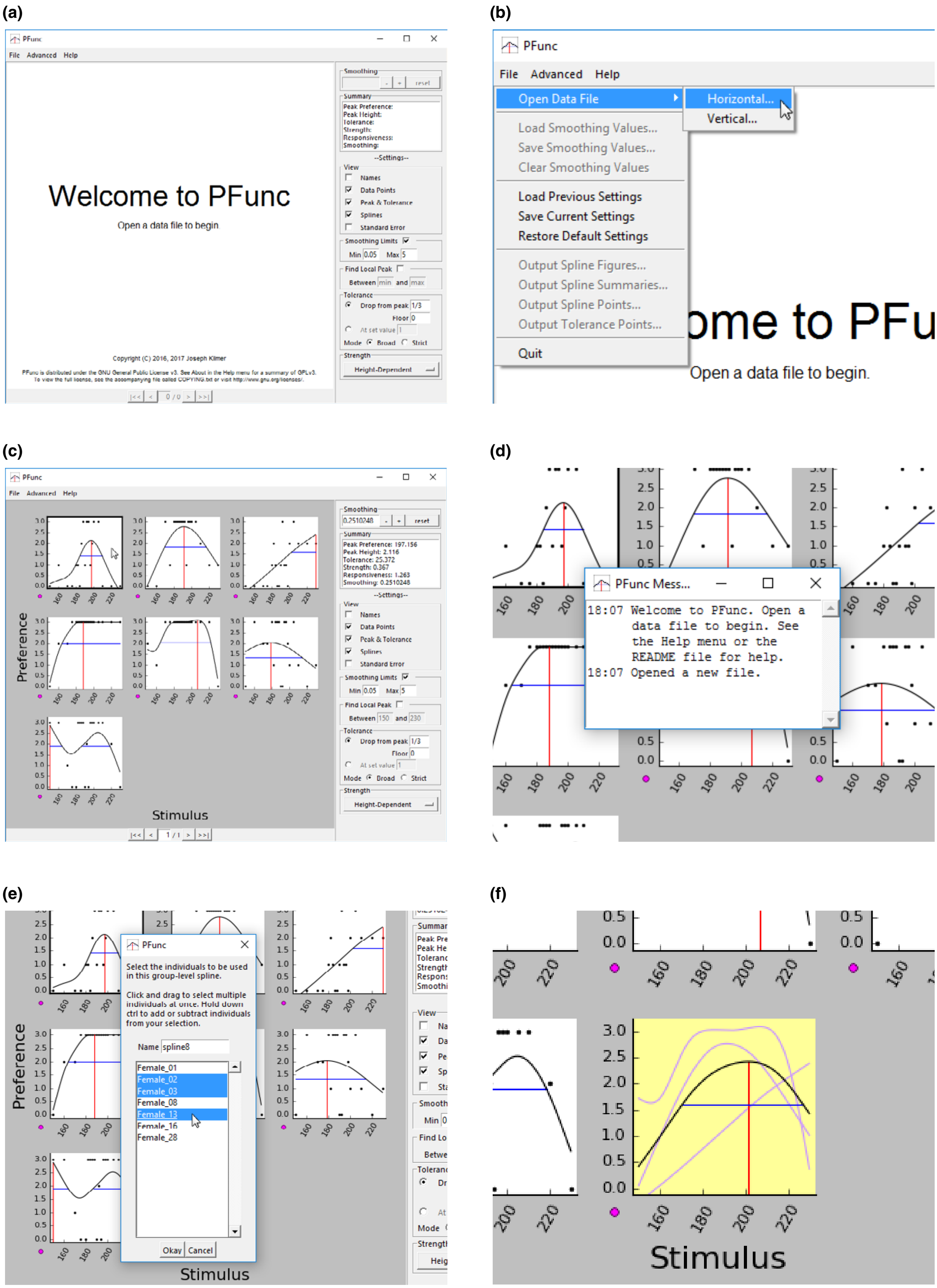
Adjusting the smoothing parameter

The gam function chooses smoothing parameters based on an algorithm that optimizes the fit of the curve using generalized cross-validation (Schluter, 1988; Wood, 2006, 2011). Sometimes, though, the default smoothing parameter is not ideal for preference functions; it may be so low that it makes the preference function very wobbly, with many peaks and troughs, or it may be so high that it makes the function perfectly linear. To restrict the likelihood of these extreme outcomes, and following the philosophy that preference functions should be neither too wobbly nor too stiff, we limit the default smoothing parameter to a range of 0.05–5. We have found that this represents a useful range of natural shapes. However, we have also included the option to freely adjust the smoothing parameter and these limits so that researchers may explore its effect (Table 2).

The box labelled 'Smoothing' shows the smoothing parameter for the currently selected curve (click to select) (Fig. 6). Users can increase or decrease this value either by pressing the +/− buttons or by typing in a new value and pressing the Enter key (user-defined smoothing parameters are not constrained by the 0.05–5.0 range). When a smoothing parameter is altered by the user, an indicator symbol next to the graph changes in colour from magenta to cyan. This helps the user keep track of which smoothing parameters have been altered and which are at their default value. Any time that a user wishes to revert to the default smoothing parameter for a particular curve, they select the curve and press the 'Reset' button in the Smoothing box. This will change the indicator symbol back to magenta.

User-defined smoothing parameters persist through single PFunc sessions and are not automatically saved if the program is closed. However, users can save their custom smoothing parameter values from the File menu (File > Save Smoothing Values…). It is important that this saved file remains in the csv format, or else it may not work in future. Users can also load previously saved smoothing parameter values from the File menu (File > Load Smoothing Values…). Note that if the user makes any changes to the data file between sessions – specifically, if they add, remove or rearrange individuals in the data file – the saved smoothing parameter file may not apply the correct values to the correct individuals. We always recommend double-checking to make sure that PFunc is handling the data correctly. Any changes made to smoothing parameter values will not be made permanent until the user re-saves the smoothing parameter file. The user also has the option of resetting all smoothing parameter values for the session from the File menu (File > Clear Smoothing Values).

The power to freely adjust smoothing parameters can be helpful in a cursory exploration of data, but it is also dangerous, because it invites temptation to interfere with otherwise objectively fit curves. Excessive fine-tuning of smoothing parameters has the potential to introduce bias into the data that should be avoided at all costs. In our work with preference functions, we take a conservative approach to adjusting smoothing parameters. We first establish our minimum and maximum smoothing values, and then we almost always keep the default smoothing values that are calculated. The few times that we have adjusted smoothing parameters for published data were instances where we saw clear trends in the data that were not reflected in the curve, and we made these changes without knowledge of the identities of those individuals nor the groups they belonged to. We urge all users to take a similarly conservative approach to adjusting smoothing



Welcome to PFunc

Open a data file to begin

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PFunc is distributed under the GNU General Public License v3. See About in the Help menu for a summary of GPLv3.
To view the full license, see the accompanying file called COPYING.txt or visit http://www.gnu.org/licenses/.

|<< < 0 / 0 > >>|

Smoothing
- + reset

Summary
Peak Preference:
Peak Height:
Tolerance:
Strength:
Responsiveness:
Smoothing:

--Settings--

View
☐ Names
☑ Data Points
☑ Peak & Tolerance
☑ Splines
☐ Standard Error

Smoothing Limits ☑
Min 0.05 Max 5

Find Local Peak ☐
Between min and max

Tolerance
● Drop from peak 1/3
Floor 0
○ At set value 1
Mode ● Broad ○ Strict

Strength
Height-Dependent

Smoothing
0.2510248 - + reset

Summary
Peak Preference: 197.156
Peak Height: 2.116
Tolerance: 25.372
Strength: 0.367
Responsiveness: 1.263
Smoothing: 0.2510248

--Settings--

View
☐ Names
☑ Data Points
☑ Peak & Tolerance
☑ Splines
☐ Standard Error

Smoothing Limits ☑
Min 0.05 Max 5

Find Local Peak ☐
Between 150 and 230

Tolerance
● Drop from peak 1/3
Floor 0
○ At set value 1
Mode ● Broad ○ Strict

Strength
Height-Dependent

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```
18:07 Welcome to PFunc. Open a
      data file to begin. See
      the Help menu or the
      README file for help.
18:07 Opened a new file.
```

PFunc

Select the individuals to be used in this group-level spline.

Click and drag to select multiple individuals at once. Hold down ctrl to add or subtract individuals from your selection.

Name spline8

Female_01
Female_02
Female_03
Female_08
Female_13
Female_16
Female_28

Okay Cancel

Fig. 6 Screenshots of PFunc's graphical user interface. (a) The starting interface. (b) Opening a data file. (c) Analysing multiple preference functions. (d) The message log (accessible from Advanced > Show Message Log). (e) Creating a group-level spline (accessible from Advanced > Construct Group-Level Spline…). (f) Visualization of a group-level spline.

parameters. To help with this, PFunc's default settings display preference functions without the names of the individuals associated with those splines. If the user wishes to view the names, they may toggle them from the View box in the sidebar.

Data layout

PFunc allows data to be formatted in either of two ways: horizontally or vertically. In the horizontal arrangement, the first column of the spreadsheet contains the stimulus values (in our treehopper example, those would be the signal frequencies of male calls that the females are exposed to). Then, each subsequent column contains the responses of a different individual to the stimuli in column 1 (Table 1a). By comparison, the vertical arrangement contains at least three columns: one for individual IDs, one for the stimulus values, and one for the individual responses (Table 1b). When choosing the vertical option, users will be prompted to tell PFunc which columns of their data correspond to each of the three types of information (individual identity, stimulus value and response score). Both data layout options work equally well, and we leave it to the user to decide which configuration works best for them. In the horizontal arrangement, new individuals are added with additional columns, whereas in the vertical arrangement, they are added with additional rows. Note that the vertical option gives users more flexibility when it comes to testing different individuals against different sets of stimulus values, because these values are specified for each individual, rather than once for the whole dataset.

For either arrangement, there must be at least three (preferably ten or more) responses per individual (or whatever the experimental unit is). Additionally, for typical studies, we recommend having no more than one response value per stimulus value per individual. If there are multiple responses per stimulus per individual, researchers should consider consolidating them into a single measure (e.g. an individual's mean response to each stimulus) or splitting the individual's data up by trial, depending on what the structure of the data allows for. This is to avoid pseudoreplication, but note that researchers can still asses the repeatability (see Boake, 1989) of individual responses and/or preference function metrics, but would do so externally to the program (e.g. with the preference function traits generated by the program; Fowler-Finn & Rodríguez, 2013).

Group-level preference functions

Researchers may wish to go beyond individual-level splines and examine group-level splines, for example, at the replicate, population or species level. Additionally, group-level functions may also be useful for studying organisms for which it is not possible to test individuals repeatedly with a range of stimuli.

Table 1 Options for arranging data for PFunc. (a) The horizontal option: stimulus values are listed in the first column, and each subsequent column contains the responses of a different individual to those stimuli (in this case, the number of times each female responded to each stimulus value). (b) The vertical option: there are at least three columns, each containing a different type of information: individual identity, stimulus values and the responses of individuals to each of the stimulus values. (Note that the use of four stimulus values per individual here is purely for the sake of example. Although it is possible to use only four values in real studies, we recommend using more.)

(a) horizontal option				(b) vertical option		
Stimulus (Hz)	Female_1	Female_2	Female_3	Individual	Stimulus (Hz)	Response
180	1	1	0	Female_1	180	1
190	2	3	2	Female_1	190	2
200	3	3	4	Female_1	200	3
210	0	1	4	Female_1	210	0
				Female_2	180	1
				Female_2	190	3
				Female_2	200	3
				Female_2	210	1
				Female_3	180	0
				Female_3	190	2
				Female_3	200	4
				Female_3	210	4

Table 2 Explanations of settings options from the PFunc control panel.

Category	Setting	Details	Default
View	Names	Toggles the display of names above each graph.	Off
	Data Points	Toggles the display of raw data points on each graph. If the user is working with group-level splines, this option also controls the view of the individual-level splines that make up the group spline.	On
	Peak & Tolerance	Toggles the display of red and blue lines marking peak and tolerance (respectively) on the graphs.	On
	Splines	Toggles the display of splines (preference functions) on the graphs.	On
	Standard Error	Toggles the display of dashed lines on either side of the spline, representing the range of ±1 standard error, as calculated by the gam function. This standard error is a measure of how well the spline fits the data, not a measure of the spread of the data.	Off
Smoothing Limits	On/off	Smoothing Limits restricts the default smoothing parameter to a certain range. This is useful for avoiding splines that are either too wobbly or too stiff, but the user has the option of toggling it off.	On
	Min	Sets the minimum value for default smoothing parameters (but the user can manually adjust the smoothing parameter beyond this limit).	0.05
	Max	Like min, but for the maximum value.	5.0
Find Local Peak	On/off	Gives users the option to focus on specific regions of the splines that contain secondary peaks of interest (e.g. the lower left-hand corner of Fig. 6c). PFunc first searches for a peak between the min and max values (below). If it finds a peak, then it uses it. If not, then it expands its search across the entire spline.	Off
	Min	Sets the lower boundary for the search for local peaks.	
	Max	Sets the upper boundary for the search for local peaks.	
Tolerance	Options	There are two options for measuring tolerance: relative or absolute. ● In the *relative* option, tolerance is the width of the curve at a certain proportion down from the peak. To select the relative option, click the radio button next to 'Drop from peak'. ● In the *absolute* option, tolerance is measured at a specified *y*-value, regardless of the height of the peak. To select the absolute option, click the radio button next to 'At set value'.	Relative
	Drop from peak	Used for calculating relative tolerance. Controls how far down on the spline that PFunc measures tolerance (the width of the curve). Drop is expressed as a proportion of the distance from the peak of the spline to the floor (see below). It can be written as either a decimal or a fraction.	1/3
	Floor	Used for calculating relative tolerance. Adjusts the height of the baseline from which tolerance is measured. In calculating tolerance, the floor is the lower limit, whereas the peak height of the curve is the upper limit, and tolerance is measured as a proportion of the distance between the two.	0
	At set value	Used for calculating absolute tolerance. This value defines the point on the *y*-axis at which tolerance for all splines is measured.	1
	Mode	Controls how PFunc handles cases where there are multiple peaks along the spline that are above the tolerance line (e.g. the lower left-hand corner of Fig. 6c). If mode is set to Broad, then tolerance is the sum of all the tolerance segments across the range of stimulus values. If mode is set to Strict, tolerance is only the width of the curve under the highest peak.	Broad
Strength	Height Dependence	Controls whether strength depends on the height of the spline (i.e. responsiveness) (Fig. 5)	Height dependent

To do this, users select Construct Group-Level Spline from the Advanced menu, and a window pops up listing all of the individuals in the data set (Fig. 6e). In this window, users can name their new group spline and select the individuals that they want to include in it (select multiple individuals by clicking and dragging or by holding down the Ctrl key while making the selection). PFunc uses the chosen splines as new input data to fit a single group-level spline; that is, it converts the individual curves into many closely-spaced points, and fits a spline through them.

The new group-level spline is added to the end of the working data set and displayed alongside the individual-level splines (Fig. 6f). Note that this does not alter the original data file. The new graph displays the group-level spline as well as the individual splines that are part of the group. The visibility of the individual splines can be toggled with the Data Points checkbox under the View options (Table 2). Group-level splines behave just as individual splines do, and all of the settings and output options that apply to individuals also apply to groups.

We recommend using lower smoothing parameters for group splines than for individual splines. As group-level splines are themselves based on smooth curves, they tend to be relatively smooth to begin with, even with very small values for the smoothing parameter. Restricting group smoothing parameters to the same range as individual splines may result in overly stiff group-level curves.

Changing settings

We have chosen the default settings that we think, based on our experience, will work best across a broad range of experimental set-ups. However, we include a variety of options in PFunc for the user to adjust according to their needs. Settings can be adjusted from the control panel on the right-hand side of the screen (see Table 2 for details). Users can save the settings configuration of their current session for future use from the File menu (File > Save Current Settings). Users can restore saved settings from a previous session (File > Load Previous Settings), or they can revert to the default settings (File > Restore Default Settings).

Saving and exporting the analyses

PFunc comes with several options for exporting data: (i) the user can export a pdf of all the preference function graphs drawn from the data file (File > Output Spline Figures…). (ii) The user can export a spreadsheet displaying the five preference traits plus the smoothing values for all the curves of every individual in the data set (File > Output Spline Summaries…). (iii) The user can export a spreadsheet containing values of points that make up the curves of the preference functions for all individuals (File > Output Spline Points…). This option is useful for those who wish to plot the splines in a separate program to make figures for publications. It is also useful for those who want to save group-level splines. (iv) The user can export a spreadsheet containing the tolerance points for all individuals (File > Output Tolerance Points…). Tolerance points are the x-values that correspond to the boundaries of where tolerance is measured. This can be useful for plotting preference functions in other programs or for studies where these values are points of interest.

Discussion

We have presented a simple and efficient method for analysing mate preference functions, and a program that implements it on many individuals at a time. We now comment on some decisions that researchers have to make for the purpose of conducting the experiments that generate the data that can be used with our method.

The assay of attractiveness

Any method for describing mate preferences is only as good as the assay of attractiveness on which it is based. The decision of which assay to use should be based on the biology and behaviour of the study species (Reinhold & Schielzeth, 2015). A good assay consists of a specialized behaviour or response performed only in the context of mate acceptance, so that there is minimal risk of counting incidental or spurious responses. A classic example of an assay used in studies of mate preference is the phonotaxis behaviour involved in pair formation in many anurans and orthopterans, whereby females approach signalling males or playback stimuli that resemble signalling males (Gerhardt & Huber, 2002; Greenfield, 2002). In species where pair formation involves male–female signal exchanges (duetting), a simple and biologically relevant assay is whether females reply with their own signals to males or to playback stimuli (e.g. Parri *et al.*, 2002; Rodríguez *et al.*, 2004; Derlink *et al.*, 2014; Reichert & Ronacher, 2015). Other species may engage in other types of back-and-forth interaction between the sexes that present cues of likely mate acceptance (e.g. the adoption of a solicitation posture; Patricelli *et al.*, 2002, 2006). Such interactions may offer useful assays for studies of mate preferences, and are drastically understudied (Rodríguez, 2015).

When it comes to quantifying acceptance behaviour, there are several options for what the response variable could be. (i) It could be the number of times an individual responds to each stimulus value (expressed either as a raw count or as a proportion of total exposures to each stimulus). (ii) In the case of choice trials (discussed below), it could be the proportion of times one stimulus was preferred over another. (iii) It could be based on the latency to mate acceptance. (iv) It could be some other score of acceptance. Note that even if the structure of the data would be described as binomial (e.g. with only 1 and 0 for response/no response) or as Poisson (e.g. with only a possibility of 0, 1, 2 or 3 responses from an individual tested three times per stimulus), the spline that PFunc fits will be in the Gaussian family. The goal is to capture the smooth function (the cognitive phenotype) that underlies the behaviour, rather than reflect the particularities of the experimental assay.

We offer a note of caution about assays based on realized mate choice decisions (e.g. whether a mating occurs or not). Such assays may have restricted usefulness in the study of mate preferences. One reason for this is that preferences and decisions are different cognitive phenotypes (Cotton *et al.*, 2006; Mendelson *et al.*, 2016): preferences influence decisions, but are distinct from them. Another reason is that whether mating occurs or not is the outcome of male–female interactions that are influenced by more variables than the preference of one individual. A mate choice decision represents not only whether a female found a male attractive but also whether he found her attractive, for how long and with what intensity he courted her, the presence and distribution of other potential mates and so on. In other words, a realized mate choice decision arises from the expression of a mate preference but is also influenced by multiple inputs from the social context in which it is expressed. Additionally, individuals may accept a mate whose features are not the most preferred (not at the peak of the preference) but fall within an acceptable range, which confounds mate preference functions with other factors that influence mate choice decisions, such as mate sampling strategies, choosiness and absolute versus relative criteria for mate acceptance (Jennions & Petrie, 1997; Widemo & Sæther, 1999; Brandt *et al.*, 2005; Cotton *et al.*, 2006).

Having said the above, we acknowledge that assays based on realized mate choice decisions may be the only suitable option for some species. For example, it may only be logistically feasible or ethically permissible to study some species in the field. Other species may not respond well to artificial stimuli, may not produce an obvious mate-acceptance response or may not thrive in laboratory settings. In such cases, researchers may only be able to observe realized decisions such as nesting pairings. One solution to the above limitation may be to observe multiple decisions for each individual, each with different potential mates, in order to isolate the contribution of that individual's preferences to the outcome of interactions with the different males.

What we wish to emphasize is that the assay of sexual response should pinpoint as much as possible the mate preference as an individual's trait, and distinguish it as much as possible from variation due to inputs other than the features of the stimuli or males that the individual is responding to. With this consideration in mind, we would argue against conceptualizing the assay of attractiveness as a measure of the resources invested in reproduction with a mate (cf. Edward, 2015). The resources invested in reproduction reflect choosiness, realized mate choice decisions and decisions made after mate choice (Jennions & Petrie, 1997) rather than the cognitive phenotypes that influence those decisions.

The range of variation in the display trait over which to assess mate preferences

Characterizing mate preferences requires assaying attractiveness over an appropriate range of variation in sexual traits. To capture the full shape of the preference, we recommend that stimuli should not only span the natural range of variation in the trait, but exceed it somewhat. This is because assessing the attractiveness of phenotypes beyond the population range provides a more complete description of the selective landscape as determined by mate preferences (cf. Schluter, 1988). Too narrow a range may underestimate the amount of variation in the attractiveness of displays – it may not capture the full shape of the preference, which might yield misleading expectations about the form of sexual selection due to mate choice. On the other hand, an excessively broad range may detract from biological interpretability, although even then it might reveal hidden preferences or supernormal responses (Arak & Enquist, 1993; Gray *et al.*, 2016). This is a decision that researchers have to make according to the biology of their study species and the scope of their research questions.

Additionally, researchers will have to decide on an appropriate number of stimuli to test. The use of more stimuli (more stimulus values) increases the resolution of the splines, but it also means more trials for each individual, which can lead to fatigue or habituation. Therefore, one must strike a balance between the resolution of their preference functions and the tolerance of individual animals to repeated testing. We recommend using at least 10 different stimuli. Although PFunc can handle data with fewer than 10 stimulus values, the resolution of the preference functions may be reduced, and users may find the need to relax the limits on smoothing parameters.

A related consideration is the distribution of stimulus values along the range to be tested. Stimuli may be spread evenly along the range (say, from 100 to 200 Hz in steps of 10 Hz) (e.g. Rodríguez *et al.*, 2006), or they may cover some parts of the range more densely than others (say, finer steps near the suspected peak in order to pinpoint it more precisely) (e.g. Fowler-Finn & Rodríguez, 2012a,b).

Researchers should also consider how best to handle a data set that has missing values. In general, function-valued approaches have the advantage of being robust to missing data (Stinchcombe *et al.*, 2012). However, we consider that the ideal situation is one in which each individual is tested on every single stimulus value (recognizing that the reality of behavioural research with live animals may make this difficult to achieve). Individuals missing many data points should probably be excluded from the analysis entirely. Individuals missing only a few data points may or may not have an impact on the final analysis, depending on where those

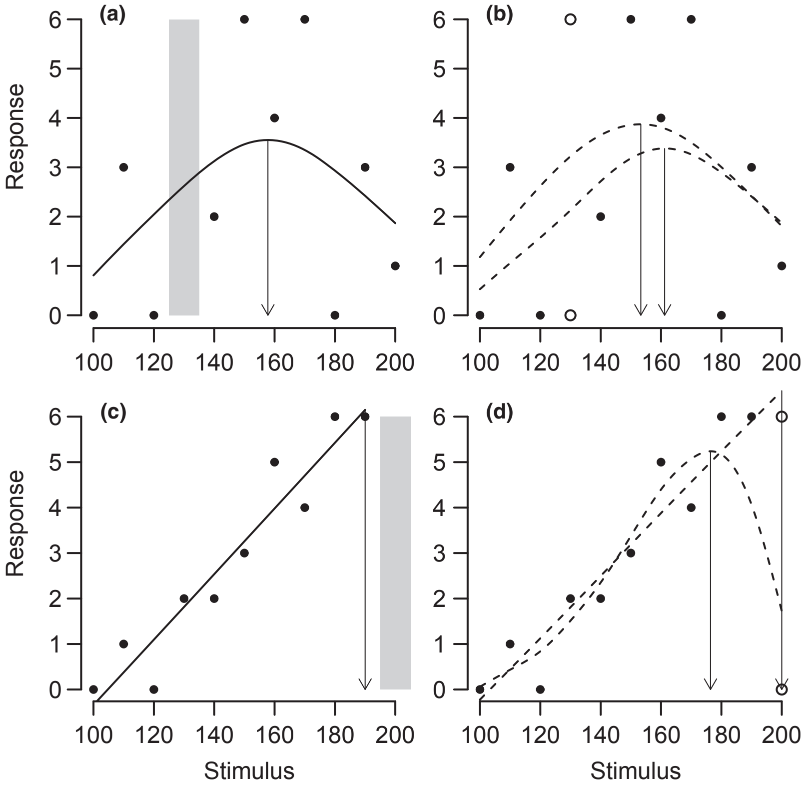

Fig. 7 Missing data points can have either a small effect (a, b) or a large effect (c, d) on the interpretation of splines. (a) A spline fit through a hypothetical data set with a single missing value in the middle of the range (represented by the grey box). The arrow indicates the peak. (b) The same data set with the highest possible and the lowest possible values filled in for the missing data point (open circles). Splines fit through the two possible extremes have the same general shape, and the peak varies by < 5% of the range in either direction compared to the peak in (a). Meanwhile, (c) shows a spline fit through data that is missing the response to the highest stimulus. This preference function appears to be open-ended, but if it is compared with other individuals in the same experiment, its peak comes out to be 190 instead of the extreme value of 200, which other open-ended preferences will have. Furthermore, this missing value holds more weight in determining the shape of the spline (d). The spline could end up being either open-ended or closed, and the peak varies by 10% or more of the range in either direction compared to the peak in (c).

points lie. If an individual is missing data in the middle of the test range, other points surrounding the missing value inform the shape of the curve (Fig. 7a,b). But a missing data point on the edge of the test range may lead to misinterpretation of the results, especially for preference functions that appear to be open-ended (Fig. 7c,d).

Design of mate preference experiments: choice and no-choice trials

A wide variety of methods are available for conducting playback experiments. So-called choice trials present individuals with two or more options or stimuli, and assemble a series of such trials to construct an overall preference function across stimulus pairs (e.g. Jang & Greenfield, 1996; Gerhardt *et al.*, 2000). Other methods use series of such choice trials to generate attractiveness scores for individual stimuli, and then use a set of those scores to construct the preference function (e.g. Ritchie, 1996; Simmons *et al.*, 2001; Brooks *et al.*, 2005). On the other hand, so-called no-choice trials (also termed single-stimulus trials) present one stimulus at a time and measure the response to each stimulus (e.g. Fig. 2; Rodríguez *et al.*, 2006).

There is reason to think that choice and no-choice designs may require different interpretation. In choice trials, the response to one stimulus depends on the relative attractiveness of the other stimulus in the pair, and this may influence the outcome of trials. Additionally, choice trials reflect not only mate preferences but also variation in investment in sampling (Wagner, 1998; Beckers & Wagner, 2011). These confounding factors may make it harder to capture the true shape of the mate preference with choice designs, making no-choice designs often preferable. (On the flip side, the sequence of stimuli in no-choice designs may influence their relative attractiveness, and sequences should be randomized.) There is evidence, however, that well-designed choice and no-choice experiments reach similar conclusions (Bush *et al.*, 2002), although they may vary in the strength of the mate preferences that they estimate (Dougherty & Shuker, 2015).

On significance testing

Here, we have focused on generating preference functions, visualizing them and extracting metrics to characterize them. One component of data analysis that our program leaves out is significance testing. For instance, to compare the mate preferences for a given signal trait between two species, PFunc will plot them and analyse them in terms of the preference traits described above. Each of the traits can then be compared between the two species with simple tests using commonly available statistical programs (e.g. one-way ANOVA to test for a species difference in peak preference, with a sample of individuals for each species). However, one may also want significance tests for a difference in the overall preference functions of the two species. For this purpose, some researchers have used statistical models with linear and quadratic terms for the stimulus features, in combination with visualization using nonparametric regression (such as with cubic splines) (e.g. Brooks & Endler, 2001; Brooks *et al.*, 2005; Bentsen *et al.*, 2006; Rodríguez *et al.*, 2006; Bailey, 2008; Gerhardt & Brooks, 2009). We have found this combination of approaches to be useful. For the above species comparison of the preferences of two species, the statistical model could include the following terms: a term for species, linear and quadratic terms for the values of the stimuli, and interactions between the species term and the linear and quadratic stimulus frequency terms (e.g. Rodríguez *et al.*, 2006). The species × linear term would test for differences in the linear components of the preferences, and the species × quadratic term would test for differences in the curvilinear components. Similar models can be used to compare preferences not only between species but also between treatments (e.g. Fowler-Finn & Rodríguez, 2012a,b). (Note that when each individual contributes a preference function to the analysis – when each individual was tested multiple times with different stimuli – individual ID should be included as a random factor in the statistical model to avoid pseudoreplication).

Univariate and multivariate mate preference functions

The method we present here is based on the manipulation of only one stimulus variable at a time, rather than multiple stimulus variables. We recognize that perception and selection often involve multiple aspects of display phenotypes, and there are methods of analysis available to explore attractiveness and fitness surfaces (e.g. Brodie *et al.*, 1995; Brooks *et al.*, 2005; Bentsen *et al.*, 2006; Ower *et al.*, 2013; Hennig *et al.*, 2016). In some cases, however, the univariate approach may be most appropriate for how the study organism processes stimuli. For example, females of some cricket species process male signal pulse rate as a single (effectively univariate) trait, despite the fact that pulse rate is in fact a compound trait determined by both pulse duration and interpulse duration (Hennig *et al.*, 2014; Blankers *et al.*, 2015). Another reason the univariate approach is broadly useful is that different display traits are often associated with mate preferences of different shapes, so that analysing display–preference relationships on a trait-by-trait basis enhances the explanatory power of tests of the contribution of mate preferences to sexual selection and display divergence (Rodríguez *et al.*, 2006, 2013a).

Broader applicability of the method

Nonbehavioural mate preferences

In this paper, we have focused on mate preferences as cognitive phenotypes (Mendelson *et al.*, 2016). However, there are many other types of sexual response that can be studied using the method that we outline here. Indeed, any response of interest may be used as the *y*-axis to characterize the causes of mate choice at any stage of the reproductive process.

As above, our main suggestion would be to use assays of sexual response that approach as closely as possible the focal individual's trait without influence from the interaction with mating partners. For example, we would consider whether females are induced to expel previously stored sperm by genitalic stimuli (von Helversen & von Helversen, 1991) to be a better assay than those same females' fertilization patterns, because the former better approximates the female function-valued trait of interest. Having said that, we recognize that this goal may be challenging for studies of mate preferences at stages of the reproductive process subsequent to pair formation, such as studies of cryptic mate choice – it may sometimes be impossible to avoid using interacting individuals in mate preference trials. In such cases, observing interactions of each female with a sample of males may help characterize her preferences (e.g. Sirot *et al.*, 2007).

Other function-valued traits

Our method is also applicable to any function-valued trait, regardless of whether it involves mate preferences or behaviour. For instance, it could be used to study variation in developmental trajectories or in plasticity across a range of environmental conditions, as long as the *x*-axis variable is continuous (Izem & Kingsolver, 2005; Kingsolver & Gomulkiewicz, 2003; Meyer & Kirkpatrick, 2005; Stinchcombe *et al.*, 2012; Murren *et al.*, 2014; Kingsolver *et al.*, 2015). We suggest that the main consideration in deciding whether to use our method should be whether researchers desire to make assumptions about the shape of the functions of interest. We hope that this approach will be broadly useful.

Acknowledgments

We thank Peter Dunn, Laurel Symes and Linda Whittingham for stimulating discussions. We are also grateful to Susan Bertram, Sarah Harrison, Damian Elias and an anonymous reviewer for their valuable feedback on the manuscript and program. This work was supported by NSF Grant IOS-0919962 to RLR and by NSF Grant IOS-1120790 to RLR and KDFF.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Appendix S1 PFunc manual.
Appendix S2 Program files.

Received 12 January 2017; revised 18 May 2017; accepted 22 May 2017