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ELECTRONIC SUPPLEMENTARY MATERIAL

METHODS

Species selected for study

T. schistaceus and *P. chloris* belong to two families (Thamnophilidae and Pipridae [or incerta sedis in some classifications], respectively) of the suboscines (Order Passeriformes, Suborder Tyranni), which include most of the species of birds in the understory of Amazonian forests. In comparison to oscines (Suborder Passeri), most suboscines, including the two species chosen here, sing songs with relatively little individual variation (for instance, Wiley 2005). In a larger study of variation in the songs of 85 understory species at Rio Cristalino, these two species, although not close phylogenetic relatives, had songs with one of the smallest differences in multidimensional acoustic space as defined by 15 timing and frequency parameters (see Table 1 for a list of these parameters). We selected them for study because they are each others' nearest neighbors in acoustic space and have populations adequate for study.

Synthesis of mean songs and three intermediates

Synthesis was based on recordings of 5 individuals of each species from Rio Cristalino obtained in May 2006 with a Marantz PMD660 digital recorder (44 kHz digitizing rate, 16-bit accuracy WAV format) and a Sennheiser ME67 ultradirectional microphone from distances of 4 - 10 m. From spectrograms and the waveforms of each song (WildSpectra1, version 051027, sampling rate 22.05 kHz, frequency resolution 172 Hz, temporal resolution 5.8 ms), we could determine the numbers of notes characteristic of the beginning, middle, and final portions of each song and identify elements of notes with constant frequency and amplitude (or relatively constant rates of change of frequency or amplitude). From these data, we calculated the mean features of songs for each species. With these means we computed the mean songs or 100% morphs of each species. The intermediate songs had parameters 33%, 50%, and 67% of the distance between the mean parameters for the two species. The program for synthesizing songs used a spreadsheet of frequencies and amplitudes at 1-ms intervals. By interpolation between the values at these intervals, the program computed the waveform of the synthesized song with an effective digitization rate of 22050 Hz.

The synthesized waveforms were normalized to maximal amplitude with Wildspectra1 (v.051027, www.unc.edu/~rhwiley). One-minute tracks were then produced with 4 songs at approximately the natural rate of singing.

General procedures for playbacks

All playbacks were conducted in the first four hours after sunrise. The playback speaker, a RadioShack amplifier speaker, was set near the center of a subject's territory, 2 m above the ground, connected with a 5-m lead to an iPod (Apple Computer). Because the range of frequencies in the playbacks was limited, the frequency response of the speaker had little influence on the fidelity of the played songs. After the speaker was in place, a playback song was selected at random (by rolling a die), subject to the constraints of the experimental design. Playback trials began when the subject had been silent for at least 5 minutes. Each trial lasted 26 min (5 min before playback, 1 min of playback, and 20 min afterwards). All playbacks to an individual were conducted within

5 m of the same location near the center of its territory. Treatments were separated by at least 48 hours to minimize habituation, and each subject received the treatments in a random order. Territorial neighbors of the same species were not tested on the same day. All playbacks had the peak sound pressure level (SPL) adjusted to approximate that of natural songs (81dB at 1 m, Realistic digital sound level meter, C weighting, fast response). It was not possible to obtain accurate SPL readings for the two species under study because they sang 5-10 m above ground. Instead the playbacks used a level measured for 4 other species of suboscines found in this region (Seddon & Tobias 2006; Seddon & Tobias 2007; see Luther 2008).

During the periods before, during, and after each playback, the experimenter recorded (1) time from the start of playback to the first visible flight toward the speaker (latency of approach in min), (2) closest distance to the speaker (in m), (3) time spent less than 5 m from the speaker (in min), (4) time from the start of playback to the first song (latency of song in min), (5) total number of minutes singing, (6) number of songs, (7) number of call notes, (8) number of duets, and (9) number of flights by the subject within 1 m of the speaker. Low values for measures (1), (2), and (4) and high values for (3), (5), (6-9) indicated strong responses to playback.

Playbacks of natural and synthesized songs

Presentations of natural songs and synthesized mean songs (100% morphs) were conducted before presentations of the intermediate morphs, in order to confirm that individuals would respond to synthesized songs. There were 10 subjects for these experiments, 6 *T. schistaceus* and 4 *P. chloris*. For each playback the experimenter randomly chose a track of a male song recorded at least 3 territories away (>500 m) or the synthesized mean song of the species.

Playbacks of synthesized mean songs and intermediates

These playbacks were presented to 16 individuals, 8 from each species. Thirteen of these 16 subjects received all 5 treatments, two mean songs (100% morphs) and three intermediates (33%, 50%, and 67% morphs). The remaining 3 individuals, 2 *P. chloris* and 1 *T. schistaceus* did not receive the treatment with 50% morphs, because rain prevented or interrupted these experiments. They did receive the other 4 treatments.

Comparison of responses to natural and synthetic songs

Since many of the behavioral responses were correlated, PCA was used to reduce the measures of response to a smaller number of independent variables. This analysis extracted 3 PCs with eigenvalues >1 which together explained 70% of the variation. PC1, which explained 29% of the variation, was used as the response variable in Wilcoxon signed-ranks matched-pairs tests to compare individual responses to natural and synthesized songs. Statistical tests were calculated with JMP 5.1.

Responses to synthesized mean and intermediate songs

We rescaled the scores on PC1 with the highest response as 1 and the lowest response as 0 so that we could fit 2-parameter and 3-parameter logistic functions, respectively $y=1/1+\exp(c+dx)$ and $y=b/1+\exp(c+dx)$. To account for multiple observations of individuals nested within bird species, we used mixed-effects models in which each parameter as well as each parameter combination was random. Mixed-effects models offer flexibility by allowing for within-group correlation, which is often present in grouped data. Separate mixed-effects logistic regressions were calculated for each species with the NLME library of R. We used NLME for the analysis because of its ability to handle grouped data in nonlinear mixed-effects models (Pinheiro & Bates 2000). Using the Akaike Information Criterion (AIC), we compared both fixed-effects and mixed-effects linear, 2-parameter non-linear, and 3-parameter non-linear models.

RESULTS

Both species responded aggressively to natural songs and 100% morphs. *T. schistaceus* showed essentially equal response toward both playbacks in latency of response, proximity to the speaker, number of minutes singing, number of duets, and the number of songs. There was no statistical difference (N = 10, T = 12.5, p = 0. 232) in responses to synthetic and natural songs.

All 8 *T. schistaceus* responded to the 100% morphs while only 7, 6, 2, and 0 individuals responded to versions with 67%, 50%, 33%, and 0% of conspecific features, respectively. All 8 *P. chloris* responded to 100% and 67% morphs, while only 5, 2, and 2 individuals responded to 50%, 33%, and 0% morphs, respectively. Both species responded more strongly to 100% and 67% morphs (with respect to their own species) than to the other 3 morphs. In general the strong responses included shorter latency of approach and song, closer approaches, a greater number of songs, calls, duets, number of minutes singing, number of flights past the speaker, and time spent closer to the speaker (Figure S1).

Each species also responded in characteristic ways. In a comparison of both species' responses to 100% morphs, *T. schistaceus* showed shorter latency of song, spent more time singing, and sang more songs and duets, while *P. chloris* spent more time close to the speaker and flew by the speaker more frequently. In responses to 67% morphs, *P. chloris* was closer to and spent more time next to the speaker, and showed a shorter latency of song, and *T. schistaceus* spent more time singing and sang more duets than *P. chloris*. In response to 50% morphs, *P. chloris* was closer to the speaker, but showed longer latency of approach than *T. schistaceus*. A comparison of responses to 33% and 0% morphs revealed that the two species responded at equally low levels. In general, strong responses by *T. schistaceus* involved more singing and more time spent singing, while responses of *P. chloris* included a closer proximity to the speaker and more time spent near the speaker.

For both species the best models for predicting responses to song morphs were 3-parameter non-linear fixed-effects models (Table S2, Figure S2). The 3-parameter and 2-parameter mixed-effects models that incorporated multiple random parameters failed to converge. The 2- and 3-parameter mixed-effects models that included one random parameter converged on fixed-effects models, with no random effects. In the best model for *T. schistaceus* and *P. chloris*, parameters c and d, associated with the inflection point of the model's curve, were highly correlated with each other (-0.93 and –0.98, respectively). Parameter b, associated with the steepness of the curve, was moderately correlated with parameters c and d in the *P. chloris* model (0.72 and –0.66, respectively) but less so in the best model for *T. schistaceus* (-0.39 and 0.64, respectively). 3-parameter model coefficient b was almost identical for *T. schistaceus* and for *P. chloris* (b = 0.68±

0.09, b = 0.68±0.08, respectively). Coefficients c and d had similar but inverse values, because the inflection points of both species' response curves were in opposite directions (c = 3.38±1.08 and d = -6.48 for *T. schistaceus* and c = -4.28±2.40 and d = 8.40±4.01 for *P. chloris*).

Song production by the two species was disjunct (Figure S3A). Ellipses of 2 standard deviations around each species' mean song left a gap between their songs approximately as wide as the radii of the ellipses. The synthesized intermediate morphs were greater than 2 standard deviations from the mean songs of each species.

In contrast, song perception, as indicated by responses to the intermediate morphs, filled the space between the mean songs. Two standard deviations around responses to each morph revealed that responses to the different morphs widely overlapped. In particular, although responses to 50% morphs averaged less than those to 67% and 100% morphs, the intensity of these responses overlapped with those to 67% and 100% morphs.

DISCUSSION

The results were analyzed with a mixed-effects model to include the individual variation within a species' behavioral responses to different morphs. Despite the inclusion of random-effects to explain the individual variation, the best models were fixed-effects models. The small sample size of 8 individuals per species combined with individual responses to 5 morphs appears to make estimating mixed-effects models impractical because the variation within each individual is greater than the variation between individuals. Thus the models settled on the population averages, the fixed-effects, rather than the individual variation, the random-effects. Despite the small sample size and large amount of individual variation, the curvilinear pattern of mean responses by both species supports the suggestion that the two species occupy perceptual acoustic space completely but with minimal overlap.

These results provide another case of a mismatch between signals and perception. Previous examples, however, have been interpreted in terms of comparisons with putative phylogenetic ancestors or of conservative psychological mechanisms (Ryan and Rand 1993, Enquist and Arak 1998, Phelps et al. 2001, Ryan et al. 2003, Lynn et al. 2005, ten Cate and Rowe 2007). In the present case, in contrast, the broader scope for perception than for production of signals fits a proposed adaptation for communication in noisy situations. Because noise is such a prevalent feature of natural communication, a mismatch between perception and production of signals is likely to be a general feature of communication.

This study of adaptations for communication in noisy conditions suggests that the neural mechanisms for the perception of signals in noise should receive more attention. In particular, the resemblance between the S-shaped curves for responses to intermediate signals and those for categorical perception require further investigation.

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TABLE S1. Factor loadings for the first three principal components derived from behavioral responses to playback experiments (see Figure 4).

	PC1	PC2	PC3
Eigenvalue	4.58	1.29	1.05
Percent of variation explained	50.86	14.38	11.71
Latency of response (min)	**-0.37**	0.05	**0.36**
Closest distance to speaker (m)	**-0.36**	0.29	-0.16
Time spent less than 5 m (min)	**0.36**	**-0.31**	0.30
Latency of song (min)	**-0.38**	-0.05	**0.37**
Number of minutes singing response (min)	**0.42**	0.24	-0.08
Number of songs	**0.38**	**0.37**	-0.04
Number of calls	0.10	**-0.49**	**-0.49**
Number of duets	0.21	**0.54**	0.21
Number of flybys	0.29	**-0.30**	**0.57**

Bold denotes variables with factor loading > 0.3 (McGarigal et al. 2000).

Table S2. Comparison of models. The AIC score with the lowest value represents the model with the best fit.

			T. schistaceus		***P. chloris***	
Model	**Description**	**Parameters**	**AIC**	**LogLik**	**AIC**	**LogLik**
1	Linear	1	-18.18	12.09	-5.68	5.84
2	Fixed-effects	2	-18.18	12.09	-7.44	6.72
3	Mixed-effects, c	2	-14.26	12.13	-4.78	7.39
4	Mixed-effects, d	2	-14.45	12.23	-4.2	7.1
5	Fixed-effects	3	-19.15	13.58	-7.98	7.99
6	Mixed-effects, c	3	-15.42	13.7	Failed to converge	
7	Mixed-effects, d	3	-15.59	13.8	-5.85	8.92

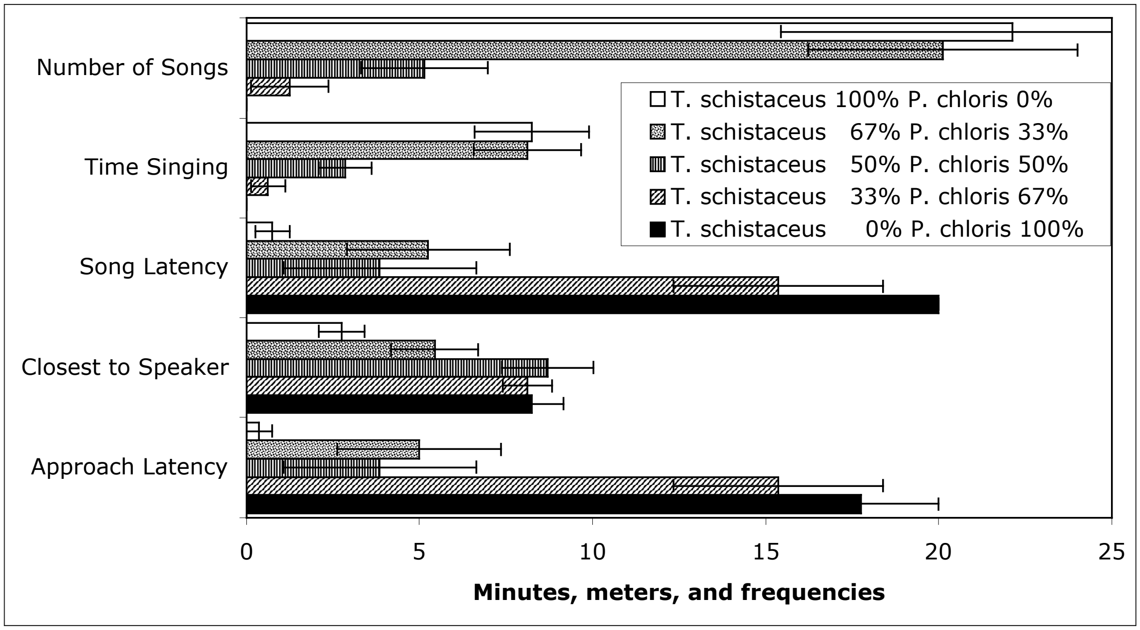
FIGURE LEGEND

Figure S1. Mean responses (±SE) of (A) *Thamnophilus schistaceus* and (B) *Piprites chloris* to different morphs. See text for description of measures.

Figure S2. Logistic regression of behavioral responses by (A) *Thamnophilus schistaceus* and (B) *Piprites chloris*, y-axis, to each morph, x-axis. 1.0 is 100% of a species' song and 0.0 is 100% of the acoustic nearest-neighbor's song. Light gray lines represent individual responses.

Figure S3. Means and 2-standard-deviation ellipses for morphs of songs in (A) signal space and in response (or perceptual) space for (B) *Thamnophilus schistaceus* and (C) *Piprites chloris*. The strongest response on PC1 in (B) is to 100% *T. schistaceus*, whereas the strongest response in (C) is to *P. chloris*.

Figure S1
(A)


Number of Songs
Time Singing
Song Latency
Closest to Speaker
Approach Latency
☐ T. schistaceus 100% P. chloris 0%
▨ T. schistaceus 67% P. chloris 33%
▥ T. schistaceus 50% P. chloris 50%
▨ T. schistaceus 33% P. chloris 67%
■ T. schistaceus 0% P. chloris 100%
0
5
10
15
20
25
Minutes, meters, and frequencies

(B)

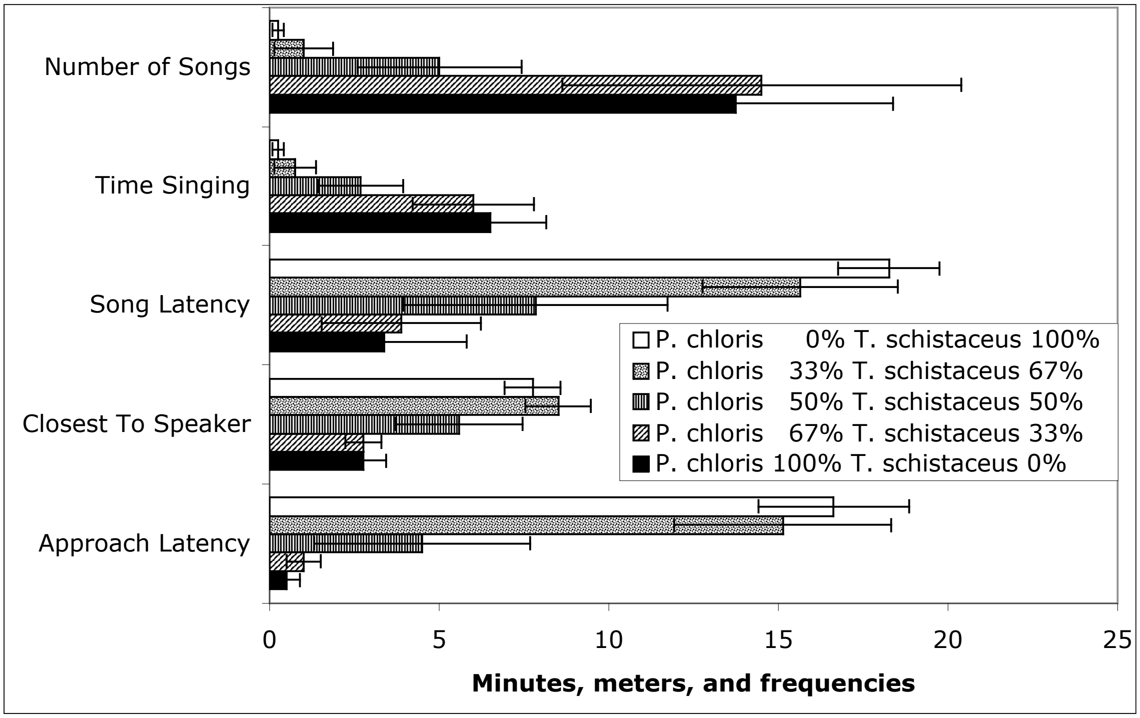

Number of Songs
Time Singing
Song Latency
Closest To Speaker
Approach Latency
☐ P. chloris 0% T. schistaceus 100%
▨ P. chloris 33% T. schistaceus 67%
▥ P. chloris 50% T. schistaceus 50%
▨ P. chloris 67% T. schistaceus 33%
■ P. chloris 100% T. schistaceus 0%
0
5
10
15
20
25
Minutes, meters, and frequencies

Figure S2

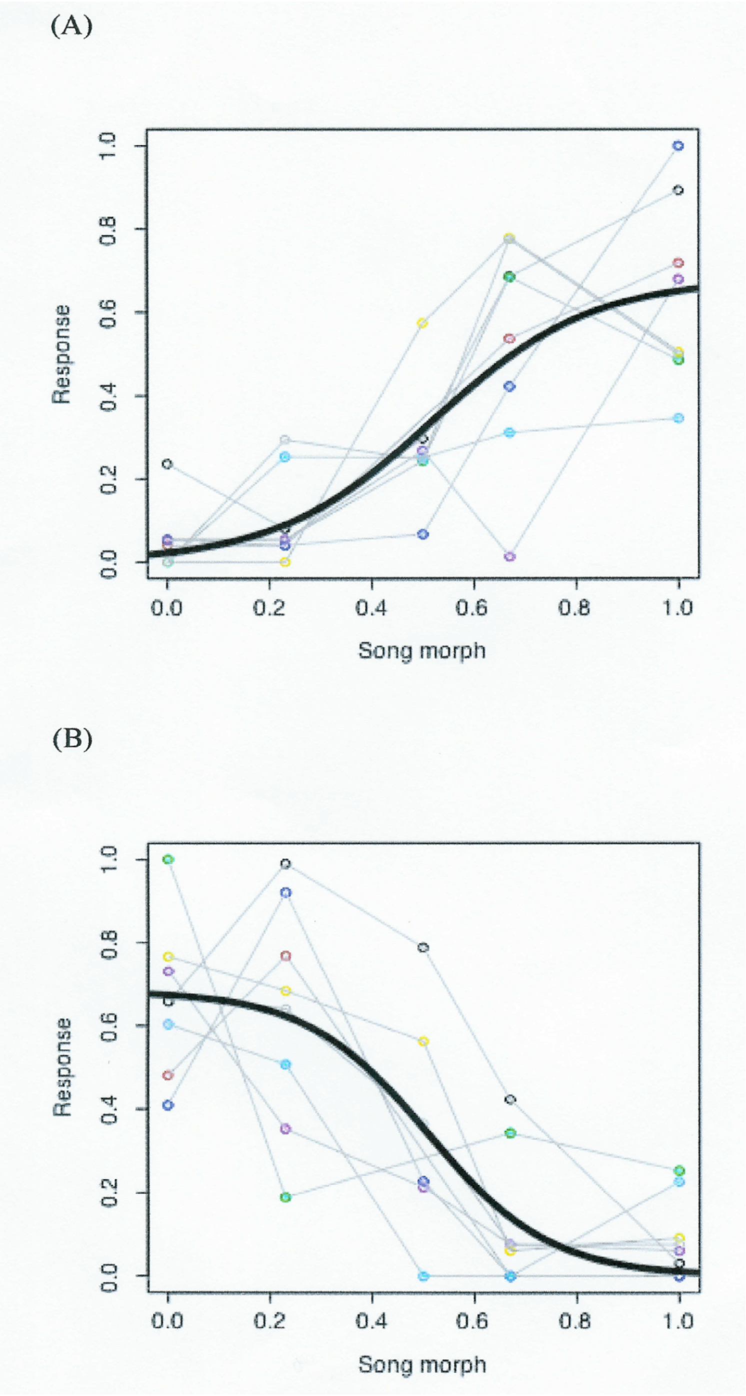
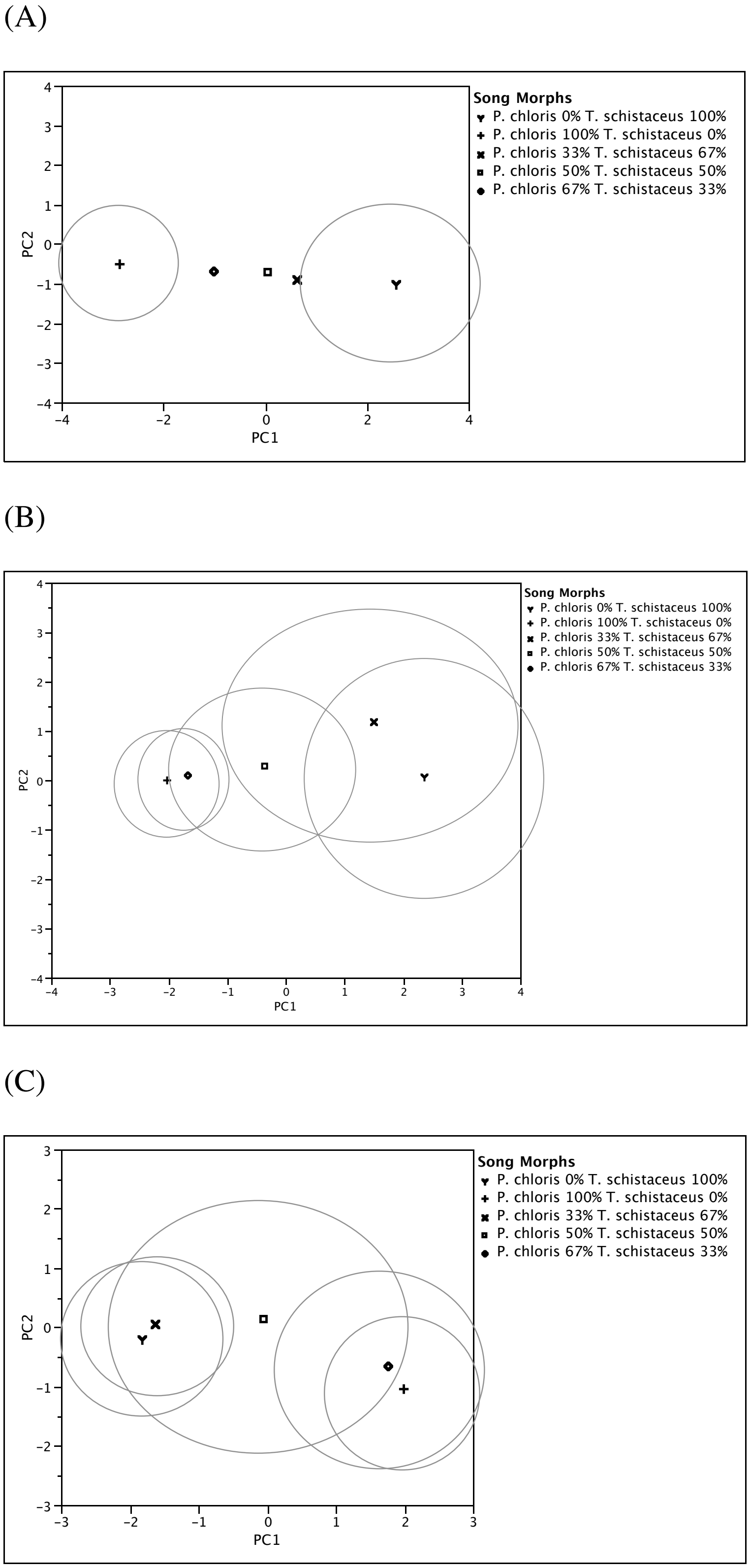

(A)
Response
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
1.0
Song morph
(B)
Response
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
1.0
Song morph

Figure S3


(A)
Song Morphs
P. chloris 0% T. schistaceus 100%
P. chloris 100% T. schistaceus 0%
P. chloris 33% T. schistaceus 67%
P. chloris 50% T. schistaceus 50%
P. chloris 67% T. schistaceus 33%
PC1
PC2
−4
−2
0
2
4
−4
−3
−2
−1
0
1
2
3
4
(B)
Song Morphs
P. chloris 0% T. schistaceus 100%
P. chloris 100% T. schistaceus 0%
P. chloris 33% T. schistaceus 67%
P. chloris 50% T. schistaceus 50%
P. chloris 67% T. schistaceus 33%
PC1
PC2
−4
−3
−2
−1
0
1
2
3
4
(C)
Song Morphs
P. chloris 0% T. schistaceus 100%
P. chloris 100% T. schistaceus 0%
P. chloris 33% T. schistaceus 67%
P. chloris 50% T. schistaceus 50%
P. chloris 67% T. schistaceus 33%
PC1
PC2
−3
−2
−1
0
1
2
3