



Song-based species discrimination in a rapid Neotropical radiation of grassland seedeaters

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Acoustic signals among newly diverged taxa have the potential to convey species identity, information that is key to reducing hybridization. Capuchino seedeaters constitute a remarkable example of recently radiated endemic species from the grasslands of South America. They are sexually dimorphic and show striking differences in male plumage coloration and song. Contrasting with this divergence in phenotype most species show extremely low neutral genetic differentiation and lack of reciprocal monophyly, which is interpreted to be a product of recent common ancestry and hybridization. Here we use field-based playback experiments to test for the first time if males of two species, *Sporophila hypoxantha* and *S. palustris*, discriminate between conspecific and heterospecific song. Using various measures of behavior we find that both species react more strongly to their own songs. The response to playback from another southern capuchino cannot be differentiated from that of a control song from a more distantly related *Sporophila* species. Additionally, we did not find evidence for reinforcement as the response of *S. hypoxantha* did not differ between individuals that co-occur with *S. palustris* and those that do not. Our finding suggests that song, a culturally inherited trait, may help maintain reproductive isolation between species in the rapid and explosive capuchino radiation.

Acoustic signals are central to avian evolution, constituting key traits involved in mate recognition and thus serving as a prezygotic barrier to gene flow between species (Catchpole and Slater 1995, Price 2008). In many oscine passerines (and in a few other avian taxa), vocalizations can undergo rapid cultural evolution (Lynch 1996, Payne 1996, Price 2008), a process with profound implications for speciation (Martens 1996, Grant and Grant 1997). Most research in bird acoustics has focused on male song as it is often used in species-specific interactions (Marler 1975, Kroodsma and Miller 1996), such as territorial defense and mate attraction (Becker 1982, Searcy and Andersson 1986, Falls 1992, Catchpole and Slater 1995). The effect of conspecific song on male territorial behavior has been tested in a large number of studies using playback (Becker 1982), male muting experiments (Peck 1972), and by removing males and substituting them with a speaker (Krebs 1977, Krebs et al. 1978). In the early stages of speciation, species-specific song recognition is essential to allow animals to effectively communicate with conspecifics, establish social links, and reduce or avoid hybridization. Recently diverged taxa have been found to show vocal discrimination (Ratcliffe and Grant 1985, Haavie et al. 2004, Greig and Webster 2013), suggesting that a culturally inherited trait such as bird song can potentially increase assortative mating.

Sporophila seedeaters are among the most species-rich genera of the Neotropics, containing numerous pairs or

monophyletic groups of recently diverged species showing elaborate male vocalizations (Meyer de Schauensee 1952, Ridgely and Tudor 1989, Ouellet 1992, Rising et al. 2011). The most striking example is an assemblage of 11 species, lacking a name in English but commonly known as capuchinos or caboclinhos in Spanish and Portuguese, respectively (Rising et al. 2011). Capuchinos are sexually dimorphic, with similar brown and olive females and males showing distinct reproductive plumage patterns based on the color cinnamon (Benites et al. 2010, Campagna et al. 2012). These taxa are diagnosed through male song and reproductive color patterns since they are otherwise extremely similar in size and shape; capuchinos can also show eclipse plumage and gather in mixed flocks when not breeding (Rising et al. 2011). Spectrophotometric analysis of female plumage has identified previously undetected differences among some capuchino females in the UV-portion of the spectrum (Benites et al. 2010). Eight of the capuchinos appear to have radiated rapidly during the Pleistocene and are predominantly sympatric and often syntopic, endemic species to central and southern South America (hereafter southern capuchinos, Lijtmaer et al. 2004, Campagna et al. 2010, 2012, 2013). Genetic analysis of these eight species (*S. cinnamomea*, *S. hypochroma*, *S. hypoxantha*, *S. melanogaster*, *S. nigrorufa*, *S. pileata*, *S. palustris*, and *S. ruficollis*) revealed extremely low neutral genetic differentiation and lack of species level monophyly as a consequence of both

recent common ancestry and ongoing hybridization and introgression (Lijtmaer et al. 2004, Campagna et al. 2010, 2012, 2013). Despite the marked phenotypic differences that exist among southern capuchinos, their phylogenetic affinities remain unresolved.

The taxonomy of the southern capuchinos is based on male plumage coloration and song, and to a lesser extent on geographic distribution and habitat use (Meyer de Schauensee 1952, Ridgely and Tudor 1989, Rising et al. 2011). Recent detailed analyses of these traits have led to the discovery of alternative color morphs within some species (Repenning et al. 2010, Areta and Repenning 2011, Areta et al. 2011), the merger of previously recognized taxa (Areta 2008), and helped identify the sister species to the core southern capuchino radiation (Machado and Silveira 2010, 2011, Campagna et al. 2013). The southern capuchino species that are currently recognized show statistically significant differences in their songs and plumage attributes, which are maintained in sympatry (Benites et al. 2010, Campagna et al. 2012). Nevertheless, the biological relevance of these differences remains untested.

To this end, we conducted a field-based playback experiment on *S. hypoxantha* and *S. palustris* males to test for the first time whether southern capuchinos can discriminate conspecific songs from those of other species in the group. We measured the behavioral response of focal males to different song stimuli with the objective of understanding if they recognize other species as potential rivals that have intruded in their territories. We find that males of both species show a stronger response to conspecific song than to that of other southern capuchino species. This result suggests that acoustic signals may help maintain reproductive isolation in the rapid and explosive southern capuchino radiation.

Material and methods

Field sites and study species

The study was conducted in two Argentine localities with stable breeding populations of southern capuchinos: the Iberá marshes near Colonia Carlos Pellegrini, province of Corrientes (28°38.52'S, 57°17.98'W, hereafter CCP), and El Bagual nature reserve, province of Formosa (26°10'S,

58°56'W, hereafter EB). These localities differ in the number of breeding southern capuchino species. While six of the eight southern capuchinos (*S. cinnamomea*, *S. hypochroma*, *S. hypoxantha*, *S. palustris*, *S. pileata* and *S. ruficollis*) are common in CCP (Di Giacomo 2005a), *S. hypoxantha* is the only species of the group that breeds in EB (Di Giacomo 2005b). We conducted playback experiments on territorial *S. hypoxantha* and *S. palustris* males in CCP during November 2010, and on *S. hypoxantha* in EB during the same breeding season in March 2011. *Sporophila hypoxantha* and *S. palustris* were chosen because of their high relative abundance (compared to other southern capuchinos) in CCP. The objective of this comparison was to test if the response of *S. hypoxantha* to playback differed between localities, given that this species breeds alone in EB and in the presence of other southern capuchinos in CCP (and therefore is exposed regularly to *S. palustris* song). Male southern capuchinos typically present complex songs, composed of various non-repetitive notes, which they start singing from perches at the beginning of the breeding season after establishing territories (Areta 2008). Males selected for playback experiments were actively singing and displaying territorial behavior. The predominant habitat in CCP and EB consists of open wet grasslands with marshes, where the vegetation is generally below 1.5 m in height.

Experimental design

We quantified the behavioral response to playback of focal adult territorial males exposed to three song treatments: homospecific song (OWN); song from a heterospecific southern capuchino (CAPUCHINO); and song from a congeneric, non-southern capuchino species (the rusty-collared seedeater *Sporophila collaris* – RUSTY). See Supplementary material Appendix 1, Fig. A1 for representative sonograms from each species' song. The OWN and CAPUCHINO stimuli used in the experiments depended on the species identity of the focal individual. If *S. hypoxantha* was the focal species, OWN was a local *S. hypoxantha* song and CAPUCHINO was a song from *S. palustris*, and vice versa. We used *Sporophila collaris* songs as a control since this species is closely related to *S. hypoxantha* and *S. palustris* but not a member of the southern capuchino radiation (Lijtmaer et al. 2004, Campagna et al. 2010, 2012, 2013, Mason and

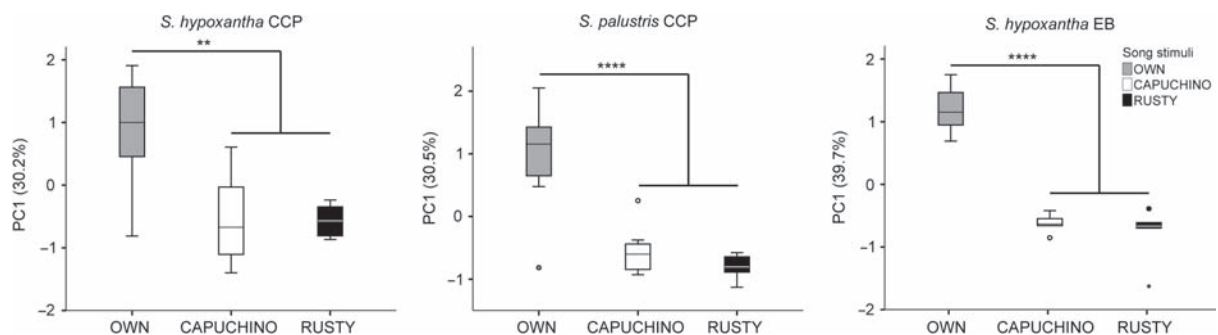


Figure 1. Results from PCA analysis of the response of *S. hypoxantha* (Colonia Carlos Pellegrini, CCP; and El Bagual, EB) and *S. palustris* (CCP) males to playback with different song stimuli. Statistical differences in PC1 scores among treatments were tested using a one-way ANOVA and subsequent Scheffé tests. The percentage of variance explained by PC1 is shown in parenthesis on the y-axis. Significant p values denoted with asterisks as follows: ** = $p < 0.01$; **** = $p < 0.001$.

Burns 2013), and because it is also sympatric with the focal southern capuchinos and commonly found across the study area. The three *Sporophila* species included in this study have similar ecological requirements (Di Giacomo 2005a, b, Vizentin-Bugoni et al. 2013) and were found in the same type of open grassland habitat in our study. To avoid testing for the effect of song from a given individual in playback experiments (Kroodsma 1990), the three treatment categories included five tracks each, recorded from different individuals in the same locality where the experiment was conducted. All tracks were obtained from previous seasons (between one and four years prior to the experiment) and/or from areas where we did not perform playback tests, thus it is likely that these recordings belonged to individuals unfamiliar to the focal birds. *Sporophila hypoxantha* individuals from CCP or EB were tested with different (local) tracks. These songs were selected for their high recording quality, presenting low background noise and no interference from vocalizations of other birds. The songs used in this study are archived at the Museo Argentino de Ciencias Naturales (MACN), the Macaulay Library of Natural Sounds (Cornell Lab of Ornithology), and the sound archive of the British Library. Test tracks were assembled using Raven Pro 1.4 into three minute stimuli with similar rates of song delivery. Test songs were broadcasted through a Sanyo CD player and amplified with a Pioneer GM-X302 onto a JBL loudspeaker.

The speaker was placed in the territory of a focal individual, within sight of the bird (generally around 10–15 m). Both the operator of the playback equipment and an observer stood at more than 20 m from the speaker. A small number of individuals did not respond to playback because they were outside their territory or abandoned the area during the experiment and could not be followed; those trials were not included in the study (3 out of 24 *S. hypoxantha* and 2 out of 24 *S. palustris* for CCP; 2 out of 21 *S. hypoxantha* for EB). Each male was presented with only one song treatment; test tracks within treatments were randomly selected. Because males were not individually banded, experiments were conducted at distances larger than 250 m from each other, which was sufficient to minimize the probability of testing the same individual twice. Playback experiments lasted nine minutes and were divided into three 3-min stages: before stimulus presentation (PrePB), stimulus presentation (PB) and post-stimulus (PostPB). We recorded the behavior of focal individuals during each period of the experiment by: 1) counting the number of songs they produced; 2) counting the number of flights they performed; and, 3) recording the proportion of time spent at a given distance to the speaker. This was done by visually estimating the distance between the individual and the speaker at 15-s intervals and assigning it to one of three distance categories: 0 to 3 m ($d < 3$ m), between 3 and 6 m ($3 \text{ m} < d < 6 \text{ m}$), more than 6 m ($d > 6 \text{ m}$).

Statistical procedure

We derived five variables from the data recorded during each stage of the playback experiments: number of flights, number of songs, proportion of time spent at $d < 3$ m, proportion of time spent between 3 and 6 m, and proportion of time spent at $d > 6$ m. These data were analyzed in two ways: 1) with a principal component analysis (PCA) that included the five

aforementioned variables in each experimental stage, and a subsequent one-way ANOVA and Scheffé tests to evaluate differences in principal component (PC) scores across song treatments. We performed independent PCA analyses (total of three) for each species/locality combination; and 2) using a general linear model (GLM) with repeated measurements (experimental stages PrePB, PB and PostPB) to evaluate the response of subjects *S. hypoxantha* or *S. palustris* to different effects (song stimuli OWN, CAPUCHINO and RUSTY). Sequential Bonferroni corrections (Holm 1979) were applied to results from the GLM tests. When statistically significant results were found, we employed Scheffé contrasts to compare among song stimuli within experimental stage or among experimental stages for a given stimulus. These analyses account for the effect of variation between individuals, experimental stages and song stimuli. Finally, we also recorded the number and species of non-focal males that modified their behavior and/or were attracted during the PB stage of the experiment; the playback stimulus that attracted these individuals was also registered. The distance to which non-focal individuals approached and investigated the speaker and the time spent exploring it varied among individuals. We searched for differences in the number of individuals attracted by a homospecific or a heterospecific song through a binomial test, considering as a null hypothesis that an individual will approach with one or the other stimuli with the same probability ($p = 0.5$) in a total of 25 trials in which non-focal individuals responded to playback.

Results

A total of 43 playback experiments were conducted in CCP: 21 corresponded to *S. hypoxantha* (eight individuals were presented with OWN, seven with CAPUCHINO, and six with RUSTY); and 22 were performed on *S. palustris* (nine individuals were presented with OWN, seven with CAPUCHINO, and six with RUSTY). Both the analysis of PC scores among treatments and the analysis of individual variables using a GLM capture behavioral differences in the response to OWN compared to CAPUCHINO and RUSTY.

The first principal component explains $\approx 30\%$ of the variance in the data for the responses of either *S. hypoxantha* or *S. palustris* (Fig. 1). In both cases, PC1 shows the highest positive correlation with the number of flights during the PB stage and $d < 3$ m during PB and PostPB, and conversely high negative correlation with $d > 6$ m during PB and PostPB (Table 1). For both species there were statistically significant differences in the PC1 scores among song treatments ($p < 0.005$), and we did not find statistically significant differences in PC2 or PC3 ($p > 0.3$). PC1 scores differed between OWN and CAPUCHINO or RUSTY (Fig. 1; $p < 0.01$ for *S. hypoxantha* and $p < 0.001$ for *S. palustris* for all comparisons) but not between CAPUCHINO and RUSTY ($p > 0.7$ for every comparison).

The GLM analysis shows overall statistically significant differences in the response of both *S. hypoxantha* and *S. palustris* males to the three playback stimuli in variables related to the number of flights and distance to the speaker but not in the number of songs (Table 2). We did not find statistically

Table 1. Loading scores for the first principal component (PC1). Loading scores for the different response variables on the PC1. Only response variables with high loading values are shown for *S. hypoxantha* and *S. palustris* from Colonia Carlos Pellegrini (CCP), and *S. hypoxantha* from El Bagual (EB). A loading value of 0.7 or larger is considered a high correlation (Tabachnick and Fidell 2001).

	<i>S. hypoxantha</i> CCP PC1	<i>S. palustris</i> CCP PC1	<i>S. hypoxantha</i> EB PC1
Flights PB	0.83	0.84	0.8
Flights PostPB	0.52	0.47	0.75
d < 3 m PB	0.72	0.74	0.77
3 m < d < 6 m PB	0.48	0.71	0.8
d > 6 m PB	-0.87	-0.94	-0.96
d < 3 m PostPB	0.81	0.72	0.83
d > 6 m PostPB	-0.85	-0.83	-0.95

significant differences among the three song treatments in the PrePB stage for any variable. While differences in the response variables during the PB stage of the experiment between treatments CAPUCHINO and RUSTY were not statistically significant ($p > 0.3$ for every comparison), the converse was true between either of these two treatments and OWN (PB stage, results are summarized in Fig. 2 and Fig. 3, see Supplementary material Appendix 1, Fig. A2 and Fig. A3 for more detail). The behavioral response elicited with OWN during PB (compared to either that of CAPUCHINO or RUSTY) included more flights (for *S. hypoxantha* and for *S. palustris*, $p < 0.005$ for all comparisons), a larger proportion of time spent at short distances from the speaker ($d < 3$ m, $p < 0.01$ for *S. hypoxantha*; $3 \text{ m} < d < 6 \text{ m}$, $p < 0.05$ for *S. palustris*), and an associated shorter time spent at larger distances from the speaker ($d > 6$ m, $p < 0.01$ for *S. hypoxantha* and $p < 0.001$ for *S. palustris*). In the PostPB stage, individuals exposed to OWN remained closer to the speaker than those presented with CAPUCHINO or RUSTY (longer time spent at $d < 3$ m and shorter time spent at $d > 6$ m, $p < 0.05$ for every comparison for *S. hypoxantha*; shorter time spent at $d > 6$ m, $p < 0.001$ for every comparison for *S. palustris*). When comparing between experimental stages within a treatment, individuals presented with OWN increased the number of flights between the PrePB and PB stages and decreased them between PB and postPB ($p < 0.005$ in both cases for *S. hypoxantha*; $p < 0.01$ in

both cases for *S. palustris*). Focal individuals also tended to approach the speaker and spend more time closer to it when comparing the PB to the PrePB stage ($d < 3$ m, $p < 0.001$ and conversely $d > 6$ m, $p < 0.001$ for *S. hypoxantha*; $3 \text{ m} < d < 6 \text{ m}$, $p < 0.005$ and conversely $d > 6 \text{ m}$, $p < 0.001$ for *S. palustris*), and remained close to the speaker when the PB stage was over (PB/PostPB comparison $p > 0.42$ for every distance category, for both species). No statistically significant differences were found for response to CAPUCHINO and RUSTY between experimental stages ($p > 0.2$ for every response variable in both species). On several occasions during the PB stage of the experiment non-focal individuals approached the speaker (25 individuals in 43 experiments); a significantly larger proportion of these were of the same species as the song stimuli presented in the experiment ($p < 0.01$; 95% CI of the proportion of non-focal individuals that matched the species of the playback stimulus = 0.59–0.93).

A total of 19 experiments were conducted on *S. hypoxantha* males in EB (seven individuals were presented with OWN, six with CAPUCHINO, and six with RUSTY). Again, the analysis of PCA scores among treatments and the analysis of individual variables using GLMs show that focal birds respond differently to OWN than to the other two stimuli.

PC1 explained $\approx 40\%$ of the variance in the data (Fig. 1) and was highly positively correlated with flights during PB and PostPB, short distances to the speaker during those same experimental stages, and conversely highly negatively correlated with larger distances to the speaker (Table 1). There were statistically significant differences among song treatments ($p < 0.001$) in PC1 scores but not in scores from PC2 and PC3 ($p > 0.4$). PC1 scores differed between OWN and both CAPUCHINO and RUSTY (Fig. 1, $p < 0.001$) but not between the latter song stimuli ($p > 0.7$).

The GLM analysis found statistically significant differences in all variables except number of songs among treatments (Table 2). *Sporophila hypoxantha* males in EB behaved in a similar way to those conspecifics from CCP (results are summarized in Fig. 4, see Supplementary material Appendix 1, Fig. A2 and Fig. A3 for details): while responses to CAPUCHINO and RUSTY did not differ ($p > 0.3$ for every variable), there were marked differences between OWN and both aforementioned stimuli. The effect of OWN differed

Table 2. Statistical analyses of playback experiments. Differences in the response of *S. hypoxantha* and *S. palustris* to playback stimuli and experimental stages. Statistics correspond to a repeated measurements GLM test for variables Flights, Songs, and proportion of time spent at three distance categories from the speaker ($d < 3$ m, $3 \text{ m} < d < 6 \text{ m}$, $6 \text{ m} < d$). Significant p values after sequential Bonferroni correction are denoted with an asterisk.

Focal individual species Locality GLM	<i>S. hypoxantha</i> Colonia Carlos Pellegrini		<i>S. palustris</i> Colonia Carlos Pellegrini		<i>S. hypoxantha</i> El Bagual	
	F ^a	p	F ^b	p	F ^c	p
Flights	9.24	<0.001*	6.67	<0.001*	5.36	<0.005*
Songs	0.91	0.469	0.95	0.446	1.62	0.192
d < 3 m	5.29	<0.005*	2.85	0.04	6.62	<0.001*
6 m < d < 3 m	0.79	0.550	3.78	<0.01*	4.98	<0.005*
6 m < d	5.26	<0.005*	9.62	<0.001*	341.95	<0.001*

Degrees of freedom: ^a = 2, 18; ^b = 2, 19; ^c = 2, 16.

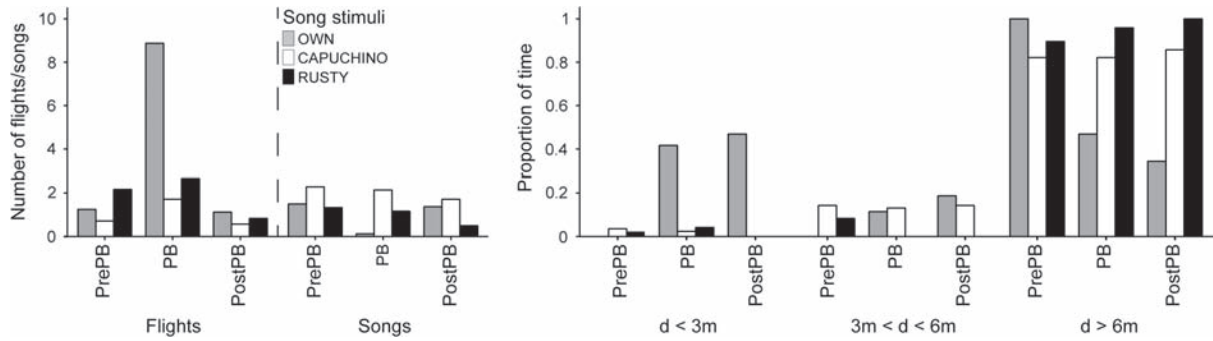


Figure 2. Response of *S. hypoxantha* males to playback with different song stimuli in Colonia Carlos Pellegrini (CCP). The left panel shows response variables Flights and Song, and the right panel shows response variables related to the distance to speaker, in PrePlayback (PrePB), Playback (PB) and PostPlayback (PostPB) experimental stages. OWN: song of *S. hypoxantha*; CAPUCHINO: song of *S. palustris*; RUSTY: song of *S. collaris*.

from CAPUCHINO or RUSTY by eliciting: a larger number of flights in the PB stage ($p < 0.01$ for both comparisons); a larger proportion of time spent at short distances from the speaker ($d < 3$ m, $p < 0.005$; 3 m $< d < 6$ m, $p < 0.001$) and an associated shorter proportion of time spent at distances larger than 6 m from the speaker ($p < 0.001$). During the PostPB stage, individuals exposed to OWN performed more flights ($p < 0.05$ in both cases) and remained closer to the speaker ($d < 3$ m, $p < 0.005$; 3 m $< d < 6$ m, $p < 0.05$) than individuals presented with either category of heterospecific song. Individuals presented with OWN also spent a shorter proportion of time at distances larger than 6 m from the speaker during PB and postPB in comparison to those exposed to heterospecific song ($p < 0.001$ for all comparisons). When comparing between stages within treatments, individuals exposed with OWN stimulus increased the number of flights between PrePB and PB experimental stages, and decreased them between PB and PostPB experimental stages. Also, they tended to approach the speaker from PrePB to PB (Fig. 4; $d < 3$ m and 3 m $< d < 6$ m, $p < 0.001$ in every case), and stayed close after PB stage was over (PB/PostPB comparison $p > 0.58$ for every distance category). No statistically significant differences between stages were found for any response measurement for individuals exposed to CAPUCHINO and RUSTY ($p > 0.3$ in every case).

Finally, we did not find statistically significant differences in the response to *S. palustris* song, during the PB stage, between populations of *S. hypoxantha* from CCP and EB for any of the response variables (two tailed t-test t_{11} , $p > 0.2$).

Discussion

Our results from playback experiments show that despite their recent origin inferred from extremely low neutral genetic variation, two sympatric southern capuchino species can discriminate between homospecific and heterospecific song. Overall, song from the same species was a stronger stimulus to attract focal individuals to the source of playback, which they investigated by conducting sequential flights towards the speaker until reaching a minimum distance. We did not observe differences in the response to a heterospecific capuchino song compared to that of a more distant *Sporophila* species, *S. collaris*. *Sporophila hypoxantha* and *S. palustris* songs are similar in overall structure (Supplementary material Appendix 1, Fig. A1 for examples), but show differences mainly in their syllable composition. The results from our playback experiments suggest that these differences are sufficient to convey species identity. Although southern capuchinos are recently diverged and there is little overall morphological differences among them, song based differentiation among males may be sufficient for species discrimination. Proximity to the sound source is considered an indirect measurement of probability of attack (Krebs et al. 1981, Vehrencamp 2001), so even though we did not directly measure attacks by the focal male, we consider that in this case proximity to the speaker indicates an aggressive response. Birds that were attracted to the speaker and actively inspecting it would not sing; although we observe a decreasing trend in the number of songs produced by the

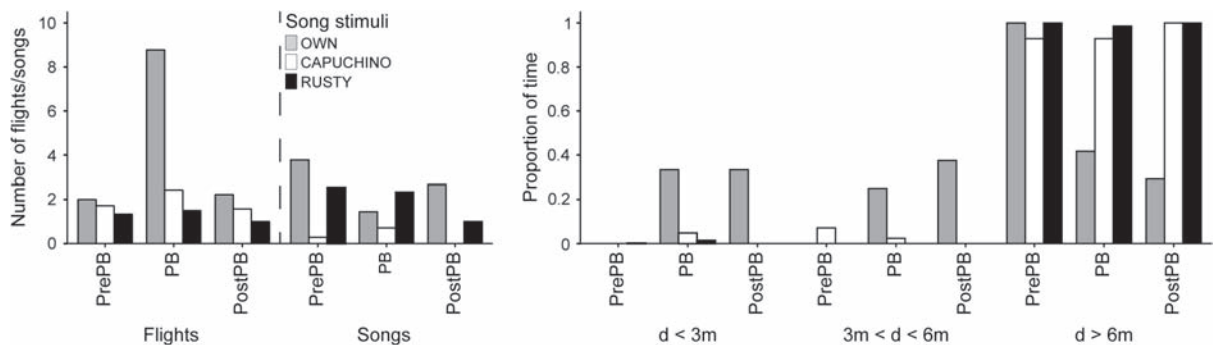


Figure 3. Response of males *S. palustris* to playback with different song stimuli in Colonia Carlos Pellegrini (CCP). OWN: song of *S. palustris*; CAPUCHINO: song of *S. hypoxantha*; RUSTY: song of *S. collaris*. Details as in Fig. 2.

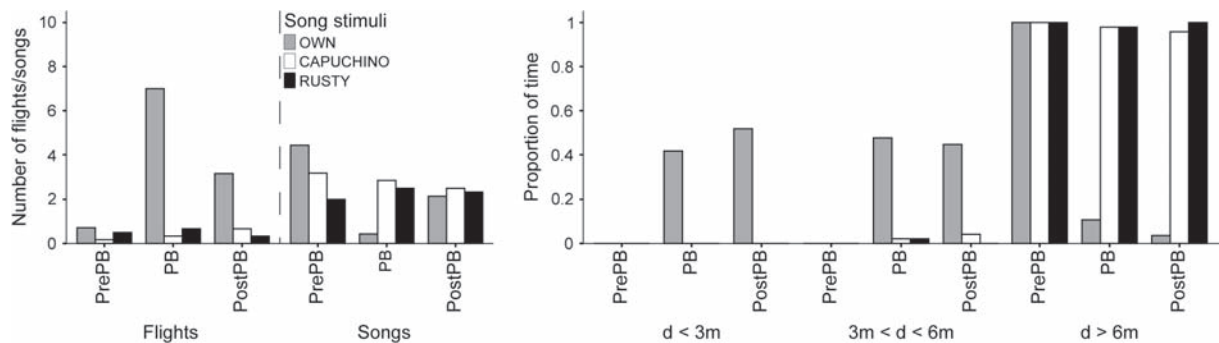


Figure 4. Response of *S. hypoxantha* males to playback with different song stimuli in El Bagual (EB). OWN: song of *S. hypoxantha*; CAPUCHINO: song of *S. palustris*; RUSTY: song of *S. collaris*. Details as in Fig. 2.

focal individual throughout the playback experiment, these differences were not statistically significant. Thus, it is possible that this variable does not accurately capture the aggressive response of males during the experiment, or that the differences are too subtle to be detected with our experimental design and sample sizes (i.e. we have insufficient statistical power). Below we discuss our findings in the context of the evolution of the southern capuchino radiation.

Implications for assortative mating

Our study suggests that males are able to discriminate between intruders from their own species and other southern capuchinos using song, and respond more strongly to the former. It remains to be tested whether these results only apply to *S. hypoxantha* and *S. palustris* or can be extended to other southern capuchino species with different degrees of overall range overlap. Establishing and defending a territory is essential for attracting mates; our results suggest that heterospecific song probably constitutes a less effective territorial and thus, possibly less female-attracting stimulus. Factors influencing territory defense might include pair formation and maintenance of the pair, female preferences for nesting sites, and securing ecological resources. Extra pair fertilizations and extra pair paternity have been observed to be high at least in *S. hypoxantha* (Facchinetti 2012); thus, in defending a territory, it is possible that males are protecting loss of paternity to other males, suggesting that territorial defense is under strong intrasexual selection.

Several studies have shown that traits used by males to recognize competitors are also used by females when choosing mates (Baker and Baker 1990, Baker 1991, Searcy et al. 1997, Patten et al. 2004). Moreover, females have been shown to have superior discrimination to conspecific song than males (Brenowitz 1982, Searcy and Brenowitz 1988). Thus, southern capuchino females may use taxon specific song cues to recognize species and choose among potential mates. Song, as in other bird species, could contribute to reproductive isolation by acting as a premating behavioral barrier to hybridization (Irwin et al. 2001, Grant and Grant 2002a, b, Patten et al. 2004, Seddon and Tobias 2007). Future laboratory controlled experiments on female choice are needed to provide a better understanding of the role of song in species discrimination and its possible function as a premating isolation barrier in the southern capuchinos. A female choice experiment has been conducted using

S. hypoxantha females and conspecific males (Facchinetti and Reboresda 2013), however the relative preference of females for homospecific over heterospecific males remains to be tested.

Comparison among different localities

We conducted playback experiments in two different localities (CCP and EB), each with different numbers of breeding southern capuchino species, to compare the aggressive response of *S. hypoxantha* individuals that had previously been exposed to *S. palustris* song (CCP) to those that had not (EB). Although both *S. hypoxantha* populations were tested four months apart, the experiments were conducted during the breeding season when males were actively defending territories (*S. hypoxantha* has an extended breeding season from November to late March in EB; Di Giacomo 2005b). In the collared and pied flycatchers *Ficedula albicollis* and *F. hypoleuca* sympatric individuals show higher divergence in male plumage and color differences than those that are allopatric, a pattern driven by female choice and interpreted as evidence for reinforcement (Sætre et al. 1997, Haavie et al. 2004). In this system, hybridization has a fitness cost (Sætre et al. 1997) and reinforcement reduces the frequency of hybridization. If a fitness cost to hybridization between *S. hypoxantha* and *S. palustris* exists, selection would favor vocal discrimination when *S. hypoxantha* and *S. palustris* males coexist in the same locality. Our results show that *S. hypoxantha* males did not respond strongly to *S. palustris* song, regardless of whether the latter species was present in the locality where the experiment was conducted (CCP vs EB). Thus in our study we do not find evidence for reinforcement between these southern capuchino species.

A role for coloration patterns

Birds often use a suite of signals, like complex songs and colorful plumages, of different sensory modalities in conspecific interactions (Ratcliffe and Grant 1983, 1985, Andersson 1994, Rowe 1999). While some signals might promote reproductive isolation, others may not, leading to complex patterns of isolation and introgression (Patten et al. 2004, Price 2008, Uy et al. 2009, Greig and Webster 2013). Song is only one type of divergent sexual signal that can lead to reproductive isolation, and thus, considering the entire phenotype in which this trait occurs is important.

The combined role of coloration patterns and song in species recognition and the generation of reproductive isolation remains to be determined in the southern capuchinos. Playback experiments in the field that combine the presentation of taxidermic mounts with song stimuli will help elucidate the relative role of these two secondary sexual characters in species recognition and its effects on territoriality. Similarly, mate choice experiments involving females in the laboratory can help to directly evaluate the relative effects of coloration patterns and song on assortative mating.

Acknowledgements – We thank Cecilia Kopuchian, Adrián Di Giacomo and Alejandro Di Giacomo for valuable help in the field. We thank Wesley Hochachka and Darren Irwin for valuable feedback on a previous version of the manuscript. Thanks to the Conservation Land Trust and Reserva Privada El Bagual for field logistics, and to the Consejo Nacional de Investigaciones Científicas y Técnicas (PIP grant to PLT) and the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2010-0805 ANPCyT grant to PLT) for funding.

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Supplementary material (Appendix JAV-00447 at <www.avianbiology.org/readers/appendix>). Appendix 1.