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Neotropical wrens learn new duet rules as adults

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Although song development in songbirds has been much studied as an analogue of language development in humans, the development of vocal interaction rules has been relatively neglected in both groups. Duetting avian species provide an ideal model to address the acquisition of interaction rules as duet structure involves time and pattern-specific relationships among the vocalizations from different individuals. In this study, we address the development of the most striking properties of duets: the specific answering rules that individuals use to link their own phrase types to those of their partners (duet codes) and precise temporal coordination. By performing two removal experiments in canebrake wrens (*Cantorchilus zeledoni*), we show that individuals use a fixed phrase repertoire to create new phrase pairings when they acquire a new partner. Furthermore, immediately after pairing, individuals perform duets with poor coordination and poor duet code adherence, but both aspects improve with time. These results indicate that individuals need a learning period to be able to perform well-coordinated duets that follow a consistent duet code. We conclude that both duet coordination and duet code adherence are honest indicators of pair-bond duration.

1. Introduction

Interactions in which individuals exchange vocal signals are an important aspect of communication in many animals [1]. In temperate songbirds, males often engage in vocal interactions such as countersinging and song matching [2], but the most complex vocal interactions occur in species that sing duets within pairs [3]. Avian duets have features analogous to ones found in human conversation, such as rules on correct temporal coordination and on which elements can be used by one participant to answer another's vocal elements [4]. The development of vocal signals such as human speech and the song of songbirds has been amply studied [5], but we know significantly less about the development of interaction rules in either group [3,4].

Some species of duetting songbirds follow 'duet codes' that specify which of a bird's own song phrases are used to answer each of its partner's phrases (e.g. own phrase A always answers partner's phrase X) [3,6]. Juvenile birds of some species have been observed to duet with their parents [3], copying their own-sex parent's replies to the phrases of the opposite-sex parent (K.D.R.-C. 2014, unpublished data). These observations strongly suggest that some birds learn a duet code as juveniles. That code might then be retained throughout life, with phrase-pairing rules remaining constant regardless of partner identity [7]; we term this idea the 'inflexible code hypothesis'. A difficulty with this hypothesis is that the phrase repertoires of same-sex individuals often show only limited overlap even within populations [8,9], so that a new mate will have many phrases that were not in the opposite-sex parent's repertoire and that were therefore not included in the parents' duet code. Furthermore, duet codes in many species are pair-specific [7,8,10–14], so that even when a new

mate shares a phrase with an individual's opposite-sex parent, the rule that individual has learned on how the phrase should be answered may not match the rule that its new mate has learned. Finally, because precise temporal coordination in some species depends on the ability of the birds to anticipate the phrase type that will be sung by their mates, an inflexible code might produce poor temporal coordination. These difficulties suggest an alternative, 'adult duet learning hypothesis', whereby individuals learn a new duet code and new timing patterns as adults each time they acquire a new mate.

Although this alternative hypothesis fits logically with the complexity of pair-specific duet codes, direct evidence on the learning of various aspects of duets is decidedly mixed. On the one hand, established pairs in canebrake wrens adhere to a duet code more consistently than do newly established pairs [9], black-bellied wrens (*Pheugopedius fasciatoventris*) are able to answer to novel phrases [6] and in magpie-larks (*Grallina cyanoleuca*) timing is more precise in established pairs than in new ones [15]. On the other hand, timing is not significantly better in established pairs than in newer pairs in canebrake wrens [9], canary-winged parakeets (*Brotogeris versicolorus* v.) [16] and California towhees (*Melospiza crissalis*) [17]. Particularly influential evidence against adult learning of duets came from a pioneering study of bay wrens (*Thryothorus nigricapillus*) by Levin [7], in which birds were experimentally removed from established pairs in order to trigger new pair formation. From comparisons of the duets of new and old pairs, Levin concluded that learning was not required 'for pair specificity and precision in the duets of new pairs of birds'. Levin, however, concentrated only on responses to phrases that were shared between the old and new mates while ignoring responses to phrases exclusive to the new mate, which are the ones more likely to require learning. Furthermore, Levin [7] focused on the stability of individual phrase repertoires rather than the consistency of phrase pairings. Finally, Levin's [7] conclusions could be based on Type II errors due to the small sample size (four individuals).

Mechanisms of development have implications for hypotheses on the function of duets. Wickler [18] assumed that adult learning was needed to duet properly with a new partner, so that a pair-specific and precisely sung duet demonstrates that both participants have invested considerable time and energy in learning to duet with each other. Duet coordination and duet code adherence could then function as a signal of commitment by one of the partners to the other [15] or as a signal by the pair to rivals in competition for territory that they are dealing with an established and committed pair [3]. Neither of these signalling functions is possible if precise duets do not require adult learning.

To test the adult duet learning hypothesis, we performed two experiments in canebrake wrens. First, we temporarily removed one member of a pair and tested the remaining bird's response to playback of its mate's phrases and of unfamiliar phrases. If learning is required to duet properly, duetting with the unfamiliar phrases should be less precise than with the familiar ones. Second, we permanently removed one member of a pair to induce new pair formation, and compared duets recorded from old pairs and new pairs. The adult duet learning hypothesis predicts (i) that new pairs immediately after pair formation should show weaker adherence to a duet code and less precise temporal coordination

than old pairs, and (ii) that both adherence to a duet code and temporal coordination should improve in new pairs with time. Our study is novel first in that we analysed the responses of individuals towards phrases exclusively in the new mate's repertoire as well as to phrases shared by the old and new mates. Second, we treated the duet codes as an individual level behaviour, and thus analysed the consistency of both female phrase pairings and male phrase pairings. Third, we measured duet code changes of new pairs with an unprecedented temporal resolution.

2. Material and methods

We performed the study at La Selva Biological Station and its surrounding areas in northeastern Costa Rica (10°26' N, 83°59' W). The area includes a mixture of lowland moist forest, swamps and cattle pasture, where canebrake wrens are common [19]. Both experiments were conducted with approval of the University of Miami's Institutional Animal Care and Use Committee (protocols 12-115 and 15-064) and under a scientific research permit (No. 05354) provided by the Ministry of Environment, Energy and Telecommunications of Costa Rica. Experiment 1 was performed between May and August of 2013 and 2014 and experiment 2 between May and July of 2015. Recordings were made throughout using a Marantz PMD660 digital recorder and unidirectional Sennheiser ME66 microphones.

Canebrake wrens sing highly precise antiphonal duets that are composed of three sex-specific categories of phrases (figure 1): I and M phrases sung by the male, and F phrase sung by the female [9] following an I(FM)_n sequence. Individuals possess a repertoire of 15–25 phrase types in each phrase category [19]. To sing a duet, canebrake wrens strictly follow a pair-specific duet code [19]. To perform coordinated duets, canebrake wrens modify their singing tempo based on the phrase types that their partners are singing [20].

(a) Experiment 1: playback experiment

We performed playbacks to 17 individuals (nine females and eight males) from 12 different territories. For the five pairs in which both individuals were tested, we performed the playback on different days with at least one week between trials. Prior to playback trials, we recorded repertoires from each subject for at least 6 h. To create a library of each individual's duet code, we created spectrograms of the recordings using SYRINX software (J. M. Burt, www.syrinxpc.com) with a Hanning window and a 512 pt FFT, and a temporal resolution of 5.8 ms. The phrase types for both males and females in each duet were determined based on visual inspection [21]. The association between mates' phrase types (duet code) was determined based on at least three instances from different recordings where the phrase types were found together.

For removals, we lured canebrake wrens to mist nets using playback of recorded duets. If no bird was captured after 10 min of playback, the experiment was stopped and another attempt was made at least 3 days later. If a member of the focal pair was captured, it was placed in a holding bag while the experiment was carried out and then released. The sex of the remaining bird was determined by its colour band combination if the bird was banded ($n = 13$) or by its sex-specific phrases if it was not banded ($n = 4$). We waited 2 min after the partner was removed to start the experimental trials.

Trials were performed between 6.00 and 9.20 (solar time), when canebrake wrens have high vocal activity [19]. Each trial included three playback treatments: (i) partner's phrase—a phrase type from the subject's mate; (ii) unique phrase—a phrase not present in the partner's repertoire but present in the

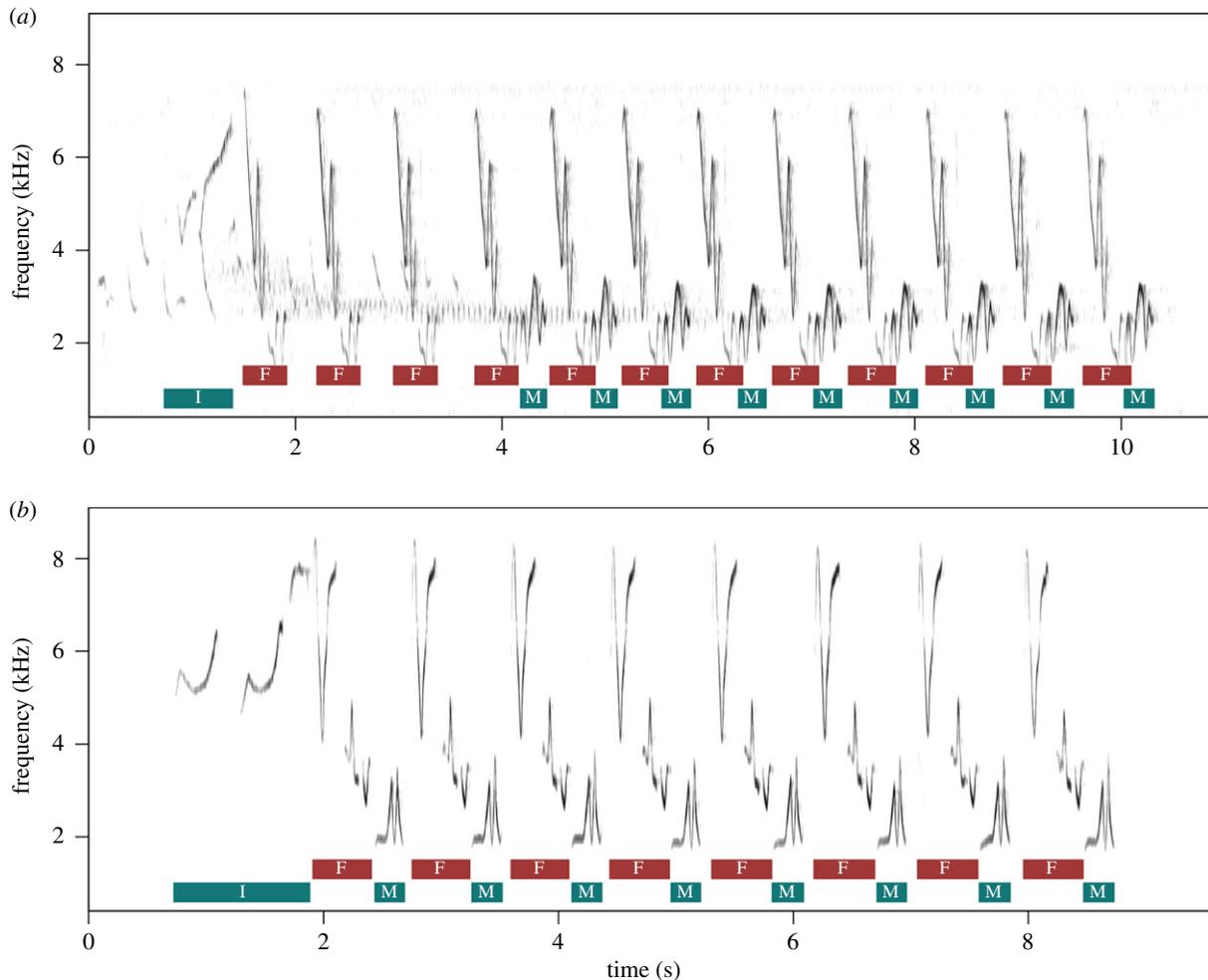


Figure 1. (a) Example of a low coordination performance duet performed by PAE3 4 days after re-pairing. Eight phrases were overlapped by the male. (b) Example of a high coordination performance duet performed by PAE3 12 days after re-pairing. No phrases were overlapped. I, introductory phrase sung by male; F, female phrase; M, male phrase. (Online version in colour.)

repertoire of one bird from the same population; and (iii) other population phrase—a phrase from a different population and that was not known from the study population. Females were exposed to M phrases while males were exposed to F phrases. Each playback included five replicates of seven phrases, repeated at 10 s intervals, followed by 90 s of silence. The order of the treatments was balanced across pairs. Information on treatment assemblage can be found in the electronic supplementary material, methods.

Subjects were recorded throughout playback trials. From the recordings, we determined (i) the proportion of phrases that were answered, (ii) the phrase type(s) that the birds used to answer, and (iii) the proportion of phrases that the birds overlapped with their own phrases.

For the shared phrase treatment, a G-test was applied [19,22] to determine whether the phrases that a focal bird used to answer were chosen randomly or followed the expected duet code. To calculate the expected values we used the inverse of the average total repertoire size recorded from each individual and multiplied that value by the number of individuals that answered the playback. We compared these expected numbers to the observed number of individuals that answered with the predicted phrase type.

A generalized linear mixed model (GLMM) was used to determine whether there was an effect of treatment on the proportion of phrases that individuals answered. A second GLMM was used to determine whether there was an effect of treatment on the proportion of phrases from the playback that the focal individual overlapped with its own phrases. We use proportion of overlap as our measure of duet coordination because optimal

coordination in this species involves little or no overlap [20] and phrase overlapping should be easy for birds to detect [23]. In both models, we also included sex and the interaction between sex and treatment as fixed factors; order of treatment was used as a covariate, and bout number and individual were used as nested random factors. Fixed factors were left in the final models if $p \leq 0.05$, and the interaction term was left in the final model if $p \leq 0.25$ [24].

(b) Experiment 2: removal experiment

We first recorded the duets of 11 established pairs. At least 4 h of recording were made per territory to get as much of the birds' repertoires as possible [19]. Eight out of the 11 pairs had been recorded during the previous 2 years, and these recordings were also used in reconstructing repertoires and duet codes. To create a library of each bird's duet code, we used the same methods described in experiment 1 (see above). Each different IF phrase pairing was considered a rule within the duet code of females and each different FM phrase pairing was considered a rule within the duet code of males.

We performed a female or male removal from two territories each day, so that either the males or the females from two different territories could be exchanged (details in the electronic supplementary material, documents). Each of the new pairs was composed of at least one member that belonged to the original 11 established pairs that were previously recorded. We monitored the birds that remained in their territories every day until they re-paired.

After a re-pairing event, we recorded the new pair for 1 h every day for an entire week and then 1 h every week for up to one month. Five pairs were recorded for the entire month, four pairs were recorded for three weeks, two pairs were recorded for two weeks and two pairs were recorded for 5 days. We separately analysed the contribution of each individual to the recorded duets.

In many cases, birds sang phrases with a new partner that we had not recorded from duets with the old partner. These phrases might represent new phrases added to the repertoire after re-pairing, or they might be phrases already present in the repertoire prior to re-pairing but missed by us due to incomplete sampling [7]. To estimate repertoire sizes before re-pairing, we used the program EstimateS 9 [25] to compute Coleman rarefaction curves [26,27] and 95% confidence intervals of unconditional variance for sample-based abundance data [28]. Details on calculation of curves can be found in the electronic supplementary material, documents.

To determine whether birds used the same answering code for phrase types that were shared between the old and new mate (shared phrases), we used a heterogeneity G -test. To calculate the expected values, we used the inverse of the repertoire size recorded from each individual. To determine whether birds incorporated the phrases exclusively present in the new mates' repertoire (unshared phrases) in a new duet code we used a GLMM, which tested whether canebrake wrens preferentially answered shared rather than unshared phrase types, and thus whether duets were formed by the shared phrases with a probability higher than expected by their frequency in the repertoires of individuals.

To determine whether the duet codes of individuals in new pairs are less consistent than those of individuals in more established pairs, we calculated the Shannon index of diversity (H') of phrase pairings used by each individual (IF pairings for females and FM pairings by males). To test for differences in H' , we used a GLMM with type of pair (established versus new) as a fixed factor, and individual and pair as nested random factors. We also controlled for the effect of the number of phrase types used by the partner by including it as a covariate (effect magnitude = 0.051, $t_7 = 4.11$, $p = 0.0063$).

To determine whether individuals in new pairs improve their duet code consistency as time progresses, we compared H' of the duet codes of individuals in new pairs using a GLMM with time (days 1–4, days 5–8 and weeks 2–4) as a fixed factor, and individual and pair as nested random factors. The number of phrase types recorded from each bird's mate was used as a covariate (effect magnitude = 0.039, $t_{25} = 3.44$, $p = 0.002$).

To measure duet coordination, we chose five duets with a clear delineation of the beginning and ending of phrases from each day, and compared the observed number of phrase overlaps with the expected number found using a Monte Carlo randomization test (electronic supplementary material, methods) in the R package warbleR [29]. Individual tests were run for each singing bout (10 000 iterations). We then calculated the proportional difference between observed and expected overlaps as a measure of coordination performance (overlap index). Positive overlap indices indicate that the observed number of overlaps exceeded those expected by chance (poor coordination), while negative overlap indices indicate less phrase overlap than expected by chance (good coordination). To determine whether coordination performance improves with time, we used a GLMM with day after re-pairing as a fixed factor, and individual and duet as nested random factors.

Sample sizes for all analyses in experiment 2 are detailed in the electronic supplementary material, table S1.

All GLMMs were analysed using the function `lme` of the package `nlme` [30] in R (v. 2.15.1). We validated all GLMMs (experiments 1 and 2) by assessing violations to homogeneity and normality based on the graphic methods suggested by Zuur *et al.* [31].

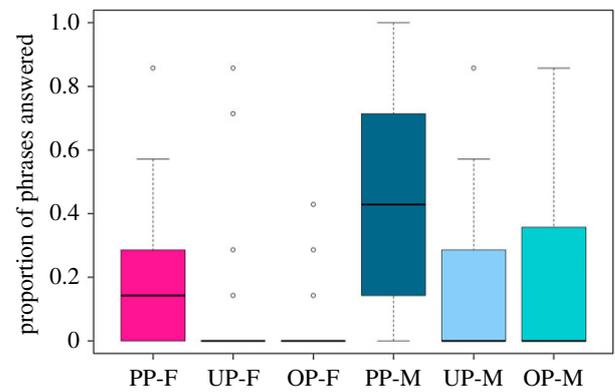


Figure 2. Proportion of phrases from playback that females (F) and males (M) answered with their own phrases for each treatment. PP, partner's phrase; UP, unique phrase; OP, other population phrase. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers), and extreme data points (open circles) are shown for each boxplot. (Online version in colour.)

3. Results

(a) Experiment 1: playback experiment

(i) Song answering

Individuals answered playback of all three treatments: phrases in their partners' repertoires, phrases that were not in their partner's repertoires but were present (though rare) in their population, and phrases from different populations that were unknown in their own population. However, birds answered significantly more often to their partners' phrases than to any of the phrases that were not in their partners' repertoires (unique phrases, effect magnitude = 0.8, $t_{217} = 3.2$, $p = 0.0016$; other population phrases, effect magnitude = 0.88, $t_{217} = 3.55$, $p = 0.0005$; figure 2). In addition, males answered a higher proportion of phrases than did females (effect magnitude = 1.81, $t_{15} = 4.03$, $p = 0.001$), but the magnitude of this effect was significantly higher only for unfamiliar phrases (unique phrases, effect magnitude = 1.25, $t_{217} = 2.43$, $p = 0.016$; other population phrases, effect magnitude = 1.04, $t_{217} = 2.10$, $p = 0.036$). The order of the treatments did not have a significant effect on the proportion of phrases produced by the individuals (effect magnitude = 0.018, $t_{15} = 0.81$, $p = 0.42$).

(ii) Duet code consistency and temporal coordination

Males and females followed the corresponding duet code when answering their partners' phrases with a probability far above chance (eight out of eight males sang the predicted phrase, $\chi^2 = 128.17$, $p < 0.001$; six out of nine females sang the predicted phrase, $\chi^2 = 75.77$, $p < 0.001$). Individuals overlapped a significantly higher proportion of phrases that were not present in their partners' repertoires, which indicates poorer coordination for any of the unfamiliar phrase types (unique phrases, effect magnitude = 0.32, $t_{12} = 3.30$, $p = 0.002$; other population phrases, effect magnitude = 0.25, $t_{12} = 0.57$, $p = 0.049$; figure 3). Sex and the interaction between sex and treatment and order of treatments had no significant effect on the proportion of overlapped phrases ($p = 0.5$, $p = 0.53$ and $p = 0.24$, respectively), and thus were dropped from the final model.

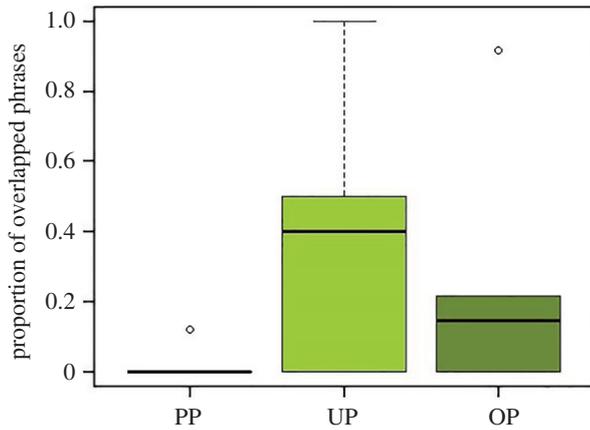


Figure 3. Proportion of phrases from playback that individuals overlapped with their own phrases. Abbreviations and boxplot parameters are the same as given in figure 2. (Online version in colour.)

(b) Experiment 2: removal experiment

(i) Individual phrase repertoires

Individuals tended to use the same phrase repertoire to create duets with old and new mates. New phrase types found after re-pairing did not exceed the 95% upper confidence interval for the estimated repertoire size in three out of four males (I and M phrase types) and six out of eight females (F phrase types), indicating that these individuals did not change their repertoires after re-pairing. However, one female and one male sang one more phrase type than expected, and another female sang two more phrase types than expected, after re-pairing (electronic supplementary material, table S2), and thus we cannot reject the possibility that a subset of individuals added a modest number of phrases after re-pairing.

(ii) Duet codes

Answers toward shared phrase types. Overall, wrens replied following their original duet codes more often than expected by chance when answering phrases from the new mates that were shared with their old mates ($G_{\text{total}} = 155.3$, d.f. = 13, $p < 0.001$; electronic supplementary material, table S3). Six out of eight females used the same phrase type to answer a phrase type that was shared between the old and new mate with a frequency significantly higher than expected by chance, whereas the same was true for one of four males. The remaining three males and two females used a different duet code than they had with their old mate to answer the same phrases of the new mate (electronic supplementary material, table S3).

Answers toward unshared phrase types. Phrases only present in the new mate's repertoire were used in duets after re-pairing at a frequency (0.63 ± 0.15) equal to that expected from the frequency of these unshared phrases in the repertoire (0.64 ± 0.11 ; effect magnitude = -0.01 , $t_{13} = 0.58$, $p = 0.59$). Thus, unshared phrases were used as often as shared phrases in duets, demonstrating that birds adjusted their duet codes to include the novel phrases of their new mates.

Duet code consistency. The diversity indices (H') of duet codes of individuals after re-pairing were significantly higher than those of individuals in established pairs (effect magnitude = -0.54 , $t_7 = -13.95$, $p < 0.0001$; figure 4). This result indicates that duet code adherence in new pairs is

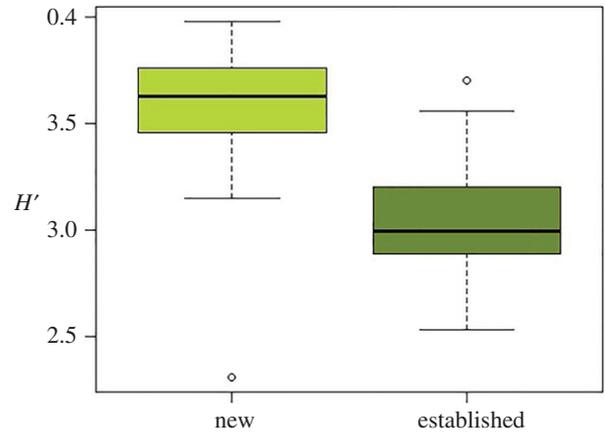


Figure 4. Diversity indices of male and female duet codes in established versus new pairs. Boxplot parameters are the same as given in figure 2. (Online version in colour.)

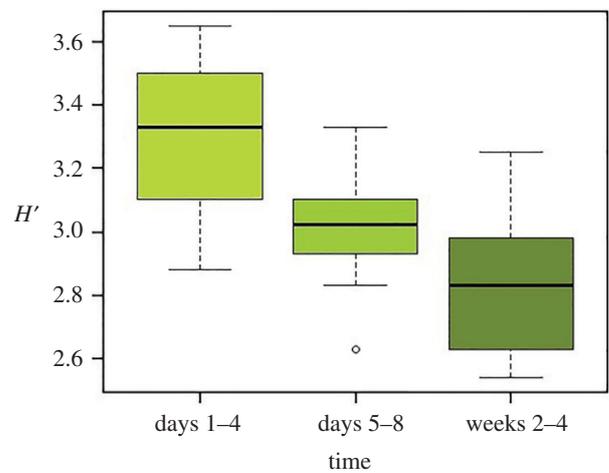


Figure 5. Diversity indices of individuals in new pairs across time. Boxplot parameters are the same as given in figure 2. (Online version in colour.)

less consistent than in established pairs. Furthermore, H' values for duet codes of new pairs during the first 4 days after re-pairing were significantly higher than H' on days 4–8 (effect magnitude = -0.277 , $t_6 = -3.84$, $p < 0.001$), and the latter were significantly higher than H' of duets recorded two to four weeks after re-pairing (effect magnitude = -0.45 , $t_6 = -5.27$, $p < 0.0001$; figure 5). These results show that individuals require time to learn a consistent new duet code after acquiring a new mate.

(iii) Duet coordination

Coordination performance of new pairs immediately after pairing was lower than that of established pairs (effect magnitude = -0.22 , $t_{19} = -2.85$, $p = 0.01$) and subsequently improved significantly as time progressed (effect magnitude = -0.0097 , $t_{288} = -2.66$, $p = 0.0081$; figures 1 and 6).

4. Discussion

(a) Duet code flexibility

The results of the two experiments together demonstrate that the duet codes of adult canebrake wrens are highly flexible after re-pairing events. In the playback experiment, adults

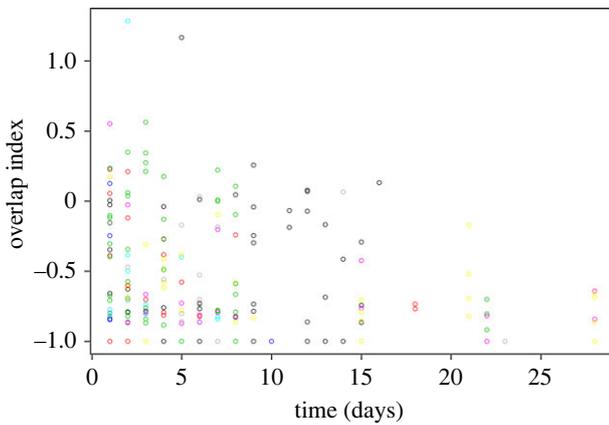


Figure 6. Coordination performances of new pairs across time. Different colours indicate different pairs. Overlap indices represent the proportional difference between observed and expected overlaps $((\text{obs} - \text{exp})/\text{exp})$. (Online version in colour.)

of both sexes responded by duetting both to their mates' phrases and to phrases not present in their mates' repertoires. Although responses to unshared phrases were relatively infrequent, the occurrence of even a low level of response to novel phrases suggests how new code elements could be created. After re-pairing events in the second experiment, all individuals significantly modified their duet codes, in that all incorporated into their duets the phrase types of their new mates that were not shared with their old mates, which required adopting new rules for phrase pairings. In addition, 5 of 12 individuals showed no overall adherence to their old duet codes in answering phrases of their new mates that were shared with their old mates.

In general, individuals that switched mates used the same phrase repertoire to answer old and new mates. These results suggest that individual phrase repertoires might be acquired during a sensitive period in early development as in other wrens [32,33]. However, because three individuals sang one to two phrase types above the 95% upper confidence interval we cannot reject the possibility that individuals possess the ability to learn new phrase types as adults [34] or that individuals memorize a larger repertoire than they sing with each partner and can decide which phrases to express depending on the identity of the partner [35].

Levin [7] found using similar methods that bay wrens do not significantly change their phrase repertoire to answer to new partners, and concluded from this that individuals do not change their duetting behaviour after re-pairing. However, here we show that despite conserving the same phrase repertoire, canebrake wrens after re-pairing develop new rules on how their own phrase types link with their mates' phrase types. In the majority of instances, birds retained their old rule when replying to a phrase of their new mate that was shared with the old mate, but three out of four males and two out of eight females answered differently towards the same phrase types present in the repertoire of both old and new mates (electronic supplementary material, table S2). A potential explanation for why individuals do not always use the same code as before is that even though codes are set at the individual level [36] the development of a code requires both individuals to agree on which phrase pairings are allowed. In experiment 1, females answered M phrases correctly, demonstrating that they know the male's code (F-M

pairings) as well as their own code (I-F pairings). Females may then use their memory of the F-M pairings developed with their former partner to influence the F-M pairings adopted by their new partner. Both females and males sometimes stop answering after their mates have answered for the first time in a given duet; for example, the female sings an F phrase, the male sings an M phrase, and the female fails to answer. After such aborted duets, birds often switch phrase types (K.D.R.-C. 2014, personal observation), which is consistent with the hypothesis that feedback from partners is important in establishing a new duet code. It is important to note, however, that individuals that did not follow the same code for phrases that we classified as shared could be interpreting these phrases as different types.

(b) Learning is needed to develop a new code

Levin [7] also proposed that learning did not play any role in the creation of new duet codes during adulthood in bay wrens. Since then it has been assumed that duet codes do not play a role in the process of pair formation and that they do not provide any information about the strength of a pair bond [3] (but see [9]). However, the design of Levin's study rather addressed the question of whether individuals retain part of their codes when they switch mates and whether coordination remains high with the retained phrase pairings. Levin's design thus focuses attention on those aspects of duetting with the new mate that ought not to require learning because they are retained from the old duet code. This study demonstrates that canebrake wrens require learning to achieve both temporal precision and pattern-specificity of duets when answering to new phrase types as (i) individuals are less coordinated when answering to unfamiliar phrases immediately after mate removal, but improve duet coordination with time, and (ii) the duet codes of individuals in newly formed pairs are less consistent than those in more established pairs and consistency improves with time.

In a previous study of canebrake wrens, Marshall-Ball *et al.* [9] found that that temporal coordination was no different for pairs that had been together for two or more years than for pairs that had formed within seven months. However, here we show that immediately after mate removal individuals overlapped significantly more than when they duetted with their established partners (experiment 2). After a low point immediately after re-pairing, coordination improved rather rapidly as time progressed (figure 5), explaining why Marshall-Ball *et al.* [9] did not find poor coordination when averaging over pairs that had been together up to seven months. Marshall-Ball *et al.* [9] also tested the consistency of the 'duet types' of pairs of canebrake wrens that had been together for more than 2 years versus pairs that had been together less than seven months. Their results suggested that codes become more stable with time, as the duet types of older pairs were more consistent than the ones of newer pairs. However, because the phrase pairings (i.e. duet types) were regarded as a pair-level behaviour [37], only FM phrase pairings were taken into account and thus only male's adherence to codes was tested. These results complement our study because it appears that even after several months of being paired together, the duet codes of males are less consistent than the duet codes of males in more established pairs.

(c) Implications for duet functions

This study establishes that both the consistency of duet codes and the precision of temporal coordination improve with time after re-pairing in canebrake wrens. These results are compatible with the hypothesis that duet coordination serves as a signal of pair quality and stability [37]. Thus, it is possible that in canebrake wrens, duet coordination could function as a pair-level signal to advertise pair quality to rivals in territory defence. This hypothesis is further supported in magpie-larks by results showing stronger territorial responses to well-coordinated displays than to poorly coordinated ones [15]. Although better coordination has been found in established pairs in some additional species, such as magpie-larks, there are some species for which this pattern does not hold, such as California towhees [17]. We suggest that the pattern of better coordination in established pairs is stronger and thus more easily demonstrated in species that have complex, highly coordinated duets than in species with simpler, looser duets.

As the development of a new pair-specific duet code requires learning, duet code adherence could also function as an honest signal that advertises an individual's commitment to a mate (pair-bond maintenance hypothesis). In black-bellied wrens, females adhere more strongly to their duet codes than do males [6]. In this study, we found that male canebrake wrens tend to answer more to unfamiliar phrases than do females, and that males are more likely to change their phrase-pairing after re-pairing than females are. These results suggest that the selective pressure of following a duet code could be biased towards one sex or another, and might depend on the level of reproductive investment from each sex and the cost of losing a mate among other factors [38].

5. Conclusion

This is the first longitudinal study that has addressed the ontogeny of pair-specific duet codes in new pairs. We found that the duet codes of adult females and males are flexible, and change when re-pairing occurs. Furthermore, individuals need a learning period to be able to perform well-coordinated duets that follow a consistent duet code. Our results show that duet coordination and duet code adherence are honest indicators of pair-bond duration in canebrake wrens.

Ethics. The research protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Miami. Research was performed under a scientific research permit (no. 05354) provided by the Ministry of Environment, Energy and Telecommunications (MINAET) of Costa Rica.

Data accessibility. Datasets are available from Dryad: <http://dx.doi.org/10.5061/dryad.c937h> [39].

Authors' contributions. K.D.R.-C. designed the study, collected and analysed data, and wrote the manuscript; E.Q.-G. collected field data and edited the manuscript; M.A.-S. performed statistical analysis of data and edited the manuscript; W.A.S. designed the study and helped write the manuscript. All authors gave final approval for publication.

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