

# The influence of developmental environment on courtship song in cactophilic *Drosophila*

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## Abstract

Closely related species often differ in the signals involved in sexual communication and mate recognition. Determining the factors influencing signal quality (i.e. signal's content and conspicuousness) provides an important insight into the potential pathways by which these interspecific differences evolve. Host specificity could bias the direction of the evolution of sexual communication and the mate recognition system, favouring sensory channels that work best in the different host conditions. In this study, we focus on the cactophilic sibling species *Drosophila buzzatii* and *D. koepferae* that have diverged not only in the sensory channel used for sexual communication and mate recognition but also in the cactus species that use as primary hosts. We evaluate the role of the developmental environment in generating courtship song variation using an isofemale line design. Our results show that host environment during development induces changes in the courtship song of *D. koepferae* males, but not in *D. buzzatii* males. Moreover, we report for the first time that host rearing environment affects the conspicuousness of courtship song (i.e. song volume). Our results are mainly discussed in the context of the sensory drive hypothesis.

## Introduction

Courtship communication involves the generation and transmission of signals and their perception and processing by the courted sex (Greenfield, 2002). As disruptions in this process lead to premating sexual isolation and hence speciation (Coyne & Orr, 2004), understanding how sexual communication systems evolve is a central topic in evolutionary biology.

Signals can be characterized by their structure and content (terminology *sensu* Endler, 1992, 1993; also see Guilford & Dawkins, 1991; Greenfield, 2002). On the one hand, signal structure affects the probability that the signal reaches the receiver, determining communication efficiency (Guilford & Dawkins, 1991; Endler,

1992, 1993; Schaefer *et al.*, 2004; Ryan & Cummings, 2013; White *et al.*, 2015; Brock *et al.*, 2017). On the other hand, signal content affects the receiver response by providing information about the signaller (Guilford & Dawkins, 1991; Endler, 1992, 1993; Candolin, 2003; Bro-Jørgensen, 2010). Both signal features define its general quality (terminology *sensu* Endler, 1993). Thus, a signal may be low in quality because it is difficult to detect (structure), or because it gives false or misleading information about the signaller (content; Endler, 1993).

Environmental conditions have the potential to affect both the content and the structure of signals and, thus, modify their quality (Boughman, 2002; Bro-Jørgensen, 2010; Gomes *et al.*, 2017). In this regard, the sensory drive hypothesis emphasizes signal structure (Endler & Basolo, 1998) and focuses on how communication systems adapt to local environments (Boughman, 2002). According to this hypothesis, signals used in sexual communication and mate recognition diverge in different environments if the easy-to-detect signals differ in each environment (Endler, 1993; Boughman, 2002). Alternatively, indirect benefits hypotheses ('good genes'

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and ‘sexy sons’ hypotheses) emphasize signal content and focus on how signals are honest indicators of male genetic quality. According to the latter hypotheses, the expression of sexual signals depends on the male’s phenotypic condition and females benefit indirectly from mate choice through increased offspring fitness (Andersson, 1986, 1994; Kokko *et al.*, 2003; Kokko & Rankin, 2006). However, if signals are part of the mate recognition system, the adaptive canalization hypothesis opposes to the condition-dependent expression of sexual traits and predicts reduced phenotypic variation by developmental mechanisms (Stearns *et al.*, 1995).

Speciation in phytophagous insects is often associated with changes in host plant use (Berlocher & Feder, 2002; Drès & Mallet, 2002; Bush & Butlin, 2004). Thus, host specificity could bias the direction of the evolution of sexual communication and the mate recognition system, favouring signal parameters and sensory channels which work best in the different host conditions (Endler, 1993).

Most species of the genus *Drosophila* are not strictly phytophagous but saprophytic, as they use decaying plant material. However, host plant chemistry has a major impact on the life history of these species (Markow & O’Grady, 2005, 2008). The cactophilic flies *Drosophila buzzatii* and *D. koepferae* provides an ideal opportunity to test the relationship between host shifts and divergence in sexual communication and mate recognition. These species have overlapping distribution ranges in the arid and semi-arid regions of north-western Argentina and southern Bolivia where they use fermenting cacti as feeding and breeding substrates (Hasson *et al.*, 1992, 2009; Manfrin & Sene, 2006). Although both species can develop to adulthood in natural necroses of several cactus species, *D. buzzatii* uses prickly pears (genus *Opuntia*) as primary hosts, whereas *D. koepferae* prefers decaying columnar cacti (genera *Cereus* and *Trichocereus*; Hasson *et al.*, 1992; Fanara *et al.*, 2006; Soto *et al.*, 2012). These hosts represent contrasting nutritional and chemical environments; *Opuntia* cacti are comparatively rich in free sugars and total fat (Stintzing & Carle, 2005; Carreira *et al.*, 2014), whereas cacti of the genus *Trichocereus* contain toxic alkaloids as mescaline and trichocereine (Reti & Castrillón, 1951; Corio *et al.*, 2013; Padró & Soto, 2013; De Panis *et al.*, 2016). Larval development in these alternative environments differentially affects survival and developmental time and also induces morphological changes, affecting body size and both wing and aedeagus size and shape (Fanara *et al.*, 1999; Fanara & Hasson, 2001; Carreira *et al.*, 2006; Soto *et al.*, 2007, 2012, 2014; Hasson *et al.*, 2009; Corio *et al.*, 2013; Padró *et al.*, 2014). Furthermore, males of both species exhibit greater mating success when they develop in their respective primary hosts (Hurtado *et al.*, 2012).

A recent study showed that sensory channels used by females for mate recognition and mate choice have

diverged in these species (Iglesias & Hasson, 2017). Mate recognition relies heavily on acoustic signals (courtship songs) in *D. buzzatii*, whereas *D. koepferae* females use courtship songs for mate choice and non-acoustic signals (e.g. olfactory) for mate recognition (Iglesias & Hasson, 2017).

To date, most research on phytophagous insects has focused on the effect of environment (e.g. host or diet) on chemical communication (Nosil *et al.*, 2007; Etges *et al.*, 2009; Geiselhardt *et al.*, 2009, 2012; Kühbandner *et al.*, 2012; Otte *et al.*, 2015, 2016; Xue *et al.*, 2016). However, only a few studies have explored the effect of host plants on nonchemical communication (Etges *et al.*, 2007; Cocroft *et al.*, 2009). In this study, we evaluate the role of the developmental environment in generating courtship song variation. We test predictions of (1) the sensory drive hypothesis, (2) the indirect benefits hypotheses and (3) the adaptive canalization hypothesis. If host-induced phenotypic plasticity influences the evolution of the mate recognition system through sensory drive, the acoustic signal should be easier to detect when flies are reared in prickly pear hosts than in columnar cacti. As *D. buzzatii* uses prickly pears as primary hosts and its mate recognition system relies on the courtship song, we expect this signal to be more conspicuous in prickly pear-reared males. Acoustic communication efficiency through host-induced phenotypic plasticity can be reached because males sing louder (Shirangi *et al.*, 2013) or because females’ auditory organs are tuned to different best frequencies; that is, females can hear a song that is at their frequency or frequencies of greatest sensitivity even at lower amplitude (Boughman, 2002; Riabinina *et al.*, 2011). Thus, the volume at which males sing should be lower or courtship songs should differ significantly in carrier frequency when males are reared in columnar cacti compared to males reared in prickly pears. In addition, if host development environment influences acoustic mate choice in line with the indirect benefits hypotheses, the first assumption that must be addressed is whether courtship songs differ significantly in content when males are reared in columnar cacti or in prickly pears (i.e. if courtship song is a condition-dependent trait). As *D. koepferae* courtship song is involved in mate choice, we expect this signal to be sensitive to the developmental environment. Adaptation to novel environments and speciation can be facilitated by condition-dependent sexual traits (Lorch *et al.*, 2003; Van Doorn *et al.*, 2009). In this context, condition may reflect environmentally determined variance in the ability to acquire or assimilate resources from contrasting nutritional and chemical environments. In contrast with traits involved in mate choice, which are expected to exhibit greater plasticity, mate recognition traits are expected to experience stabilizing selection favouring canalization and thus reduced plasticity (Boughman, 2007; Fernandez-Montraveta & Moya-Laraño, 2007; Stillwell *et al.*,

2010; Svensson *et al.*, 2014). In this sense, we expect *D. buzzatii* song to be protected (i.e. canalized) against the influences of environmental fluctuations, in line with the adaptive canalization hypothesis.

## Materials and methods

### Experimental design

Wild inseminated females were collected in San Agustín del Valle Fértil Natural Reserve (30°41'26.5"S 67°29'45.5"W, San Juan Province, Argentina) where both species coexist. As females of these species are morphologically indistinguishable, the intromittent organ (aedeagus) of males in their progenies was inspected to determine the species (Vilela, 1983). Seven isofemale lines (lines hereafter) of each species were established and used in the experiments outlined below. Lines were kept separately in a rearing chamber at  $25 \pm 1$  °C and a 12-h light: 12-h dark cycle for 24 generations before the experiments started.

In the sampled area, two cactus species, *Opuntia sulphurea* and *Trichocereus terscheckii*, are used as primary hosts by *D. buzzatii* and *D. koepferae*, respectively. Fresh material of both cactus species was also collected in the sampling area and stored at  $-20$  °C until their use in the preparation of 'semi-natural' media. To this end, cactus tissues were mixed in a blender and 1% of dehydrated commercial yeast (*Saccharomyces cerevisiae*) and 0.8% of agar were added. Dehydrated commercial yeast was used as protein source and agar to control the consistency of media. Once prepared, vials were autoclaved.

To obtain the experimental male flies used for song recording, batches of 40 first-instar larvae were seeded in vials containing 6 mL of 'semi-natural' medium prepared either with *O. sulphurea* or *T. terscheckii*. Eight replicated vials were run for each combination of species, cactus and line [with the exception of one *D. koepferae* line with low fecundity (few eggs laid) for which two replicates could not be set up]. Vials were incubated under the same conditions as described above until the emergence of adult flies.

The courtship song of one 5- to 6-day-old male per replicate was recorded in an acoustically isolated room at  $25 \pm 0.3$  °C using a SONY ICD-SX712 recorder. Songs were recorded in an uncompressed linear PCM wav file, and using a sampling rate of 44.1 kHz and 16-bit precision. For song recording, each virgin male was placed in a mating chamber together with a virgin female whose wings had been removed to avoid interferences. Mating arenas consisted of polymethyl methacrylate cylinders 0.5 mm height and 1.2 mm in internal diameter. The floor of the chamber was the microphone itself and had a foam cap in the opposite extreme where flies were introduced. For more details, see Iglesias & Hasson (2017).

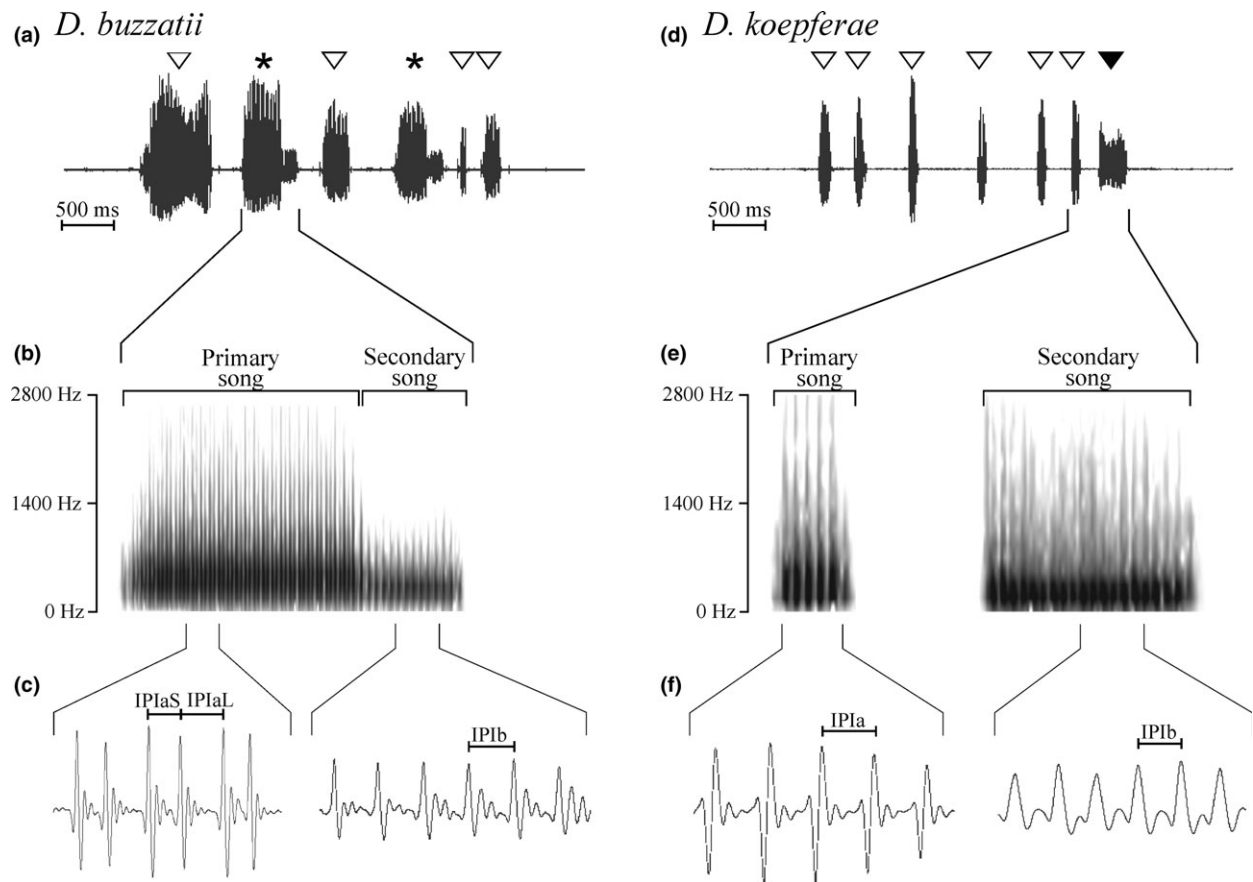
Body size can influence the evolution of communication systems through its correlated effect on signal frequency (Cocroft & De Luca, 2005). As previous studies have shown that wing size (that it is also used as a proxy of body size) differs between flies reared in *O. sulphurea* and *T. terscheckii* (Carreira *et al.*, 2006; Soto *et al.*, 2008), it is important to control for this variable in song analysis. Therefore, after successful song recording, males were conserved in alcohol 96° and right wings were removed with a pair of microsurgical scissors from the base of the wing and measured (see below).

### Song measurements

Courtship songs of *D. buzzatii* and *D. koepferae* consist of two different components, a primary (*sensu* Oliveira *et al.*, 2013) or 'A' song (*sensu* Ewing & Miyan, 1986), that is produced during most of the courtship sequence, and a secondary or 'B' song that is produced later in courtship and immediately before copulation (Ewing & Miyan, 1986; Oliveira *et al.*, 2013). Each component is characterized by low-frequency pulses arranged into pulse trains that have different temporal and spectral features (Ewing & Miyan, 1986; Oliveira *et al.*, 2013). In this sense, only *D. buzzatii* courtship song has primary and secondary songs with significantly different interpulse intervals (IPI; Oliveira *et al.*, 2013) and primary song with a bimodal distribution of IPIs ('doublet pulses'), that is alternate pulses with long and short IPIs in the primary song (Iglesias & Hasson, 2017; Fig. 1). Also, both courtship song components can be distinguished by the carrier frequency (CF) of their pulses in both species (Oliveira *et al.*, 2013; Fig. 1).

A group of pulses which together make up a repeated unit is called 'burst' (*sensu* Ewing & Miyan, 1986). Consequently, there are bursts containing only the primary or the secondary song component, here referred to as 'simple bursts' and bursts containing both components, here referred to as 'composite bursts' (Fig. 1). *Drosophila koepferae* males produced simple bursts of primary or secondary song, and sporadically composite bursts. However, *D. buzzatii* males produced simple bursts of primary song and composite bursts.

Three parameters that characterize both components of courtship songs were measured: the carrier frequency (CF) of pulses, the interpulse interval (IPI) and the duration (D) of each component in a burst. In the case of *D. buzzatii*, we take into account the 'doublet pulses' and we divided the IPIs of the primary song in short (IPIaS) and long (IPIaL; Fig. 1c). We also measure the volume at which males emitted each component (V) following Shirangi *et al.* (2013). The CF was taken as the highest peak frequency from a fast Fourier transformation (FFT) analysis, IPI as the time interval between one peak of a pulse and the next (Fig. 1c,f), and V was estimated by calculating the square root of



**Fig. 1** Sonograms and spectrograms of male courtship song of *D. buzzatii* (a–c) and *D. koepferae* (d–f). (a,d) Six seconds of a courtship song illustrating simple bursts (arrowheads) of primary (white) and secondary (black) songs, and composite bursts (asterisks). (b,e) Spectrograms showing frequency differences between primary and secondary songs. (c,f) Expanded view of bursts indicating the interpulse intervals (IPI) measured.

the mean of the squares (rms) of all pulses as in Shirangi *et al.* (2013).

Each parameter was measured five times for each song using the Raven sound analysis software (Raven Pro-1.4 Build 48; Cornell Lab of Ornithology, Bioacoustics Research Program), and the means of these measures were taken. As these parameters have different units of measurement, individual means were standardized (converted to z-scores) before analysis when appropriate (see Statistical Analysis below).

### Wing size quantification

We used landmark-based geometric morphometrics to quantify wing size. Ventral views of wing images were captured using a digital camera attached to a binocular microscope (10 $\times$ ) connected to a computer. We scored 10 landmarks, following Soto *et al.* (2008), using tpsDig (Rohlf, 2004). Centroid size (CS) was computed in MorphoJ 1.06a (Klingenberg, 2011) and used as isometric estimator of overall wing size. Centroid size is

calculated as the square root of the sum of squared distances between the centre of the configuration of landmarks and each individual landmark (Rohlf & Slice, 1990; Bookstein, 1997).

### Statistical analysis

To include wing size as a covariate in song analysis, we used an ANOVA to test for wing size differences between flies reared in different cactus media and among lines.

For song analysis, we subdivided song measurements in three subsets of data: (1) the subset ‘volume’ was composed of the amplitude of primary (Va) and secondary (Vb) songs; as the amplitude of both components were highly correlated in *D. buzzatii* (Pearson’s correlation:  $r = 0.82$ ), we only used Vb in *D. buzzatii* analysis. (2) The subset ‘primary song’ was composed of the carrier frequency (CFa), the interpulse interval (IPIa) and the duration (Da) of primary song components; in the case of *D. buzzatii* songs, IPIa was subdivided into IPIaS and IPIaL. (3) The subset ‘secondary

song' was composed of CFb, IPIb and Db of secondary song components.

The effect of cactus media on 'volume' was investigated by means of an ANCOVA in *D. buzzatii* and a MANCOVA in *D. koepferae*, with cactus (two levels: *O. sulphurea* and *T. terscheckii*) and line (seven levels) as main sources of variation and CS as a covariate to control for size. To investigate the effect of cactus media on both 'primary' and 'secondary' songs, we used the same MANCOVA model as above. When covariate CS was non-significant, it was removed from the models and ANOVAS or MANOVAS were run when appropriate. To determine which song parameters were driving the patterns revealed by multivariate analyses, we also ran *post hoc* ANCOVAs/ANOVAS for each parameter. All analyses were conducted in R (version 3.2.5) using the function *Adonis* of the *vegan* package which performs an analysis of variance based on distance matrices and permutations (Oksanen *et al.*, 2013).

## Results

### Wing size variation

The ANOVAS testing the effects of line and cactus on wing size showed that both factors were significant in both species (Table 1). However, the cactus-by-line interaction was significant only in *D. koepferae*, suggesting that lines respond differently to the rearing conditions. On average, *D. buzzatii* and *D. koepferae* flies emerged from *O. sulphurea* medium were larger than those emerged from *T. terscheckii* medium (Fig. 2).

### Song variation

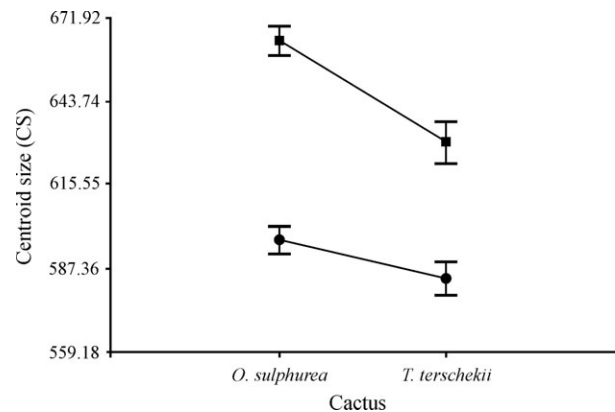
Mean values and standard deviations for each of the variables measured in both species are shown in Table 2.

Male wing size (which is also used as a proxy of body size) was not a significant predictor of song parameters ( $P > 0.05$  in ANCOVA/MANCOVAs across the three subsets of song data). This suggests that differences in the main effects are not driven by allometric

**Table 1** ANOVAS testing for differences in wing size (centroid size).

Species	Effect	df	MS	F	P
<i>D. buzzatii</i>	Cactus	1	0.00357	23.96	<b>&lt;0.001</b>
	Line	6	0.00227	15.24	<b>&lt;0.001</b>
	Cactus × Line	6	0.00015	1.01	0.4169
	Error	98	0.00014		
<i>D. koepferae</i>	Cactus	1	0.01956	90.02	<b>&lt;0.001</b>
	Line	6	0.00163	7.51	<b>&lt;0.001</b>
	Cactus × Line	6	0.00053	2.45	<b>&lt;0.05</b>
	Error	94	0.00022		

df, degrees of freedom; MS, mean squares; F, F statistic; P, P-value. Significant P values ( $P < 0.05$ ) are in bold.



**Fig. 2** Mean wing size (centroid size) and 95% confidence intervals in *D. buzzatii* (circles) and *D. koepferae* (squares) reared in *O. sulphurea* and *T. terscheckii*.

relationships with wing or body size. Thus, the size term was removed from the models in subsequent analyses.

### Volume variation

The mean volume of courtship songs differed significantly among lines in both species (Table 3a). The MANOVA showed a significant effect of the rearing cactus and a significant interaction between line and cactus only in *D. koepferae* (Table 3a). *Post hoc* ANOVA's in *D. koepferae* revealed a significant line effect on Vb ( $F_{6,94} = 3.44$ ;  $P < 0.01$ ) and a significant cactus effect (Va:  $F_{1,94} = 3.71$ ;  $P < 0.05$ — Vb:  $F_{1,94} = 14.42$ ;  $P < 0.001$ ) and the interaction line by cactus (Va:  $F_{6,94} = 1.99$ ;  $P < 0.05$ — Vb:  $F_{6,94} = 2.96$ ;  $P < 0.01$ ) on both components of the courtship song. These results suggest that volume variation has a genetic basis in both species, but only in *D. koepferae*, the rearing cactus affects the quality of courtship song by changing its structure (i.e. conspicuousness). On average, *D. koepferae* males reared in *O. sulphurea* produced louder songs than those reared in the primary host, *T. terscheckii* (Fig. 3). Primary and secondary song components were produced at different volumes by *D. buzzatii* males, being louder the primary song (Fig. 3). However, *D. koepferae* males produced both components at the same volume. Song volume of *D. koepferae* males reared in *O. sulphurea* approximately equalled the volume at which *D. buzzatii* males produced the primary song (Fig. 3), whereas song volume of *D. koepferae* males reared in *T. terscheckii* approximately equalled the volume at which *D. buzzatii* males produced the secondary song (Fig. 3).

### Primary song variation

The MANOVA showed significant variation in the primary song among lines in both species (Table 3b). *Post hoc* ANOVA's revealed a significant line effect on all four song

**Table 2** Mean  $\pm$  SD of courtship song parameters of *D. buzzatii* (Db) and *D. koepferae* (Dk).

	Va (RMS)	Vb (RMS)	Da (ms)	Db (ms)	CFa (Hz)	CFb (Hz)	IPIa (ms)	IPIaS (ms)	IPIaL (ms)	IPIb (ms)
Db	1323.41 $\pm$ 665.35	826.68 $\pm$ 494.35	457.71 $\pm$ 180.18	241.03 $\pm$ 73.64	378.78 $\pm$ 22.35	228.12 $\pm$ 16.23	–	8.36 $\pm$ 0.5	10.36 $\pm$ 0.84	15.45 $\pm$ 0.97
Dk	1105.40 $\pm$ 658.30	1156.38 $\pm$ 671.41	114.30 $\pm$ 57.36	259.57 $\pm$ 97.37	341.70 $\pm$ 35.72	194.24 $\pm$ 18.59	11.36 $\pm$ 0.55	–	–	10.04 $\pm$ 0.50

*Db*, *Drosophila buzzatii*; *Dk*, *Drosophila koepferae*; a, primary or A song component; b, secondary or B song component; V, volume; D, duration; CF, carrier frequency; IPI, interpulse interval (L, long/S, Short).

parameters in *D. buzzatii* (CFa:  $F_{6,98} = 17.03$ ,  $P < 0.001$ ; Da:  $F_{6,98} = 3.37$ ,  $P < 0.01$ ; IPIaS:  $F_{6,98} = 2.80$ ,  $P < 0.05$  and IPIaL:  $F_{6,98} = 7.77$ ,  $P < 0.001$ ) and on CFa ( $F_{6,94} = 7.33$ ,  $P < 0.001$ ) and IPIa ( $F_{6,94} = 10.68$ ,  $P < 0.001$ ) in *D. koepferae*. These results suggest that primary song variation also has a genetic basis in both species. However, only the primary song of *D. koepferae* was significantly influenced by the rearing cactus (Table 3b). *Post hoc* ANOVA'S revealed no significant effect of the cactus medium on any of the individual parameters. This result indicates that the significant cactus term in the MANOVA is a consequence of a correlated response between parameters. In this respect, primary song seems to be more sensitive to the developmental environment in *D. koepferae* than in *D. buzzatii*.

### Secondary song variation

Significant differences were found among lines of both species in the secondary song component (Table 3c). *Post hoc* ANOVAS showed a significant line effect on all three song parameters in *D. buzzatii* (CFb:  $F_{6,98} = 10.60$ ,  $P < 0.001$ ; PERb:  $F_{6,98} = 13.54$ ,  $P < 0.001$  and Db:  $F_{6,98} = 4.13$ ,  $P < 0.001$ ), and only on CFb ( $F_{6,94} = 7.95$ ,  $P < 0.001$ ) in *D. koepferae*. In addition, differences in the secondary song component between flies reared in different cactus media were not significant in either species (Table 3c). These results suggest that there is genetic basis for secondary song variation and also that this component is consistently produced in both species.

## Discussion

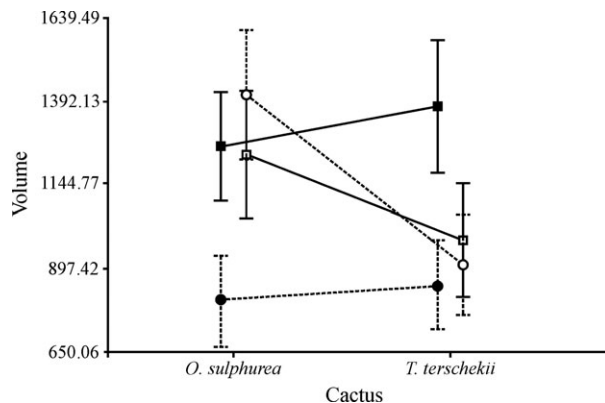
Many closely related species differ in the signals involved in sexual communication and mate recognition (Byrne, 1999; Isoherranen *et al.*, 1999; Rafferty & Boughman, 2006; Secondi *et al.*, 2010; Gleason *et al.*, 2012; Giglio & Dyer, 2013; Weiss *et al.*, 2015). Determining the factors affecting signal quality provides an important insight into the potential pathways by which these interspecific differences evolve. In this study, we focused on a pair of cactophilic sibling species that has diverged not only in the sensory channel used for sexual communication and mate recognition but also in the cactus species that use as primary hosts. On the one hand, *D. buzzatii* uses prickly pears as primary hosts and its mate recognition system relies heavily on the courtship song. On the other hand, *D. koepferae* primarily exploits columnar cacti and uses nonacoustic cues for mate recognition and the courtship song for mate choice (Hasson *et al.*, 1992; Fanara *et al.*, 1999; Iglesias & Hasson, 2017). Our results show first that variation in song parameters are not driven by allometric relationships with wing or body size; second that much of the variation in courtship song would be genetically determined; and third that host environment during development alters the quality of courtship songs in *D. koepferae*, but not in *D. buzzatii*.

**Table 3** Results from ANOVA/MANOVAS testing for volume and both primary and secondary song component differences between *D. buzzatii* and *D. koepferae* males reared in two cactus media.

Effect	ANOVA <sup>1</sup> /MANOVA <sup>2</sup> <i>D. buzzatii</i>				MANOVA <i>D. koepferae</i>			
	df	MS	F	P	df	MS	F	P
(a) Volume <sup>1</sup>								
Cactus	1	0.03803	0.59	0.5009	1	0.02059	13.47	<b>&lt;0.001</b>
Line	6	0.24164	3.74	<b>&lt;0.001</b>	6	0.00392	2.56	<b>&lt;0.05</b>
Cactus × Line	6	0.09839	1.52	0.1456	6	0.00444	2.90	<b>&lt;0.001</b>
Error	98	0.06456			94	0.00153		
(b) Primary or A song component <sup>2</sup>								
Cactus	1	0.00334	2.20	0.1003	1	0.00533	4.08	<b>&lt;0.05</b>
Line	6	0.00953	6.29	<b>&lt;0.001</b>	6	0.00875	6.71	<b>&lt;0.001</b>
Cactus × Line	6	0.00142	0.94	0.5109	6	0.00183	1.4	0.1528
Error	98	0.00152			94	0.00130		
(c) Secondary or B song component <sup>2</sup>								
Cactus	1	0.00197	1.54	0.2056	1	0.00334	2.14	0.1035
Line	6	0.01043	8.15	<b>&lt;0.001</b>	6	0.00525	3.36	<b>&lt;0.001</b>
Cactus × Line	6	0.00108	0.84	0.6456	6	0.00168	1.07	0.3818
Error	98	0.00128			94	0.00156		

df, degrees of freedom; MS, mean squares; F, F statistic; P, P-value. Significant P values ( $P < 0.05$ ) are in bold.

<sup>1,2</sup>type of statistical analysis carried out for each song subset in *D. buzzatii* (see Material and methods for details).



**Fig. 3** Mean volume (in rms) and 95% confidence intervals of primary (squares and solid lines) and secondary (circles and dashed lines) song components of *D. buzzatii* (filled) and *D. koepferae* (open) males reared in *O. sulphurea* and *T. terscheckii*.

### Signal structure variation: evidence for sensory drive hypothesis

The sensory drive hypothesis regards diversity in mating signals as a by-product of adaptation to different environments (Endler, 2000; Boughman, 2002). Thus, direct ecological selection on signal conspicuousness (i.e. signal structure) can lead to changes in the sensory channel used in mate recognition because different forms of male traits are the most stimulating in alternative environments (Endler, 2000). Easy-to-detect signals may benefit both sexes by increasing male mating success and reducing the time that females require to detect potential mates (Dawkins & Guilford, 1997; Boughman, 2002). In this regard, we found that the

rearing cactus affects the quality of courtship songs by changing its volume in *D. koepferae* but not in *D. buzzatii* (Table 3a). Moreover, we found that *D. koepferae* males emerged from its primary host sing lower songs than males reared in the secondary host (Fig. 3). The fact that courtship song of *D. koepferae* males becomes less conspicuous when they are reared in its primary host could help to explain why *D. koepferae* females rely on nonacoustic cues for mate recognition. A recent study in *D. melanogaster* showed that females are less willing to mate with males that produce the ‘sine’ component of its song with reduced volume and also demonstrated that size variation in the thoracic wing muscle *hgl* is responsible for this volume variation (Shirangi *et al.*, 2013). However, it is important to recall that *D. koepferae* females still use courtship songs for mate choice (Iglesias & Hasson, 2017). In this regard, we showed that the reduction in *D. koepferae* song volume equals the volume at which secondary songs of *D. buzzatii* males are produced (Fig. 3). We hypothesize that a reduction in song volume may have a greater impact at the beginning of courtship, when the male follows the female and the distance to the sound perception organ (i.e. the arista) is greater, rather than at the end, when the female reduces her locomotor activity and the male moves around the female (Spieth, 1974; Markow & Hanson, 1981). In this sense, Morley *et al.* (2012) demonstrated that the mechanical sensitivity of the arista changes as a function of the angle of incidence of the acoustic stimulus. Suggestively, males of *D. buzzatii* produce secondary songs later in courtship and immediately before copulation (Oliveira *et al.*, 2013; Iglesias & Hasson, 2017).

Female perception can also change as a by-product of adaptation to a new host. For instance, females can become more sensitive to some sound frequencies allowing its detection even at low volume (Boughman, 2002). In this sense, it has been shown that the receiver tuning is correlated with high-frequency pulses of conspecific courtship songs in the *D. melanogaster* species group (Riabinina *et al.*, 2011). Given that post hoc contrasts revealed no significant effect of cactus media considering only CFa, rearing environment would affect the quality of primary songs of *D. koepferae* males by changing its content rather than its structure.

The ability to switch from one sensory channel to another in sexual communication has been shown to be a beneficial strategy when animals face environmental changes that impair signal transmission in particular channels (Grafe *et al.*, 2012; Partan, 2017). Moreover, a rapid evolutionary response to the loss of the acoustic male signal was shown in the field cricket *Teleogryllus oceanicus* (Bailey *et al.*, 2007; Tinghitella & Zuk, 2009). A wing mutation eliminates the singing ability of more than 90% of *T. oceanicus* males in a Hawaiian population in less than 20 generations (Zuk *et al.*, 2006). However, pre-existing variation in the propensity of females to accept silent males accommodated this rapid morphological change (Bailey *et al.*, 2007; Tinghitella & Zuk, 2009). Thus, selection for females with relaxed acoustic requirements was proposed to account for the widespread loss of the acoustic signal (Tinghitella & Zuk, 2009).

### Signal content variation: indirect benefits and adaptive canalization

Signals that convey different kinds of information differ in terms of the nature of selection acting on signallers, patterns of phenotypic variation, developmental mechanisms and evolutionary consequences (Tibbetts *et al.*, 2017). Thus, traits involved in mate choice are expected to experience strong directional selection and to exhibit greater sensitivity to environmental conditions, that is to be more plastic (condition-dependent traits of indirect benefits hypotheses; Boughman, 2007; Fernandez-Montraveta & Moya-Laraño, 2007; Stillwell *et al.*, 2010; Svensson *et al.*, 2014). On the other hand, traits involved in mate recognition are expected to experience strong stabilizing selection and to be more canalized, that is to be less plastic (adaptive canalization hypothesis; Fernandez-Montraveta & Moya-Laraño, 2007; Stillwell *et al.*, 2010; Svensson *et al.*, 2014). Consistent with such predictions, we found that courtship song seems to be more sensitive to the developmental environment when functions as mate choice signal (in *D. koepferae*) rather than when functions as mate recognition signal (in *D. buzzatii*). However, given the subtle correlated effect of the rearing environment on the primary song of *D. koepferae* males, future work should

focus on female preferences for the host-induced primary song variation found in this species. In this respect, variation is only biologically meaningful if females are able to detect such variation and if female preferences are not affected by the rearing environment.

The model system studied in this work relies on three major interactors: (a) the cactus, whose cladodes and stems are used as substrata, (b) the saprotrophic yeast-like community that participate in the decomposing process of cactus tissues and (c) the cactophilic *Drosophila*. Given that the focus of our study relied solely on the effect of the host cacti, there is a possibility that dissimilar evaluations would have arisen if the focus had been on the complete system, including the yeast community. Thus, further experimental work is required to determine whether, and in what sense, the yeast community modifies the results herein reported. At the moment, the effect of our 'semi-natural' media on wing size was consistent with those of previous studies that do use natural yeast-inoculated cactus media (Soto *et al.*, 2008).

### Conclusions

A new environment can rapidly lead, through plasticity, to the simultaneous expression of new phenotypes in many individuals that increase the likelihood of survival in the new environment (Pfennig *et al.*, 2010). However, when the quality of sexual signals is also affected, the relationship between signal variation and mating success can be altered promoting diversification and the evolution of both sexual communication and the mate recognition system (Candolin & Heuschele, 2008; Crocroft *et al.*, 2009). Despite we only examined the effect of the cactus hosts, our findings demonstrate that the rearing environment may have important implications in the evolution of courtship song. We report host-induced changes in the acoustic signals in *D. koepferae*, but not in its sibling *D. buzzatii*. Interestingly, we found that host environment during development can change the conspicuousness of courtship song, highlighting the host rearing environment as a potential factor affecting the effectiveness of acoustic communication. Thus, our findings provide a fruitful starting point to understand the evolution of sexual communication and the mate recognition system in cactophilic species.

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