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**Full title: Collective singing dynamics in a competition context drive song plasticity**

**Short title: Collective singing dynamics drive song plasticity**

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**Author contributions**:

PA designed research, performed experiments, analyzed data, drafted, edited the manuscript.

SM performed experiments, analyzed data, edited the manuscript.

MG contributed to project conceptualization, edited the manuscript.

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**Abstract**

Animals need to adapt their motor production to challenging social conditions at behaviorally-relevant time scales. Here, we use telemetric recording technology from freely-behaving canaries in natural-like social conditions in which male canaries compete for females. We report that male canaries influence each other's singing during 'duels' characterized by temporal overlaps of their songs, which are often followed by physical aggression. Duels evolve in time and both canaries can lead or follow the other canary's song on a song-to-song basis. Remarkably, overlapping behavior induces singing plasticity: both song length and its variability increase when canaries overlap their songs. Furthermore, song acoustic properties reveal a link between dueling and song similarity. Altogether, results show that canary singing behavior is plastic in social environments.

**Keywords**

birdsong, songbird, social interactions, canary, competition, behavioral coupling, behavioral plasticity.

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## Introduction

During the last decades, the link between neural networks and behavior has been extensively studied in simplified laboratory settings, departing from the complex natural environments in which animals and their brains have evolved (*1*). A key component of natural environments is the social context in which individual behavior takes place. The social context has been shown to influence the production and fine-tuning of learnt motor skills (*2-5*). The interaction of animals through vocal communication, a complex motor skill, embeds them in social networks that are critical to understand individual vocal behavior (*6*). Singing by songbirds is one such motor skill influenced by con-specifics in both developing and adult animals (*7, 8*). In songbird species where only males sing, not only do females listening to singing males influence their singing directly or indirectly (*8, 9*). Singing males do also influence each other (*10, 11*). In fact, singing by songbirds is involved in a plethora of functions, including mate attraction, pair bond formation, parental investment, territorial behavior and signaling of social hierarchy (*6, 12-16*). A number of these functions directly or indirectly involves signaling between males. Although the social context may affect song properties, whether songs change during natural counter-singing interactions of songbirds in social environments remains elusive.

Songs are fine tuned throughout learning until they become stable or 'crystallized'. In 'close-ended' songbirds like zebra finches (*Taeniopygia guttata*), songs crystallize during development. In contrast in seasonal songbirds like canaries (*Serinus canaria*), the song circuits become plastic yearly, and songs crystallize before each breeding season. As a result, songs strongly differ across seasons (*17, 18*). Once song is crystallized, singing typically occurs with increased stereotypy in terms of temporal, spectral, grammatical structure and length (*19, 20*). Whereas most studies have focused on the zebra finch model, whose songs are highly stereotypical in adults, canary songs are, on the contrary, complex and variable in their acoustic properties, length and grammar (*20, 21*), even when stereotypy is increased during the breeding, crystallized season. Canary songs therefore constitute an excellent model to study the impact of social contexts on the plasticity of 'crystallized' songs (*20*).

The ecological, behavioral and evolutionary frameworks in which singing interactions occur in songbirds have been extensively characterized (*6*), making of songbirds an excellent model to study behaviors relevant for the ecology and evolution of species. Singing interactions have been described in a number of species, where the relative timing and acoustic properties of male songs relative to each other can encode aggressive information (*22-25*). Most studies have however used song playbacks, that is, songs played on a loudspeaker and not in contexts in which birds vocally interact. Whereas existing studies have allowed deepening our understanding on how playbacks

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alone are able to evoke behavioral responses, they lack the interactive component between singing birds. The study of directly-interacting birds has been hurdled by the necessity to disambiguate signals from individual birds, particularly when birds overlap their songs. A first attempt to overcome this difficulty consists in the use of microphone arrays (*22*). However, they lack the temporal and acoustic precision to characterize in greater detail singing interactions due to their distance to sound sources. An alternative solution to record individual vocalizations that lacks these caveats is the use of individual microphones mounted on each animal (*26*). This technique, that we opted for here, allows the precise monitoring of individual songs.

In canaries, song playbacks have revealed that female canaries prefer overlapping to overlapped playbacks (*27*). Overlapping another canary's song may be interpreted as a signal of dominance (*27, 28*). Furthermore, female canaries allocate greater yolk amounts in their eggs in response to overlapping songs, highlighting the importance of overlapping behavior for biological fitness (*16*). Because of all these reasons, canaries are a model species for which the behavioural and ecological implications of singing overlaps are best studied. However, whether countersinging occurs in social groups of canaries, what are the relative dynamics of singing and how they impact songs has not been studied due to the technical difficulty to record individual songs in a group. Here, we combine recordings in the field and in the laboratory. In the laboratory, we use microphones mounted on individual canaries. These allow us to record their songs with telemetry with high temporal precision (*26*) in mixed aviaries and characterize the relative singing dynamics of males and their impact on song properties.

## Results

### *Multiple-microphone recordings in the field reveal temporally-overlapping songs*

We first asked whether canaries overlap their songs in their natural environment. For that purpose, we recorded wild canaries during the breeding season in Pico island (Azores, Portugal). Recordings were simultaneously performed with two directional microphones oriented towards different directions in fields where canaries had been located. Song spectrograms revealed canary song temporal overlaps, as shown by songs from different canaries preferentially recorded in one microphone (Fig. 1).

In the same fields where canaries could be recorded, the vocal production from a number of other bird species was also recorded, resulting in a complex and 'noisy' recording of canary songs. Additional frequently recorded species included chaffinches (*Fringilla coelebs ssp. moreletti*), blackbirds (*Turdus merula ssp. azorensis*), green finches (*Carduelis chloris*), blackcaps (*Sylvia atricapilla*) and robins (*Erithacus rubecula*). The vocal production of other bird species also

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happened to overlap canary songs, as can be appreciated in the syllables labeled with a star in Fig. 1, which correspond to non-canary calls and songs.

In order to further characterize the intraspecific singing dynamics of populations of male canaries with improved temporal detail and in better-controlled social environments that exclude inter-specific interactions, we designed a reduced social setting in an aviary kept in breeding conditions with three males and two females, reproducing sex ratios previously reported in the wild (*29*). That is, we reproduced a competition context in which males compete for females during the breeding season.

***Telemetric recordings in natural-like social environments***

We mounted custom-made 'backpack microphones' (*26*) on the back of freely-moving male canaries. This allowed us to record the individual vocal production simultaneously from all males with the help of a telemetric recording system (Fig. 2A,B). Our results show that male canaries influence each other's singing and tend to overlap their songs when they spontaneously interact with each other. Fig. 2C (left) illustrates the songs from a canary aligned to the song onset for all songs of another canary in the same aviary during one day. Fig. 2C (right) shows the corresponding histogram of the times at which songs were recorded centered at song onset from the reference canary. Inspection of the distribution of songs aligned to the onset of the reference canary songs revealed a bimodal distribution. The distribution at negative values in Fig. 2C corresponds to songs in which singing started before the reference canary started singing, whereas the distribution at positive values corresponds to songs occurring after the other canary started singing. In five out of six groups recorded, and in 2 to 3 birds per group, the histogram of the times at which songs were recorded from one bird centered at song onset from another bird showed an increased singing activity relative to the histogram generated by shuffling randomly the onset of songs during the recording period (dashed lines in Fig. 2D). The sixth group ('Group 1', not shown) was excluded from analysis due to the low number of songs that did not allow us to perform statistical analysis on singing interactions.

To quantify whether canaries increase their overall singing activity when they overlap their songs, we examined the relationship between the fraction of daily overlaps and the number of songs sung each day by all recorded birds. The fraction of song overlaps and the number of songs were positively correlated (two-tail linear correlation test, $P = 0.002$, $r = 0.27$), suggesting that canaries that show overlapping behavior sing more frequently.

Finally, in order to confirm the aggressive signaling nature of canaries singing together, we quantified the relationship between singing and physical fights. Singing temporal overlaps were

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followed by fights in 51.4 ± 0.1 % of the cases, whereas individual singing evoked fights in only 13.1 ± 0.1 % of the cases (average from video recordings in n = 3 groups, $P < 0.05$ for each group, Fisher exact test). Thus, song overlapping interactions are aggressive signals predictive of physical fights. We will henceforth call 'duels' the interactions in which canaries influence each other's singing, establishing pairwise singing contests.

To summarize, a social competition context induces song overlapping between male canaries, influencing both the singing activity and the relative timing of singing. Moreover, duels are predictive of aggressive interactions.

***Leader and follower dynamics***

We observed in the five groups overlapping behavior from two male canaries, and occasionally from a third canary. The third canary adopted otherwise a predominant soloist strategy, potentially eventually joining the group. Songs were recorded continuously for days, revealing overlapping behaviors in which both singers could lead or follow on a song-to-song basis. Remarkably, singing dynamics were plastic on a temporal scale of days, as can be appreciated in the three examples depicted in Fig. 3A-C.

The histogram of the times at which songs were recorded centered at song onset from the reference canary are shown in Fig. 3 for several canaries in each group during several days. The two birds from group 3 (Fig. 3A1, B1) start in a configuration in which bird 0 leads the singing duel and bird 2 follows. On the following days, leader/follower roles reconfigure: whereas bird 2 is only acting as a follower during the first days, singing dynamics evolve to mixed leader/follower dynamics in which the two canaries both lead and follow. In group 5 (Fig. 3A2, B2) during the first days, singing activity of bird 5 shows a single mode of singing activity at short latencies (0.2 to 0.8 s.) relative to the reference bird 4 singing onset, mostly corresponding to a leader behavior: most songs start at negative time values before song onset of bird 4 and stop at positive values, after song onset of bird 4. With time, the distribution becomes wider and bimodal. The temporal dynamics of group 6 (Fig. 3A3, B3) show plasticity in the timing of singing activity from bird 4 relative to bird 2: singing from bird 4 shifts from loosely following bird 2 (peak singing at 7.6 s-delay from song onset in bird 4) on day 1 to a tight, 0.0 s-latency between the peak of singing activity of bird 4 relative to the song onset of bird 2 on day 8. Interestingly, shortening of this latency occurred when the third male in the group, bird 5, also started to vocally interact with the two birds that initially overlapped their songs. Remarkably, bird 5 behaves as a leader, reconfiguring the relative singing dynamics in the whole group.

In summary, the relative timing of overlapping singing interactions in a group evolves on a

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time scale of days.

***Leader's, follower's and soloist's songs differ in length***

We next examined the influence of the social singing context on a song-to-song basis. We quantified song length in different overlapping conditions. Songs produced by the two canaries mediating most singing interactions in each group had a significantly longer duration during overlapping interactions than when the same birds sang solo songs (5.66 ± 0.07 s. vs 4.86 ± 0.05 s., $P << 0.001$, mixed model, n = 3661 solo songs, n = 2501 temporally-overlapping songs from n = 10 canaries).

Interestingly, song length differed depending on the relative timing of singing interactions: leader songs were on average longer than solo songs, systematically for all birds. In contrast, follower songs were significantly longer than solo songs in only three out of ten birds (Fig. 4A), revealing a common strategy to sing longer-lasting songs when canaries lead singing interactions, compared to a strategy only taken by some of them to also sing longer songs when they behave as followers. Overall, across birds, leading songs were 51.0 % longer than solo songs (7.46 ± 0.54 s vs. 4.95 ± 0.41 s, P = 0.002, two-tail Wilcoxon Signed-Rank test, n = 10 canaries, Fig. 4B1) and 33.5 % longer than following songs (5.59 ± 0.51 s, P = 0.002, two-tail Wilcoxon Signed-Rank test, n = 10 canaries, Fig. 4B1). Solo songs were not significantly shorter than follower's songs, comparing mean lengths for all birds (P = 0.12, two-tail Wilcoxon Signed-Rank test, n = 10 canaries, Fig. 4B1).

We further examined whether the variability of song length was changing during singing interactions (Fig. 4 B2). Remarkably, the standard deviation of song length also significantly increased by ~27 % for leading songs relative to solo songs (3.6 ± 0.5 s vs. 2.8 ± 0.2 s respectively, P = 0.004, two-tail Wilcoxon Signed-Rank test, n = 10 canaries), but was not significantly different for follower songs (3.2 ± 0.3 s, P = 0.12, two-tail Wilcoxon Signed-Rank test, n = 10 canaries). Thus, canaries sing songs that are more variable in length when they lead singing duels.

In summary, song length and its variability depend on the relative timing in the singing interactions within a duel.

***Dueling canaries sing similar songs***

In order to further investigate the link between songs and singing overlapping behavior, we finally asked whether canaries that tend to duel in each group share specific song properties relative to the 'soloist' canary in the group. We performed principal component analysis (PCA) from the songs of all canaries in the four groups in which we had enough songs from all the canaries to perform this analysis. Performing principal components analysis (PCA) on song features shows a

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separation between the dueling canaries and the soloist canary (Fig. 5).

A representative example of songs in a group is shown in Fig. 5A. The corresponding representation in the three main projection vectors is shown in Fig. 5B, where the song of the three canaries in the group is represented by different colors. It can be appreciated in Fig. 5B that the clouds representing the three first components from the two overlapping canaries are closer to each other than to the soloist. We quantified the average distance between points in this 3D projection for all four groups, finding a significantly smaller distance between song acoustic properties of dueling canaries than between dueling canaries and the predominantly-soloist canary (Fig. 5C) ($0.50 \pm 0.04$ vs. $0.61 \pm 0.07$, $P = 0.008$, Wilcoxon Signed-Rank Test, $n = 8$ paired comparisons, 12 birds). Since the songs for this analysis were solo songs, results suggest that the singing repertoire used by dueling canaries is closer to each other than it is to the soloist's, independently of the singing interaction.

## Discussion.

### *Socially-induced plasticity of crystallized songs*

The first major conclusion of our study is that interactive singing dynamics influence both singing activity and song properties of male canaries. Our results show that the decision of male canaries to sing depends on the decision to sing by other males embedded in a social competition network. Remarkably, song interactions evoke singing plasticity: song length and its variability differ depending on the singing onset relative to the other canary (Fig. 4). This study adds to previous evidence that crystallized songs are plastic (*30*).

Here, we have shown that direct interactions between male canaries through singing induce singing plasticity and they are a good predictor of physical aggression. This type of influence on songs from other males adds therefore to the classically-studied direct influence by females on male song ('directed' songs). Birdsong should therefore be studied in mixed groups of birds containing males and females. Songs from male songbirds in isolated contexts, or from a male housed with a female, as is often done, may bias the type of songs recorded for a specific function, whereas songs in natural contexts involve multiple signaling modes, and complex eavesdropping interplays (*6, 11, 12, 24, 31*). Singing interactions and their impact on songs should in the future be addressed in the field, where functions of songs are likely more diverse than in laboratory settings. Altogether, our results confirm the importance of studying song production and more broadly, behaviors that involve socially-influenced actions and decision making, in their social context.

### *Neurobiological implications*

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Motor behaviors are acquired throughout plastic developmental periods during which pre-motor programs are stored in the connectivity and properties of neural networks (*32*). This is the case of rhythmically-activated neural networks or central pattern generators (CPGs), which retain throughout adulthood the ability to be activated in specific cellular sequences while remaining plastic (*33*), ensuring the temporal control of movements. Our results shed light into the socially-induced plasticity of crystallized songs. Singing has been suggested to be controlled a CPG found in song nucleus HVC (*34*). Whether song timing and duration is encoded in a CPG in HVC or as has been more recently suggested, in a recurrent network across song nuclei in the songbird brain (*35*), the behavioral plasticity of songs found here suggests that premotor networks may undergo a reconfiguration of their activity in social contexts. Neural recordings from canaries in natural social contexts should allow in the future studying the neural basis of how crystallized behaviors reconfigure at fast behavioral time scales in response to challenging social environments. The recording of brain nuclei and in particular of HVC during canary duels will be of great benefit to understand whether and how birdsong premotor networks are reconfigured in social environments.

We find that the relative timing of singing influences song properties in a song-to-song basis, suggesting that environmental cues feed into the motor commands controlling singing. This feedback occurs as fast as ~ 100s of ms time at which the latency of maximal singing activity of one canary aligns to the singing onset in a dueling canary (Fig. 3). Interestingly, it is known that there is a powerful inhibitory gating of HVC auditory responses during singing (*36, 37*). However, our results suggest that it may be suppressed in social contexts. It would be interesting to further investigate whether inhibitory gating of auditory responses in HVC is released during singing duels.

***Coordinated singing strategies: behavioral ecology and song evolution implications***

Our results show that there is a diversity of behavioral strategies in canaries that are already apparent in small controlled groups of three males and two females: some canaries tend to perform singing duels and aggressive displays, whereas other birds adopt predominantly soloist strategies. Songs are therefore a complex signaling system with marked individual differences and strategies. Remarkably, these strategies are highly plastic in animals with crystallized songs. This is particularly relevant since leader/follower relationships control song properties (Fig. 4). The link of singing strategies with biological fitness and with the evolution of birdsong cultures are additional exciting future directions to pursue. Further studies should elucidate the specific links between singing interactions in canaries, the underlying social structures and their evolution. Our experiments have shown the emergence of leader/follower dynamics, reminiscent of direct bidirectionally-coupled systems. (*28*). It is tempting to speculate that the preference of females for

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overlapping songs in natural social competition contexts (*28*) may have shaped the abilities of male canaries to sing long songs, and the neural circuit mechanisms allowing them to integrate social contextual information, reshape activation patterns in the song system and sing longer-lasting songs. Further analysis of songs should reveal additional traits that change during singing interactions and what they signal. In terms of evolutionary ecology, how song and physical duels impact fitness is at this stage unclear. Indeed, females prefer overlapping singers, but also non-fighting canaries (*28*). However, in our conditions, singing duels were often accompanied by physical aggression. Future experiments in the field should reveal whether physical aggression also occurs in natural conditions between dueling birds, and the biological fitness associated to different singing strategies of leaders, followers and soloists. How dominance expresses itself in a group in the singing dynamics strategies also needs to be investigated.

### *Acoustic properties of dueling birds: ecological and cultural implications*

Results in Fig. 5 suggest that singing similarity may predispose canaries with more similar repertoires to perform singing duels. However, canaries may alternatively adapt very fast, with few song exposures from the first canary singing, to the repertoire and properties of another canary, thereby avoiding or forcing song feature matching and temporal overlaps. Only knowing the full repertoire before the onset of singing interactions will allow disambiguating whether canaries use a repertoire that predisposes to compete or they instead make an adaptative choice of song properties among a library of available features in a social competition context. This raises the fascinating question of how singing duels may have shaped the evolution of song culture, both regarding song length and similarity of songs in a population, and whether the co-existence of both strategies, solo singing and duels, combine complementary strategies which ensure a large song repertoire at the population level.

## Materials and Methods

### *Ethics permits*

The use of audio transmitters, bird housing and all experimental procedures were approved by the government of Upper Bavaria (Ethical approval ROB-55.2-1-54-2532. Vet_02-17-211) and performed according to the directives 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes.

### *Animals and housing conditions*

A total of 30 adults canaries (six groups formed by three males and two females each) were part of

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this study. Animals were randomly selected from larger aviaries and housed together in 2m*1m*1m indoor aviaries or alternatively 1.68m*0.78m*1.68m boxes. In both cases, birds were not within hearing range of other birds. Birds were kept in breeding conditions under a 13/11 Light/Dark cycle (fluorescent lamps), at 24 °C and 60–70% humidity. Food (mixed seeds, and "egg food"), fresh water and cuttlebone were provided *ad libitum*, as well as nesting material and nests.

***Audio recordings on Pico Island***

Two Sennheiser MKH 70 P48 directional microphones pointed in different orientations in field where canaries had been observed. Recordings were performed with a Marrantz PMD661 recorder with an audio bit depth of 24 bit.$s^{-1}$. The location of the recording areas (Fig. 1) in Arieiro and São João were 38°26'29.5''N28°28'40.6''W and 38°25'10.9''N28°20'05.0''W.

***Audio recordings at the Max Planck Institute for Ornithology***

The five birds for each group used for telemetric recordings were moved from larger aviaries to smaller indoor aviaries in our institute. Custom-made wireless microphones (0.6g, including battery) (*26*) were used for sound recording. The wireless microphone was placed on the back and fixed with an elastic band around the upper thighs of the bird. The frequency modulated radio signals were received with the communication receivers (AOR5000, AOR, Ltd., Japan). Audio signals were fed into an eight channel audio A/D converter (Fast Track Ultra 8R, Avid Technology, Inc. U.S.A.) and recorded with custom-made software.

***Video recordings***

Videos used for behavioural quantifications were recorded in aviaries with a CX405 handycamcorder (Sony).

***Song analysis***

Song onset and duration were manually determined on song sonograms. Analysis were performed with Matlab and Igor Pro Software. Songs recorded in the field were high-pass filtered for display using Audacity software, with a cutoff frequency of 2kHz and a roll-off of 12 dB per octave.

We used Sound Analysis Pro 2011 software (Version 2011.104) (38) for segmentations and analyses of vocal sounds to generate Fig. 5. The program segments the song in an automatic process and measures the sound features of each syllable including syllable duration, mean amplitude, mean pitch, mean frequency modulation, mean entropy, mean pitch goodness, mean average frequency. We measured the sound features of all retained songs for each bird. Then we used the generic

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function "pca" in Matlab (Mathworks, U.S.) to calculate the principal components (PC1, PC2 and PC3) for each bird based on all the measurements.

***Statistical analysis***

Data along the manuscript are provided as mean ± S.E.M. Statistics were performed with Igor Pro Software or Matlab. The specific tests used are specified along the manuscript.

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**Competing interests**

The authors declare no competing interests.

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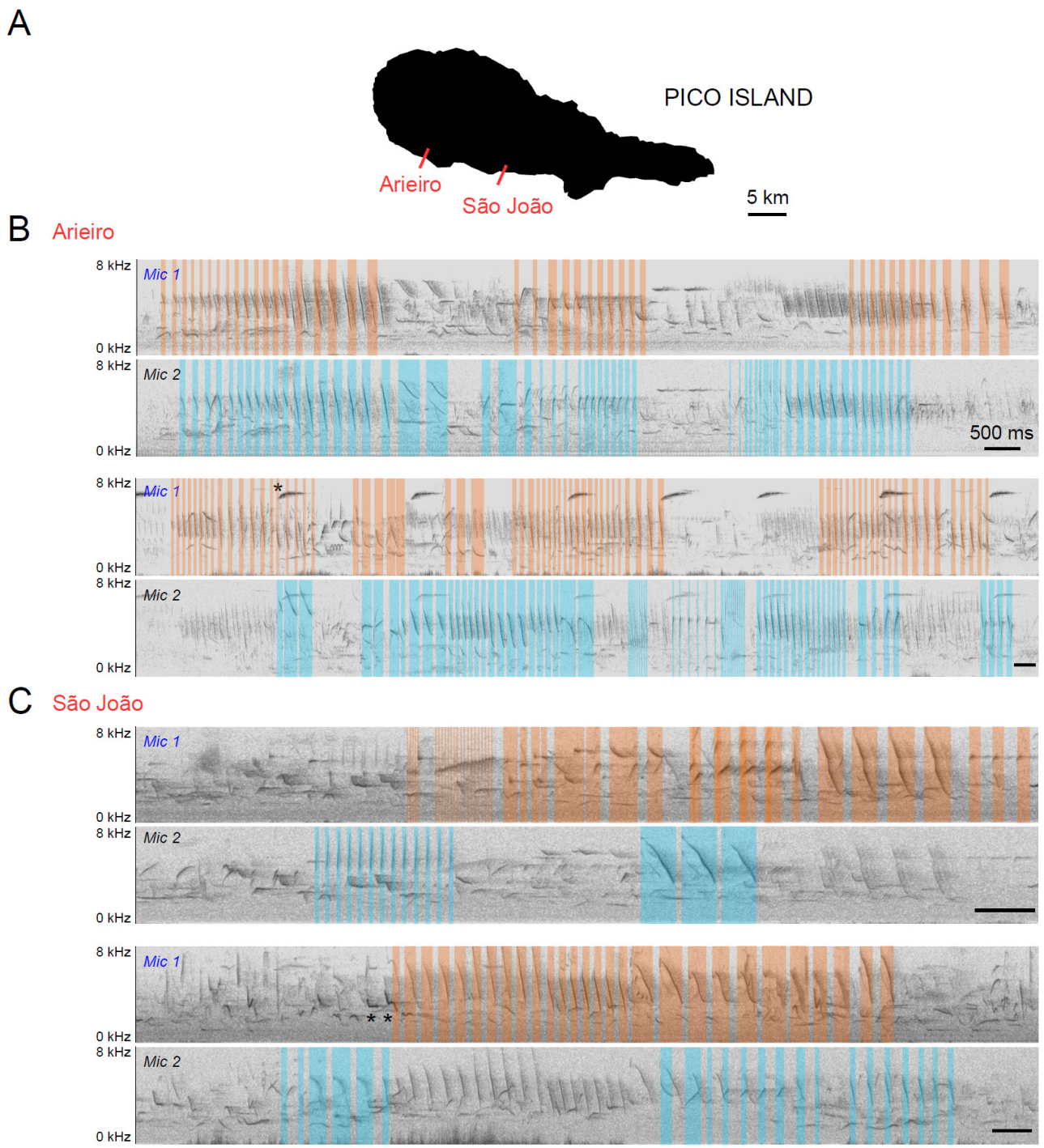
455 **Figures**




456

457 **Fig. 1. Multiple microphone recordings reveal overlapping songs from wild canaries. A.**
458 Recording sites are indicated on a map of Pico island. **B.** Spectrograms of two example overlapping
459 singing periods in Arieiro and **C.** two example overlapping singing periods in São João. Syllables
460 from putative canary songs from two different canaries recorded preferentially in microphone 1
461 (Mic1) or microphone 2 (Mic 2) are labelled in red and blue respectively. Scale bars, 500 ms. Note
462 that recordings also show additional distant canary songs (top), and the vocal production of
463 additional species (some examples being annotated with stars).

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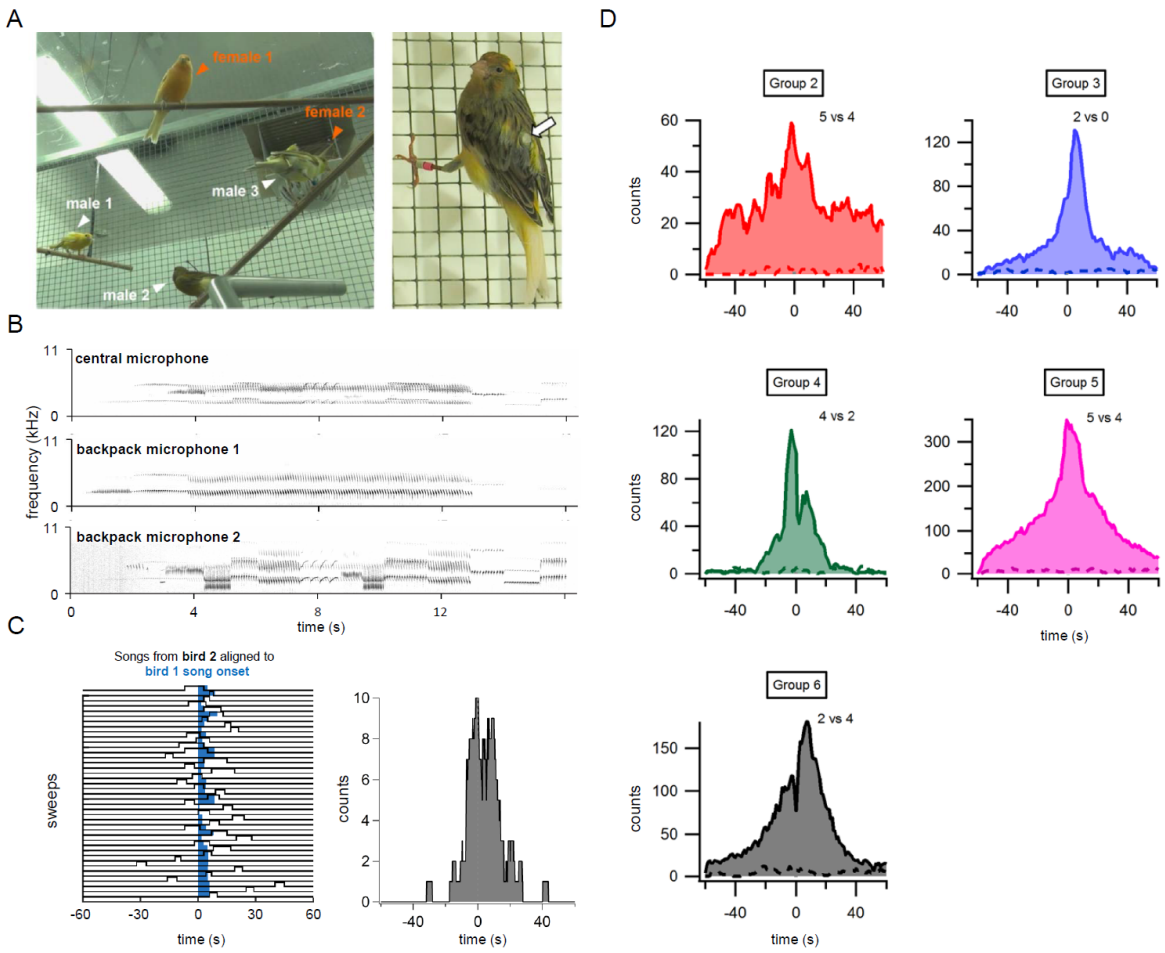


**Fig. 2. Telemetric recordings with backpack microphones reveal temporally-overlapping songs. A.** Left, experimental design: mixed groups comprising two female and three male canaries, all individually equipped with a backpack microphone. Right, male canary equipped with an individual microphone, pointed by the arrow. **B.** Representative simultaneous recording from a central microphone and from two backpack microphones confirms the identification of the individual vocal production by backpacked microphones. **C.** Left, example raster plot for all songs sung in a day by a canary (blue) and aligned vocal activity of a second canary (black). Right, corresponding histogram of the times at which songs were recorded in canary 2 centered at song onset from the reference canary 1. Bin size: 1 s. **D.** Data for all days in each group show increased singing when the singing activity of one bird was aligned to a second bird. Histograms were also generated from shuffled data (dashed lines), confirming that distributions correspond to increased firing of canaries in the 60 s time-window relative to random overlaps. Bin size: 1 s.

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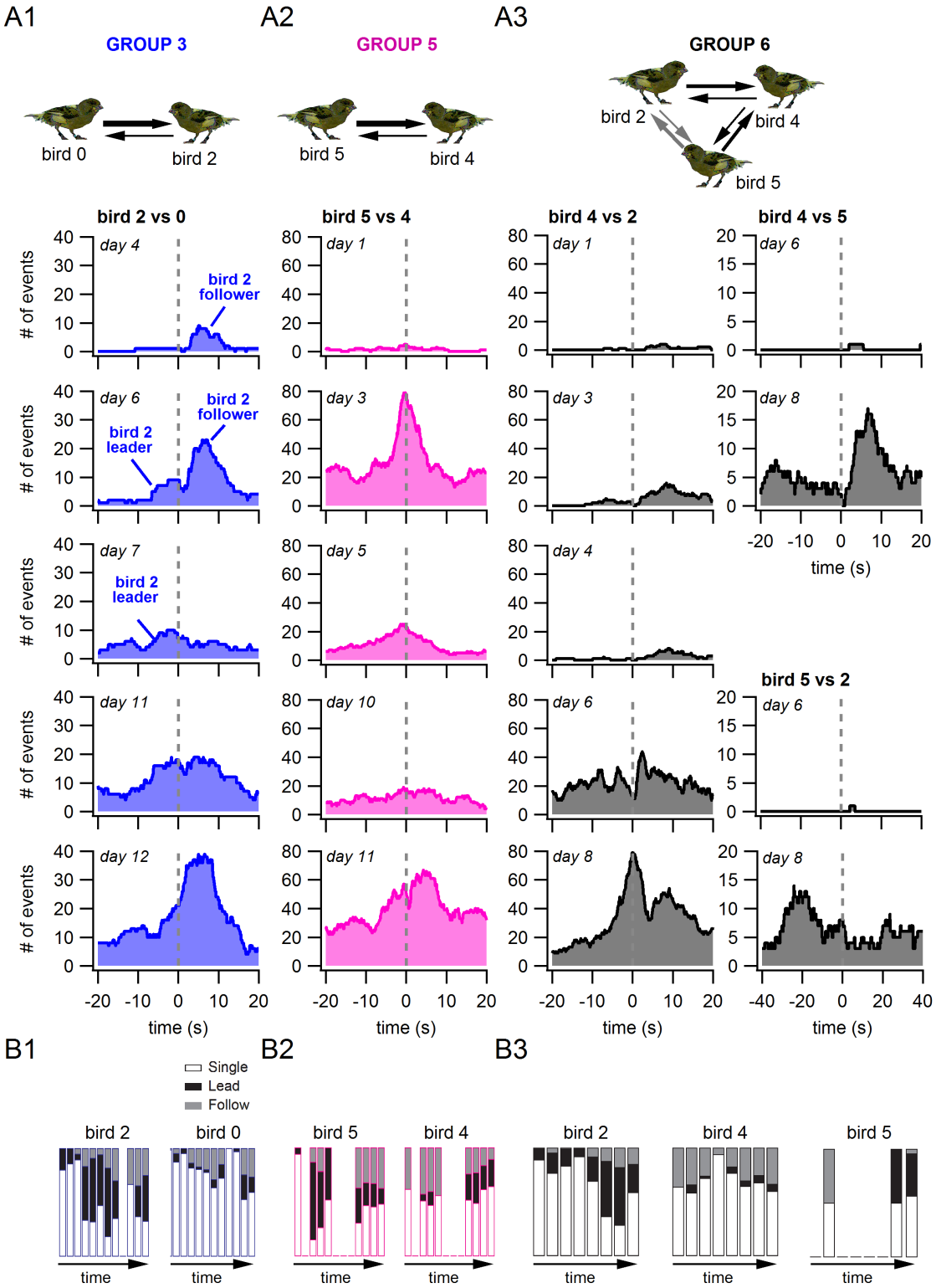


**Fig. 3. Singing dynamics reveal temporally-evolving follower and leader roles. A1, A2, A3.** Examples of singing interactions in three groups and their time course across several days. Note the change of scale in the bottom right histogram of the times at which songs were recorded centered at song onset from the reference bird. Bin size: 100 ms. **B1, B2, B3** illustrate the fraction of solo, overlapping and overlapped songs in each group. Note that empty entries shown in figure 3B

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correspond to the absence of singing activity detected during the whole day.

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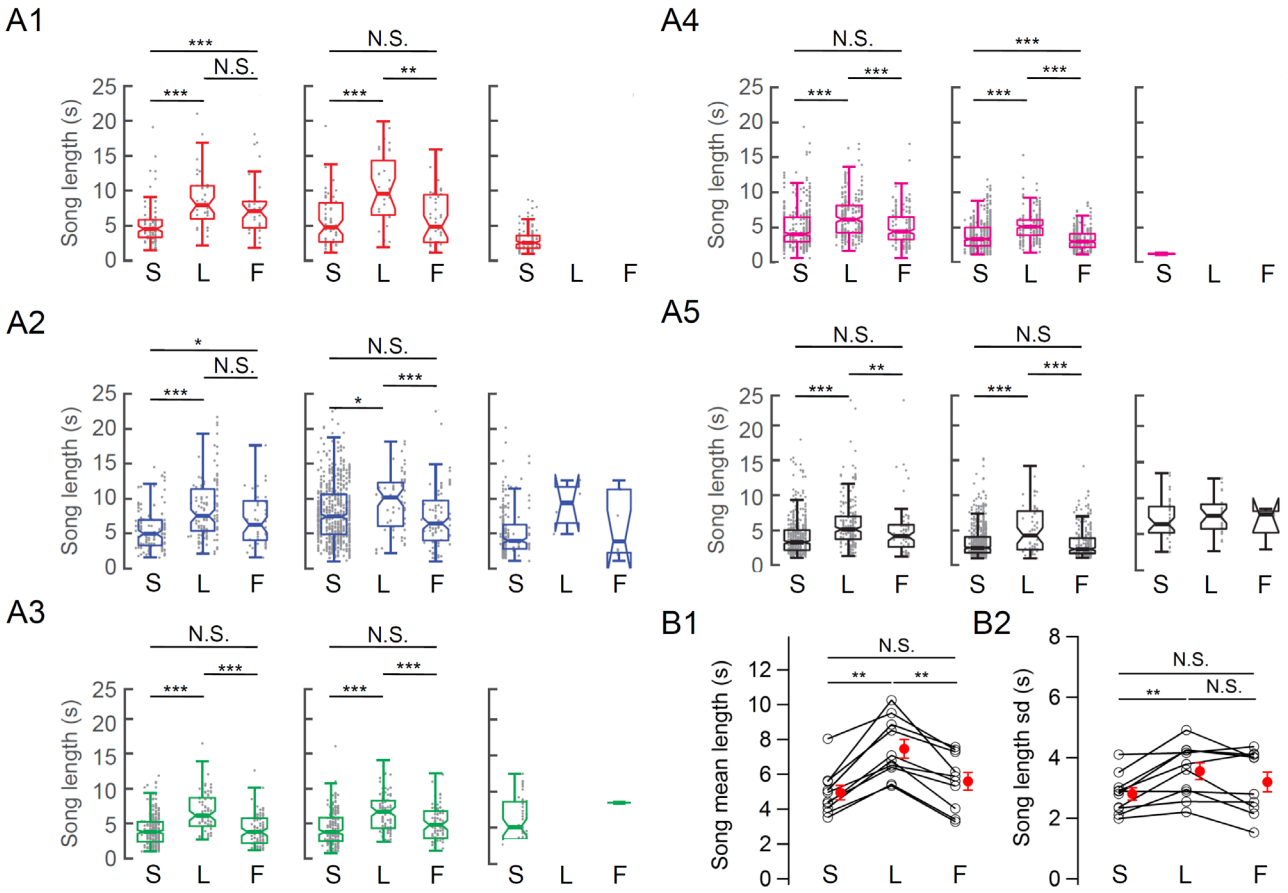


**Fig. 4. Song length and its variability depend on the relative timing of singing onset from male canaries.**

**A1-A5.** Song length for the three canaries in each of the five groups were plotted in panels A1 to A5. S, solo songs; L, leading songs; F, following songs. Multi-comparison Dunn-Holland-Wolfe test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **B1.** Summary data show significant differences in average song length for solo, leader and follower songs. Each line links average song lengths from the same bird in different singing conditions (S, solo songs; L, leading songs; F, following songs). * $P < 0.05$, ** $P < 0.01$, two-tail Wilcoxon Signed-Rank test. **B2.** Summary data for all four groups show significant differences in the standard deviation of song length for solo, leader and follower songs. $P < 0.05$, ** $P < 0.01$, two-tail Wilcoxon Signed-Rank test.

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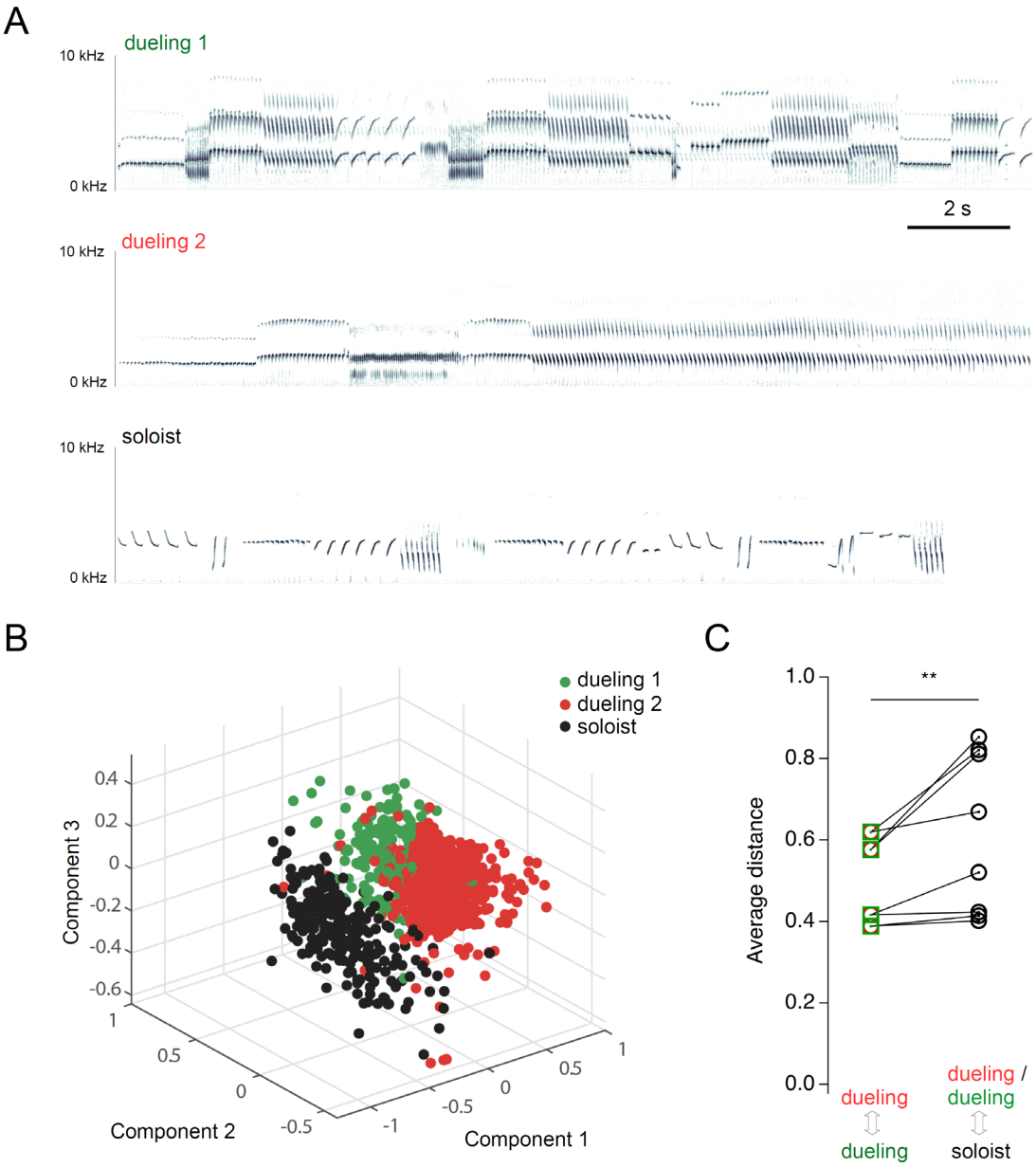


**Fig. 5**. **Dueling singers sing similar songs. A.** Representative example of songs from the three canaries in a group. **B.** PCA reveals closer songs in the three first components describing song parameters between dueling birds than between a dueling bird and the soloist. Data represents all individual songs (dots) of the three males in a group. **C.** Summary results for all four groups comparing the distance in the 3-component vector space between songs of the two dueling birds with each other and songs of each dueling bird with the soloist bird of the group. ** $P < 0.01$, two-tail Wilcoxon Signed-Rank test.