# Female preference functions drive interpopulation divergence in male signalling: call diversity in the bushcricket *Ephippiger diurnus*

F. BARBOSA*, D. REBAR*† & M. D. GREENFIELD*

*UMR 7261, Faculté des Sciences et Techniques, Institut de Recherche sur la Biologie de l'Insecte (IRBI), Tours, France
†Department of Zoology, University of Cambridge, Cambridge, UK

## Abstract

Female preferences play a major role in the elaboration and diversification of male traits: as a selective pressure on males, variation in female preferences can generate population divergence and ultimately, speciation. We studied how interpopulation differences in the shape of female mate preference functions may have shaped male advertisement signals in the bushcricket *Ephippiger diurnus*. This species is distributed as geographically isolated populations with striking interpopulation variation in male acoustic signals, most notably in the number of syllables per call. Here, we asked whether differences in the shape of preference functions exist among populations and whether those differences may have driven male signal evolution resulting in the observed differences in syllable numbers. Our results reveal fundamental differences in female preferences among populations, with differences in the overall preference function shape corresponding to differences in male signals. These differences in female preferences best explain the major differences in male signals among populations. The interpopulation variation in signals and preferences potentially reflects the evolutionary history of the species and may contribute to further divergence among populations and subsequent speciation.

## Introduction

Female mate preference is often assumed to exert a major influence on the elaboration of male traits (West-Eberhard, 1983; Gunnarsson & Andersson, 1996; Kokko *et al.*, 2006) because several different components of female preferences can exert separate influences on corresponding male traits. For example, the population mean female preference for a male signal may affect the direction of sexual selection acting on that signal, whereas the amount of variability around that mean may determine the strength of selection on that signal (Wagner, 1998; Edward, 2015). Within a population, high variation among the preferences of individual females is expected to yield

weaker selection (Jennions & Petrie, 1997). The strength of selection also depends on how 'tolerant' females are, that is, the likelihood that a female will accept males that deviate from her most preferred trait value (Fowler-Finn & Rodríguez, 2012; Rodríguez *et al.*, 2013). If females express low tolerance and are thus more likely to reject less preferred mates, selection on male signals is expected to be stronger. Notably, the match between mean female preference and mean male trait may also determine the nature of selection that acts on the male trait: a close match is associated with stabilizing selection, whereas a mismatch leads to directional selection (Ritchie, 1996; Gerhardt *et al.*, 2000; Rodriguez *et al.*, 2006). Because several attributes of female preferences may differ between populations, variation in female preferences can drive variation in male traits and thereby generate trait diversification among populations and ultimately, speciation (West-Eberhard, 1983; Houde & Endler, 1990; Endler & Houde, 1995; Mendelson & Shaw, 2002).

*Correspondence*: Flavia Barbosa, UMR 7261, Faculté des Sciences et Techniques, Institut de Recherche sur la Biologie de l'Insecte (IRBI), Avenue Monge, Parc Grandmont, Tours 37200, France.
Tel.: +33 07 85 64 12 54; fax: +33 02 47 36 69 66;
e-mail: flavia.barbosa@univ-tours.fr

Determining the relationship between interpopulation divergence in male signal traits and differences in female preference demands quantitative analysis of the attributes of both signals and preferences (Wagner, 1998; Dougherty & Shuker, 2015). An effective way of analysing and depicting preference is as a continuous response function: female response level to the male signal is measured in a series of no-choice tests for a range of signal values sampled along one of the relevant signal axes, for example call rate, frequency, duration (Lande, 1981; Meyer & Kirkpatrick, 2005; Stinchcombe & Kirkpatrick, 2012; Edward, 2015). A species or population-level 'preference function' can then be specified by several critical attributes or parameters. For example, peak preference is the male signal value that elicits the greatest response from a female, whereas tolerance describes the shape of the curve as it moves away from peak preference and shows how quickly female response drops for signal values above and below the peak (Fowler-Finn & Rodríguez, 2012). Various studies have reported a simple match between female peak preference and mean male signal value across populations and species (Ryan, 1985; Seehausen & van Alphen, 1998; Siva-Jothy, 1999). However, less attention has been paid to the question of whether and how the entire female preference function, that is its overall shape, might influence male signal evolution (but see Rodriguez *et al.*, 2006). Consequently, we have an incomplete picture of how preferences may affect the signals we observe in nature.

Here, we test the hypothesis that diversification of male signals among populations has been driven by differences in female preference functions. We studied the bushcricket *Ephippiger diurnus* (Tettigoniidae: Bradyporinae), a European species, that shows striking interpopulation variation in several morphological and behavioural characters, notably acoustic features of the male advertisement call. *Ephippiger diurnus* males broadcast rhythmically repeated calls that consist of 1–8 'syllables' and are attractive to receptive females (Busnel *et al.*, 1956). Due to specific habitat requirements, flightlessness and an absence of migration, *E. diurnus* is distributed in isolated populations that are separated by distances as short as 5 km (Spooner & Ritchie, 2006). Importantly, the various populations are distinguished by having rather specific – and highly repeatable – numbers of syllables in the male call. In the context of mate preference, females evaluate several characters of the male call: they prefer faster rhythm and higher syllable numbers, and within a chorus of calling males, they prefer leading calls (Ritchie, 1991, 1992; Party *et al.*, 2014). Several studies have shown that female preference for syllable number in male calls also exhibits considerable interpopulation variation (Ritchie, 1996; Party *et al.*, 2014).

Phylogenetic analyses of *E. diurnus* have revealed a branching topology of the various populations (Spooner & Ritchie, 2006; Party *et al.*, 2015). This structure, which is consistent with the ecological and behavioural features of *E. diurnus*, indicates that interpopulation gene flow is probably negligible to low. On a broader phylogeographic scale, populations in the *E. diurnus* complex may result from origins in separate refugia along the Mediterranean coast during the height of the Pleistocene glaciation (Kidd & Ritchie, 2000; Ritchie *et al.*, 2001). Recent glaciations have played a major role generating variation and population divergence in European species (Hewitt, 1996, 1999, 2000, 2004; Taberlet *et al.*, 1998), and this is hypothesized to also be the case for *E. diurnus.* These populations group into two clades (Party *et al.*, 2015); one of them includes populations in the Mediterranean littoral south of Narbonne and extends west on southern slopes of the Pyrenees Mountains. This group is distinguished by male song having 3 or more syllables per call. Another clade is found in the Mediterranean littoral north of Narbonne, Dept. Aude, France, and extends west in valleys and on northern slopes of the Pyrenees as well as north into the Massif Central and east to the Rhone River. These populations are distinguished by male song having only 1 or 2 syllables per call.

We relied on interpopulation variation in *E. diurnus* to ask the following questions: (i) Do preference functions differ among populations? (ii) Where preference functions differ, are they likely to have driven male signal evolution, resulting in calls with different syllable numbers? (iii) Does the tolerance parameter of a preference function influence the degree of mismatch between female preference and male signal? We predicted that there would be differences in the preference functions between populations, and that these differences would be commensurate with differences in the syllable number of male calls. Further, we predicted a particular effect of preference function shape in which elevated tolerance would yield a greater mismatch between peak preference and mean syllable number in the male signal.

## Materials and methods

### Study species

*Ephippiger diurnus* are found in open, natural habitats (garrigue) in southern France and north-eastern Spain. We studied adult males and females from 10 different populations in two clades (Party *et al.*, 2015). Four of these populations are in clade 1, where male signals have more than 3 syllables per call: Col de Mantet (CDM), Font Romeu (FR), Peyriac-de-Mer (PEY) and Col de Puymorens (PUY). The other six feature are in clade 2, where male signals have only 1–2 syllables per call: Col de Chioula (CHI); Merèns-les-Vals (MER); Mireval (MIR); Pouzol (PZL); Vias (VIAS); and Vilamòs (VIL). We will henceforth refer to these populations by their abbreviations. Geographic locations of these

populations are given in Fig. 1, and male call parameters are listed in Table 1. We chose populations that incorporated both phylogenetic and geographic diversity as well as to provide a range of male calls as measured by variation in syllable number.

### Collection and rearing

Most study animals were collected in August 2013 and August 2014 as either late-instar nymphs or recently emerged adults and brought to the laboratory. Two of
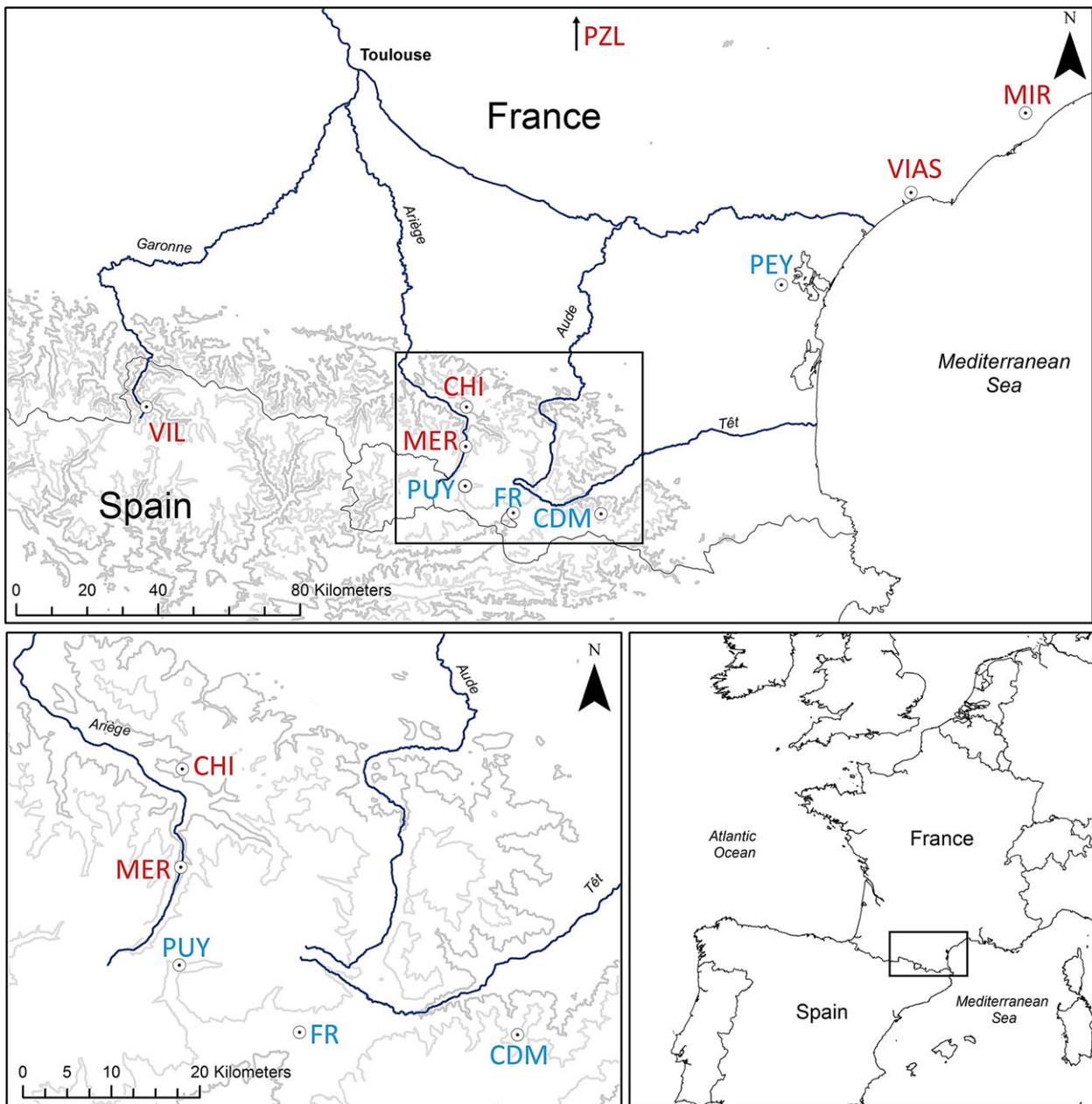


**Fig. 1** Map of southern France and north-eastern Spain showing the geographic distribution of *Ephippiger diurnus* populations tested in this study. Blue lines represent major rivers, and solid grey lines represent relief isolines for 1200 and 1800 m. Different colours denote differences in the syllable number of male signals (red = 1–2 syllables per call; blue = > 3 syllables per call), and black circles show the locations of each collecting site. Geographic coordinates and altitudes of collecting sites are as follows: CDM, Col de Mantet (42°29′N, 2°18′E, 1700 m); FR, Font Romeu (42°29′N, 2°00′E, 1620 m); PEY, Peyriac-de-Mer (43°04′N, 2°55′E, 50 m); PUY, Col de Puymorens (42°33′N, 1°50′E, 1750 m), CHI, Col de Chioula (42°45′N, 1°50′E, 1430 m); MER, Merèns-les-Vals (42°38′N, 1°49′E, 1090 m); MIR, Mireval (43°30′N, 3°46′E, 80 m); PZL, Pouzol (45°12′N, 3°10′E, 785 m); VIAS, Vias (43°18′N, 3°22′E. 27 m); VIL, Vilamòs (42°44′N, 0°44′E, 1240 m).

**Table 1** Mean (±SD) male signal and female preference traits and sample sizes of the study populations.

| Population | Call syllable number | Call rate (calls min$^{-1}$) | Syllable rate (syll min$^{-1}$) | Male $n$ | Peak preference | Tolerance | Female $n$ |
|---|---|---|---|---|---|---|---|
| 1–2 syllables per call | | | | | | | |
| Col de Chioula | 1.98 ± 0.05 | 40.85 ± 8.23 | 78.76 ± 13.43 | 12 | 6.87 ± 0.53 | 6.77 ± 0.62 | 7 |
| Merèns- les-Vals | 2.01 ± 0.19 | 36.42 ± 7.09 | 74.42 ± 14.22 | 30 | 7.96 ± 2.72 | 6.09 ± 2.04 | 21 |
| Mireval | 1.03 ± 0.09 | 44.97 ± 8.49 | 46.18 ± 8.49 | 19 | 1.93 ± 1.29 | 3.28 ± 2.65 | 22 |
| Pouzol | 1.07 ± 0.22 | 46.79 ± 6.72 | 52.34 ± 13.09 | 19 | 2.07 ± 3.88 | 3.45 ± 3.93 | 19 |
| Vias | 2.14 ± 0.09 | 43.35 ± 7.82 | 71.16 ± 11.07 | 16 | 3.87 ± 0.81 | 5.56 ± 0.79 | 11 |
| Vilamòs | 1.37 ± 0.33 | 42.69 ± 8.39 | 57.92 ± 11.38 | 27 | 1 ± 0 | 3.08 ± 2.93 | 18 |
| > 3 syllables per call | | | | | | | |
| Col de Mantet | 4.79 ± 0.58 | 22.09 ± 3.21 | 106.96 ± 22.93 | 42 | 9.84 ± 0.65 | 6.66 ± 1.84 | 17 |
| Font Romeu | 4.28 ± 1.21 | 32.45 ± 6.71 | 116.19 ± 24.17 | 26 | 8.44 ± 1.76 | 6.55 ± 1.32 | 27 |
| Peyriac-de-Mer | 5.28 ± 0.85 | 14.65 ± 3.45 | 78.13 ± 18.51 | 14 | 9.38 ± 1.05 | 5.12 ± 1.03 | 20 |
| Col de Puymorens | 3.74 ± 0.48 | 28.98 ± 6.14 | 110.48 ± 23.24 | 28 | 8.73 ± 2.32 | 6.21 ± 2.03 | 21 |

the populations were obtained from eggs collected from field individuals and hatched in the laboratory (CHI and PEY). Animals were kept in climate-controlled chambers maintained at 25 °C and an L : D 16 : 8 h photoperiod, housed in individual plastic containers (146 mm height, 117 mm diameter) and fed cabbage, pollen and fish flakes *ad libitum*. We kept males and females in separate chambers to limit female exposure to male calls. We misted individuals with water daily.

## Male signals

We recorded and characterized calls from 15 to 36 males in each of our 10 study populations. Males were recorded individually inside a mesh container. Because temperature affects both male calling behaviour and female call preference in orthopterans, and in ectotherms in general (Gerhardt & Mudry, 1980; Pires & Hoy, 1992), we recorded males in a climate-controlled room at 25 °C (range from 24 to 25.5 °C). In the field, males from all tested populations experience a similar temperature range during the hours in which they are active, approximately between 20 and 30 °C. Males were recorded in the presence of a chorus that served to prompt males to call, since most call little or not at all when in isolation. This chorus was composed of 10–16 live males from the 10 populations tested in the study. For logistic reasons, we could not present a chorus of constant composition at all times. We used a digital recorder (Tascam HD-P2; 96 kHz sampling rate, 16 bits) and an electret microphone (LinearX M51, LinearX Systems, Tualatin, OR, USA; corrected frequency response ±1 dB from 10 to 40 kHz) which was placed directly above the male's container and recorded each male for three, 2-min intervals during which the male was calling regularly.

We analysed the recorded calls using digital sound processing software (Adobe Audition 3.0, Adobe Systems Incorporated, San Jose, CA, USA). We counted the total number of calls produced and the number of syllables per call. We then used this information to calculate a male's call rate (calls min$^{-1}$), average number of syllables per call and syllable rate (syllables min$^{-1}$) in each of the recording intervals; we then averaged the three measurements. Finally, we performed a repeatability analysis on syllable number for each population, using 10 calls from each male. Repeatability of syllable number was equated with the intraclass correlation coefficient (Lessells & Boag, 1987) calculated as $S_A^2/(S^2 + S_A^2)$, where $S^2$ is the within-group variance component (error MS), $S_A^2$ is the among-groups variance component (group MS – error MS)/$n$, and $n$ is the number of repeated measures of syllable number per male.

## Female preference tests

To investigate the differences in preference function shapes among populations, we evaluated preference for call syllable number in 7–27 females in each of the 10 populations. We used the male recordings to select a typical syllable from each population, one with peak frequency and length equivalent to the mean in that population, as calculated from measurements of calls from a minimum of 20 males per population. We then constructed synthetic calls from the selected syllable at a sampling rate of 96 kHz and 16 bits with BatSound Pro 4.0 (Petterson Elektronik, Uppsala, Sweden). We created 10 stimuli per population, each stimulus consisting of 10 advertisement calls of a given syllable number that ranged from 1 to 10. Stimuli were all 30 s in length and presented at a rate of 20 calls min$^{-1}$. Although call rate varies among populations (see Table 1), this parameter has markedly less repeatability than syllable number. Thus, we chose to test all populations with the same call rate, and we used a value that fell within the natural range for all populations.

Female preference was tested in a climate-controlled room at 25 °C. Females were presented with the 10 acoustic stimuli in randomized sequence, thus limiting the possibility that order effects might generate artifactual results. For a given test female, each of the stimuli was made from syllables from her own population. Our range of 10 stimuli extends beyond the known range of syllable numbers in male calls across all populations of *E. diurnus*, allowing us to evaluate the full potential for directional selection on male signalling. We broadcast stimuli through a loudspeaker (Scanspeak; Avisoft Bioacoustics, Glienicke, Germany) and calibrated all broadcasts to an amplitude of 85 dB SPL (0 dB = 20 μPa) at the female's position. This level approximates that of males singing 1 m distant in the various populations.

We tested female preferences with a locomotion compensation sphere (TrackSphere LC-300, Syntech Equipment and Research, Kirchzarte, Germany) that recorded female movement in response to the stimuli. Females were placed on top of the sphere and presented with a signal from the loudspeaker. The apparatus mechanism compensates for female movement, retaining the test female on the top of the sphere throughout the trial. In each trial, we obtained the female's trajectory as reconstructed with Syntech TrackSphere software from the series of her *x*, *y* coordinates in virtual space sampled at 100-ms intervals. Female *E. diurnus* walk towards preferred stimuli (Busnel *et al.*, 1955); thus, we used her net movement towards the stimulus over the 30-s duration of the trial as measure of her preference. Her net movement, in centimetres, was calculated from the female trajectory recorded by the TrackSphere software, and this movement was treated as the female's response to a given stimulus.

### Preference functions

We used the data on response level to the series of 10 call stimuli tested on the locomotion compensation sphere to construct a continuous preference function for each female. The continuous function was built with a cubic spline, a nonparametric regression that makes no assumptions about the shape of the curve other than its smoothness (Meyer & Kirkpatrick, 2005). We calculated cubic splines using a custom-written script and the mgcv package in R (R Development Core Team, 2014), allowing the program to choose the smoothing parameter for each individual spline. We built a preference function for each female and calculated her peak preference (i.e. the stimulus value that elicited the highest female response). We also created a mean preference function for each population by averaging the responses of all individuals tested. We described variation in female mate preferences in terms of peak preference and tolerance, measured as the

width of the preference function at 66.7% of the height of the peak.

### Statistical analyses

We used a function-valued approach (Meyer & Kirkpatrick, 2005; Stinchcombe & Kirkpatrick, 2012) to describe female preferences in the several populations. Thus, the entire preference function is the trait of interest, and each female contributes one preference function to the analysis. We tested for overall differences in preference function shape among populations using a linear mixed model with female response to male stimuli as the dependent variable. The model included population as a fixed effect, female identity as a random effect nested within population, as well as a linear and a quadratic stimulus term, a population × linear stimulus term, and a population × quadratic stimulus term. The stimulus term measures the number of syllables, ranging from 1 to 10, and the interaction terms measure variation in the shape of the preference functions: The population × stimulus term describes female response as a linear function of increasing or decreasing syllables per call, that is open preference, whereas the population × quadratic term describes female response as a curvilinear function of syllables per call, that is closed preference (Fowler-Finn & Rodríguez, 2012). All interaction terms were of particular interest to us, as significant values for interaction terms allowed us to explore how the preference functions varied among populations.

We described variation in female preference functions in terms of two traits: peak preference and tolerance. We used these two traits as response variables in a one-way ANOVA with population as a fixed effect. We then conducted a *post hoc* test to determine which populations differed in peak preference and tolerance (Tukey–Kramer HSD). Finally, to test whether differences in preference drive signal variation and whether tolerance affects the match between female preference and male signal, we investigated the relationship between (i) tolerance and signal-preference mismatch (defined as the difference between preference peak and the mean call syllable number of the population); (ii) call syllable number and preference peak; and (iii) call syllable number and tolerance, using least squares linear regression. All statistical analyses were carried out with JMP software (JMP 7; SAS Institute Inc., Cary, NC, USA).

### Results

The mean call syllable number, call rate and syllable rate for the study populations are presented in Table 1. Repeatabilities for the number of syllables per call were quite high in all 10 populations. (CHI = 0.55, MER = 0.46, MIR = 0.96, PZL = 0.98, VIAS = 0.68,

**Table 2** Linear mixed model testing for differences in the shape of preference functions among populations of *Ephippiger diurnus*.

| Source of variation | d.f. | F | P |
|---|---|---|---|
| Whole model | 49 | 56.9186 | **< 0.0001** |
| Population | 9 | 67.4189 | **< 0.0001** |
| Stimulus | 1 | 0.3999 | 0.53 |
| Population × Stimulus | 9 | 195.8734 | **< 0.0001** |
| Stimulus$^2$ | 1 | 17.4237 | **< 0.0001** |
| Population × Stimulus$^2$ | 9 | 195.8734 | **< 0.0001** |
| Individual | 26 | 6.8394 | **< 0.0001** |

The model includes linear and quadratic stimulus syllable terms, along with interactions of both with population. The interaction terms test for variation among populations in the shape of female preference functions, with the stimulus interaction testing for linear (e.g. directional) preference and the quadratic stimulus interaction testing for curvilinear (e.g. stabilizing) preference. Significant values are in bold ($P < 0.05$).

VIL = 0.83, CDM = 0.71, FR = 0.73, PEY = 0.55 and PUY = 0.85).

### Do preference function shapes differ among clades and between populations?

We found significant differences in preference function shape among populations (Table 2). Notably, there are major, qualitative differences across the populations: those with more than 3 syllables per call had directional preference functions; that is, peak preference was much higher than the mean male trait. They were also open functions for the range of calls we tested. Among populations with 1–2 syllables per call, preference functions were closed. They were stabilizing in three populations (close match between peak preference and mean male signal) and directional in the remaining three (peak preference was considerably higher than mean male signal) (Fig. 2). The significant differences in overall preference function shape prompted us to investigate differences in two specific traits of the function: peak preference and tolerance. The trait-by-trait analysis showed differences in both traits (one-way ANOVA, $F_{9,172} = 2329.11$, $P < 0.0001$ for peak preference and $F_{9,172} = 365.67$, $P < 0.0001$ for tolerance), and overall, peak preferences and tolerances were lower in populations with 1–2 syllables per call (Fig. 3).

### Do differences in preference functions drive signal variation among populations?

Peak preference was close to the population mean for call syllable number in 3 of the populations with 1–2 syllables per call: MIR, PZL and VIL. On the other hand, there was a considerable amount of mismatch between female preference and male signal in MER, CHI and VIAS, where males also call with 1–2

syllables per call. Preference functions were open in populations with more than 3 syllables per call (Fig. 4), and peak preferences were much higher than the mean male syllable number in each population. These findings support the hypothesis that the difference in male signal structure among populations was driven by female preferences: there are major differences in two measures of female preference, and both differences are congruent with the observed signal divergence.

### Does tolerance affect the match between female preference and male signal?

As predicted, there was a positive relationship between call syllable number and preference peak (Pearson product-moment correlation; $r = 0.76$, $P < 0.001$, Fig. 5a), and a positive but nonsignificant relationship between call syllable number and tolerance (Pearson product-moment correlation; $r = 0.32$, $P = 0.09$, Fig. 5b). We also observed a positive relationship between the signal-preference mismatch and tolerance (Pearson product-moment correlation; $r = 0.78$, $P < 0.001$, Fig. 5c), supporting the hypothesis that tolerance influences male signalling: when tolerance is low, there is stronger selection for the male signal to match female preference.

## Discussion

Our results reveal fundamental differences in female preferences among the 10 *E. diurnus* populations studied. Female preference functions for syllable number are directional in populations with 3 or more syllables per call, with peak preferences being much higher than the mean male syllable number, and with females being very tolerant of calls that deviate from their peak preference. Such directional selection in these populations indicates that males can greatly increase their reproductive success by increasing their call syllable number in these populations. On the other hand, preference functions varied among populations with 1–2 syllables per call: they were directional in three populations and stabilizing in the remaining three (i.e. there was a close match between preference and male signal). The stabilizing functions also exhibited low tolerance. Males in these populations would not benefit from producing calls with a higher syllable number, and in fact, such calls would be selected against. These results support the hypothesis that differences in the shape of female preference functions drive male signal evolution, resulting in the major differences in male calls we observe among populations. In concert with the limited gene flow between *E. diurnus* populations, these differences may yield increasing divergence, with a potential for eventual speciation, among populations of the *E. diurnus* complex.

It should be pointed out that we did not control for the phylogenetic relationships between the study populations, and for this reason, these populations cannot be viewed as independent data points. The most recent phylogenetic tree for the *E. diurnus*, which is based on mitochondrial markers and is not expressed with a single branch per population (Party *et al.*, 2015), does not allow the formal application of phylogenetic independent contrasts. Nonetheless, this phylogeny shows that the populations group into two clades, and some of the differences in female preferences among the populations likely reflect the rather unique phylogeography
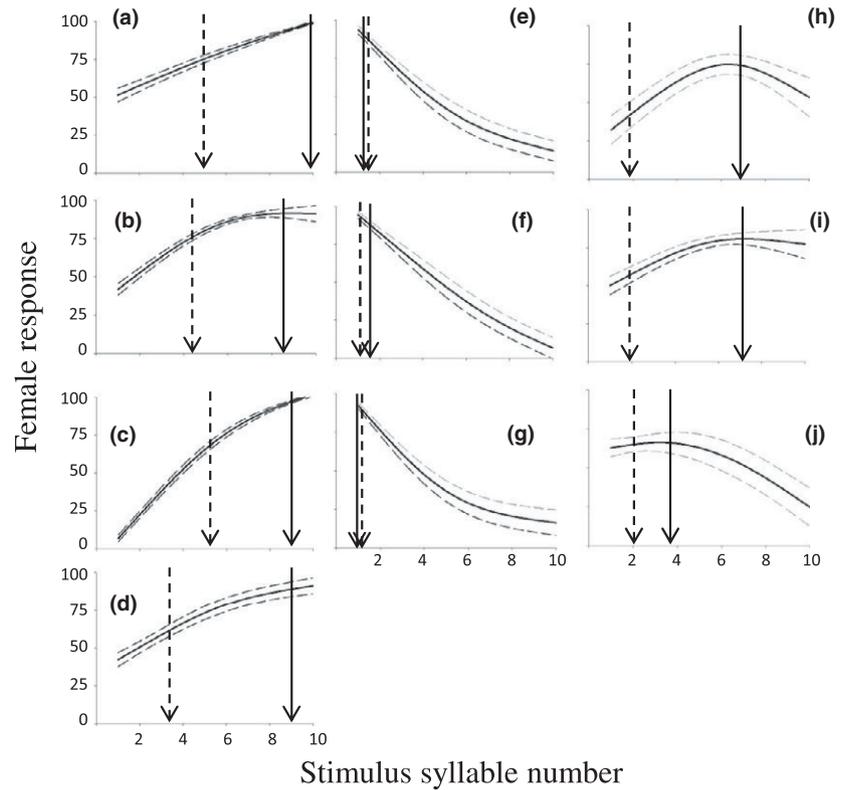


**Fig. 2** The shape of female mate preference functions of *Ephippiger diurnus* populations with > 3 syllables per call (a–d) and 1–2 syllables per call (e–j): (a) Col de Mantet, (b) Font Romeu, (c) Peyriac-de-Mer, (d) Col de Puymorens, (e) Mireval, (f) Pouzol, (g) Vilamòs, (h) Col de Chioula, (i) Merèns- les-Vals, (j) Vias. The solid line is the population mean preference function, and dotted lines denote one standard error. Solid arrows denote female peak preference, and dashed arrows denote mean male syllable number for that population.
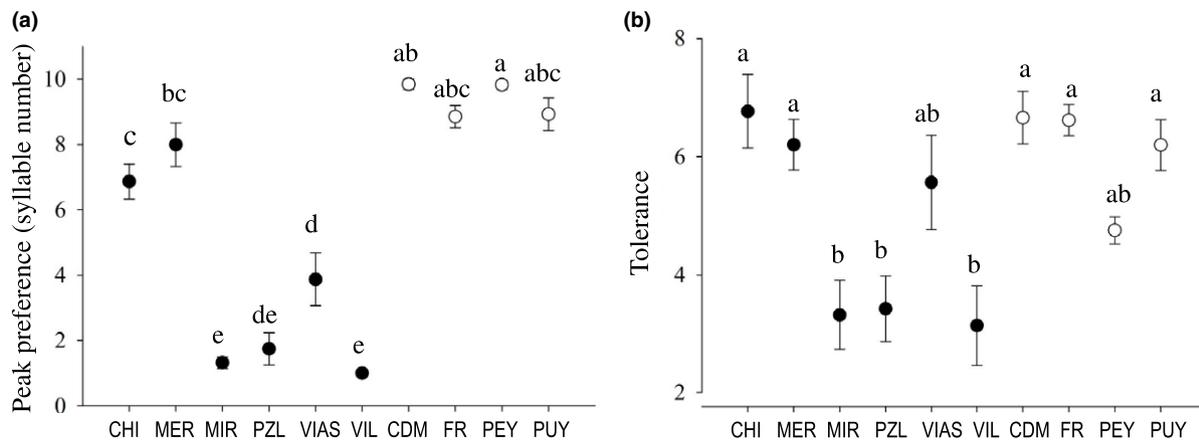


**Fig. 3** Multiple paired comparisons (Tukey–Kramer HSD) of (a) peak preference and (b) tolerance among populations. Populations not connected by the same letter are significantly different. Solid and open circles denote populations with 1–2 and > 3 syllables per call, respectively. CHI, Col de Chioula; MER, Merèns- les-Vals; MIR, Mireval; PZL, Pouzol; VIL, Vilamòs; VIAS, Vias; CDM, Col de Mantet; FR, Font Romeu; PUY, Col de Puymorens; PEY, Peyriac-de-Mer.
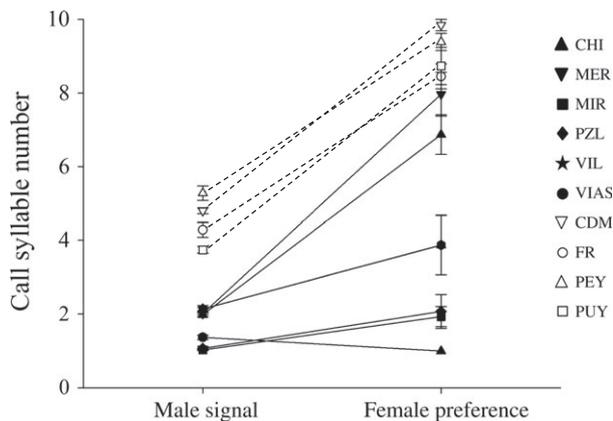
**Fig. 4** Mean call syllable number and female peak preference for the study *Ephippiger diurnus* populations. Solid symbols and lines denote populations with 1–2 syllables per call, and open symbols and dashed lines denote populations with > 3 syllables per call. CHI, Col de Chioula; MER, Merèns- les-Vals; MIR, Mireval; PZL, Pouzol; VIL, Vilamòs; VIAS, Vias; CDM, Col de Mantet; FR, Font Romeu; PEY, Peyriac-de-Mer; PUY, Col de Puymorens.

of the complex. It is hypothesized that these two clades originated in separate refugia along the Mediterranean coast during the Pleistocene glaciation (Kidd & Ritchie, 2000). In this case, populations in those refugia may have experienced bottlenecks such that the distinct preference function shapes we observe today reflect that historic genetic divergence (Armbruster *et al.*, 1998; Avise *et al.*, 1998; Hewitt, 2000; Clegg *et al.*, 2002; Lovette, 2005). It should be noted that three populations, MER, CHI and VIAS, have signals with an average of 2 syllables per call, but have preference functions with high tolerance and a considerable mismatch between peak preference and male signal. Interestingly, male calls in these populations have a lower call rate and a higher syllable rate than the other populations producing calls with 1–2 syllables in this study. Two of these populations are geographically closer to populations with more than 3 syllables per call (Fig. 1), but further investigation in the phylogenetic relationships within the *Ephippiger* complex is needed to reveal whether the signal and female preference traits in these three populations are a result of phylogeography (Party *et al.*, 2015).

Although we were unable to remove phylogenetic signal from the various pairwise relationships in our study (Fig. 5), an *ad hoc* examination of one of these relationships (Fig. 5c) suggests that tolerance is probably influenced by signal-preference mismatch independently of phylogeny. The six Clade 2 populations that we sampled encompass a wide range of signal-preference mismatch, but nonetheless they exhibit a similar correlation with tolerance (Pearson product-moment correlation; $r = 0.78$, $P < 0.05$) as observed for all 10 populations in both clades. As we only sampled four
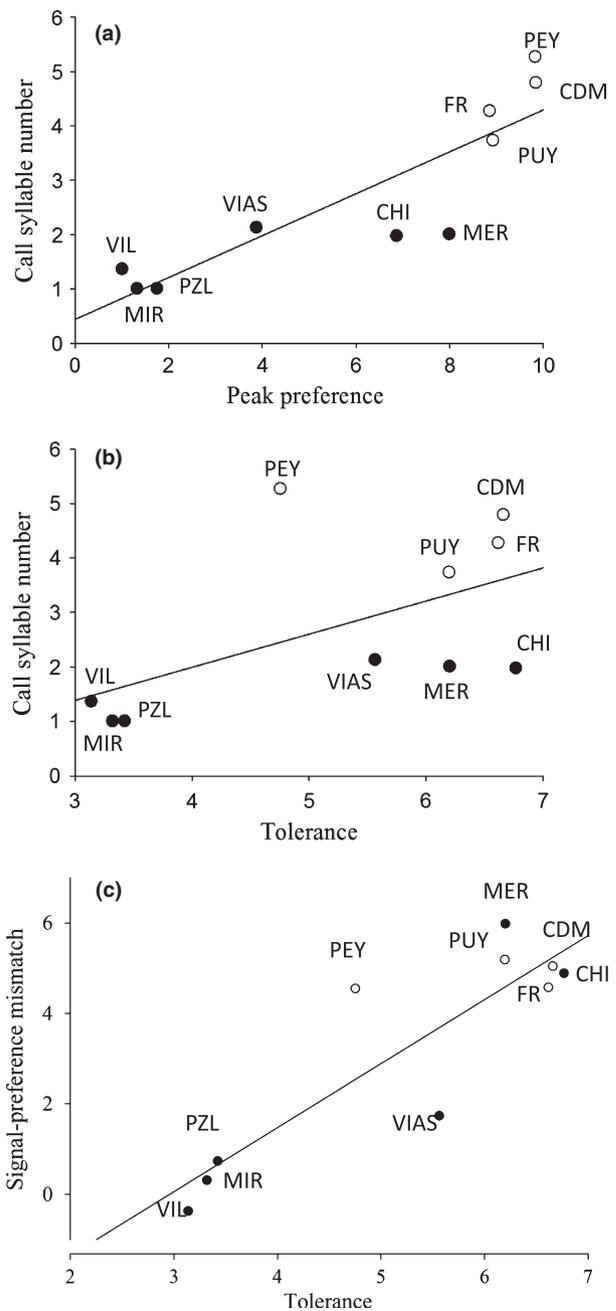


**Fig. 5** Relationship between (a) tolerance and signal-preference mismatch, (b) call syllable number and peak preference and (c) call syllable number and tolerance in the studied populations. Solid and open circles denote populations with 1–2 and > 3 syllables per call, respectively.

Clade 1 populations, we were unable to examine the relationship between tolerance and signal-preference mismatch within this clade. Nonetheless, this suggests that phylogeny accounts for some portion of the overall relationship between tolerance and signal-preference mismatch that we report in Fig. 5c.

Ecological and physiological factors may have also played a major role in shaping the two types of preference functions we observed, as well as the differences in the degree of mismatch between male signal and female preference across populations. The general energetic expense that acoustic insects incur when producing regularly repeated calls (Prestwich, 1994; Gerhardt & Huber, 2002) suggests that signal production may be limited by a male's energy budget. In fact, a recent study of the CDM population showed distinct trade-offs between three major reproductive and survival traits among the males: (i) signalling, as measured by mean call syllable number, (ii) size of the spermatophore transferred at mating and (iii) strength of an immune response (Barbosa *et al.*, 2016). Such trade-offs likely result from energy limitations, and certain traits may be favoured more than others in particular environments. Limitations on available energy and the need to allocate some energy to activities other than calling may account for the striking signal-peak mismatch in polysyllabic populations. This could then account for the great variation in preference functions – and male calls – seen among *E. diurnus* populations: Where energy is severely limited and transfer of a large spermatophore or maintaining a strong immune response remains critical, the syllable number in male call may be particularly reduced. Because female preferences likely coevolve with male traits such as call and spermatophore size, a diversity of preference functions is generated and maintained across the various populations.

Previous studies of female preference in *E. diurnus* populations (Ritchie, 1996; Party *et al.*, 2014) have also examined relationships between preference and male call parameters. Although some of their basic findings parallel those described here, they did not analyse the major difference in preference function shape across a full range of populations. Consequently, they could not focus on the different forms of selection imposed by female preference (Fig. 2) and the way in which female tolerance may drive the nature of male calls (Fig. 5). These added findings in the current study highlight the importance of the preference function approach and appropriate testing in studies of mate choice: We would not have been able to discern stabilizing vs. directional selection among the various populations without testing a full range of synthetic call stimuli, and for practical reasons, these stimuli had to be presented in a series of no-choice playback trials (Wagner, 1998; Dougherty & Shuker, 2015). We also would not have acquired our current understanding without taking a comparative approach that necessitated sampling of a wide range of populations.

In conclusion, preference functions differ among *E. diurnus* populations in overall shape, peak preference and tolerance. These differences potentially reflect evolutionary history of the species and explain differences in male signals among populations. The several *E. diurnus* populations are currently geographically isolated from each other, and the differences in signals and preferences may further reinforce these differences, contributing to divergence (West-Eberhard, 1983). The substantial repeatability of male syllable number indicates that this trait is potentially heritable. If female preferences are also heritable in this species, Fisherian coevolution may arise and further increase population divergence in this species, further driving population divergence in this species.

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