Repertoire size and composition in great tits: a flexibility test using playbacks

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ARTICLE INFO

In many bird species, song repertoire characteristics play an important role in intersexual competition and intrasexual attraction. Studies on the importance of having a large repertoire typically assume the available song types as a fixed trait. However, repertoire composition may be flexible. We explored the effects of playbacks on the composition and use of the song type repertoire in great tits, *Parus major*. Playbacks simulate a nearby competitor male, vocally advertising territoriality. We compared the effects of novel song types with two control groups, one in which we played back one of the song types from the repertoire of the focal individual, and one without playback. The repertoire size for individual great tits of actively used song types remained more or less constant, but playbacks led to a significant turnover in repertoire composition over a relatively short period (about a week). The repertoire assessment across three experimental phases led to significantly larger accumulated repertoires compared to the more traditional short-term assessment method. The appearance of new song types was especially prominent among birds treated with a novel song type, suggesting the process is socially mediated. None of the 'new' song types were accurate copies of the novel unfamiliar song types played back. We discuss the potential origin of these 'new' song types and the impact of repertoire plasticity on signal value for sexual selection.

Many bird species sing a variety of songs making up a repertoire of distinct song types. Vocal repertoires can also comprise a set of different song notes or syllables producing a set of variable songs. Such song repertoires can play an important role in mate attraction and territorial defence (Searcy & Andersson 1986; Collins 2004). Many studies on repertoires have focused on size, although composition and rate of use could also be important. Moreover, repertoire composition may be flexible and being able to make changes in the repertoire throughout life may permit birds to adjust to changing environmental conditions, such as new neighbours or acoustic characteristics of the local habitat (McGregor & Krebs 1989; Nordby et al. 2001; Slabbekoorn & den Boer-Visser 2006; Nicholson et al. 2007).

Individual repertoire composition and its potential for modification will depend on when songs can be memorized in life. In most songbirds learning of song features is largely restricted to one or more sensitive periods for song memorization (Hultsch & Todt 2004). Based on the timing of these sensitive periods, species can be broadly divided into two groups: one for which song learning is age limited (closed-ended learners, e.g. Marler 1970; Jones et al. 1996) and one for which learning can continue throughout life

(open-ended learners, e.g. Kiefer et al. 2006; Nicholson et al. 2007). Closed-ended learners have been studied in detail, whereas only a few species have been shown to be open-ended learners. Some examples of open-ended learners are canaries, *Serinus canaria* (Nottebohm & Nottebohm 1978), European starlings, *Sturnus vulgaris* (Eens et al. 1992) and nightingales, *Luscinia megarhynchos* (Todt & Geberzahn 2003). However, anecdotal evidence and field observations suggest that a long-lasting ability to pick up new songs is more common than previously thought (e.g. Laskey 1944; Derrickson 1987; McGregor & Krebs 1989; Kroodsma 2004).

Individual repertoire composition and potential for modification will also depend on from whom birds learn their songs. Songbirds do not copy all that they hear; several studies have shown that there is a predisposition to copy vocalizations from conspecifics (Thorpe 1958; Marler 1976), or even to copy vocalizations from their own subspecies in favour of those of a related subspecies (Nelson 2000a). Furthermore, among conspecifics there is a variety of individuals from whom it is possible to learn. Depending on when the sensitive phases for song learning occur and the kind of interactions, social parents, neighbours in the natal territory, birds encountered during dispersal or neighbours in the breeding territory may serve as tutors (see e.g. Baptista & Petrinovich 1984; Bell et al. 1998; Freeberg 1999; Liu & Kroodsma 2006; Bertin et al. 2007).

Great tits, *Parus major*, are an ideal species for repertoire-related studies, given their moderate but variable repertoire size of clearly

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distinct stereotypically produced song types (Gompertz 1961; McGregor & Krebs 1982; Lambrechts 1996). The repertoire sung by individual males consists of two to six, up to nine, song types, and although males within a locality share song types, there is considerable variation in repertoire composition between males (McGregor et al. 1981; McGregor & Krebs 1982, 1989; Slabbekoorn & Peet 2003). A large song type repertoire in great tits is important in territorial defence as shown by speaker replacement experiments (Krebs et al. 1978), and song type sharing among males is a critical factor for male success during vocal interactions (Krebs et al. 1981; Falls et al. 1982; McGregor et al. 1992). A repertoire can also affect male attractiveness as larger repertoires seem also more effective in triggering great tit females to show copulation solicitation displays (Baker et al. 1986).

The repertoire size of adult great tits appears rather stable across years, but changes in song type composition have been reported (McGregor & Krebs 1989). Some song types may be dropped while others are incorporated into the repertoire, leading to a turnover in repertoire composition. In McGregor & Krebs's (1989) study, new song types added to the repertoire of adult males were similar to those of newly arrived neighbours. This led the authors to suggest that males directly copied these song types from these new neighbours when adult, potentially after having heard them for the very first time. However, an alternative explanation for this learning of song types anew is recalling them from memory. Early in life, young birds could have heard and memorized a wide variety of song types, which may all be sung at some point, but only some of which may be culled from this original repertoire during vocal interactions at a later stage in life. This phenomenon has been labelled 'selective attrition' (Marler & Peters 1982; Marler & Nelson 1993; Nelson 2000b). Song types that have dropped out of a repertoire in this way, or song types that were memorized but were never even sung, might be retained in memory as 'silent song types' and then be (re)activated when triggered by external stimulation (Hough et al. 2000; Geberzahn et al. 2002; Geberzahn & Hultsch 2003).

Our objective in this study was to test the flexibility of the song type repertoire of adult territorial great tits and to test experimentally whether they would copy novel, unfamiliar song types when adult. We explored the effects of playbacks of novel song types on the repertoire composition and use of individual great tits (treatment Novel). The effect of playing back novel song types was compared with the effect of two control sets: one in which we played back a song type from the repertoire of the focal individual (treatment Own), and one without any playbacks (Control). With the novel song type we intended to simulate a new neighbour, and the two controls allowed us to single out the effects of conducting a playback in general and of song type novelty in particular. Our main aim was to confirm the hypothesized ability to modify the song type repertoire in response to new neighbours in an experimental set-up. More insight into flexibility of repertoire size and composition for this model species is likely to affect our understanding of signalling potential in the context of sexual selection in general.

METHODS

Study Site and Species

We conducted the study in the contiguous urban area of Leiden and Oegstgeest (52°10′N, 4°27′E), the Netherlands, between 7 March and 15 June 2006. We selected 27 great tit territories with vocally active birds; only nonimmediate neighbours were included in the analysis. Consistent singing perches were indicated on detailed maps and used as identifiers for revisiting birds on 6–12 consecutive or semiconsecutive days. Male great tits hold territories and sing year-round, but song activity increases significantly during spring, especially at dawn (Mace 1987). They produce song strophes, which usually contain three to six (Slabbekoorn & Ripmeester 2008), but up to 20, repetitions of the same phrase (i.e. song type). Males sing several strophes of the same song type before changing the song type (eventual variation; see Collins 2004). Song types mostly contain two, three or four discrete notes, but may contain more (McGregor & Krebs 1982; Lambrechts 1996) or fewer (Slabbekoorn & den Boer-Visser 2006). A previous study on great tits in Leiden and Leiderdorp, immediately adjacent to the current study area, indicated that these song characteristics are also typical for Leiden and the surroundings (Slabbekoorn & Peet 2003).

Experiment Set-up

We tested subjects in sets of three individuals, and the different sets were tested during partially overlapping periods of time dispersed throughout the breeding season. We assigned each of the three individuals within a set to a different treatment (Novel, Own or Control). Experiments for each individual were carried out in three phases. During phase 1 we recorded at least 15 min of spontaneous singing (not triggered through playback) on 2–5 consecutive or semiconsecutive days. When phase 1 was completed for a set of three individuals, we initiated phase 2 for this set. During phase 2, we treated the first individual to be recorded for at least 15 min with playbacks of a novel song type (Novel), the second with playbacks of one of the song types in his own repertoire, recorded during phase 1 (Own), and the last individual was not treated with playbacks (Control). The songs sung in response to playbacks during phase 2 were recorded (only individuals in treatments Novel and Own). During phase 3, we recorded again at least 15 min of spontaneous songs from each individual on 2–5 consecutive or semiconsecutive days.

Recordings

For recordings we used a Sennheizer ME67/K6 directional microphone connected to a portable Marantz PMD670 solid-state digital recorder. All recordings were made between 1 h before sunrise and 1 h after sunrise. Each day during phases 1 and 3 we visited different territories. If the male in a territory was found singing we recorded him for as long as he continued singing; if not, we waited approximately 15 min before moving to another territory. During phase 1 of each set we focused on individuals recorded on previous days, to complete at least 15 min of recordings for three individuals. During phase 3 we focused on the individuals of the focal set, but we also started recording other individuals for phase 1 of the next set. Successive phases were separated by 0, 1 or 2 days depending on weather conditions.

Playbacks

We conducted playbacks to individuals in treatments Novel and Own between 45 min before sunrise and 45 min after sunrise. Each day, on 3 consecutive days, the same song type loop was broadcast three times in the vicinity of the territory of the focal individual. Each loop lasted 2 min, with a 3 min gap of silence between loops. The first loop started 3 min after the equipment was put in place, and was left running for a total of 12 min per experiment. We broadcast song type loops through a Visaton SC4ND speaker placed on an extendable pole extended into some available vegetation (pole height ranged from 1.5 to 5 m), and connected through a cable 10 m long to a Sony CDX-S2000 CD player. All song type loops used during playbacks were played at a sound pressure level of 90 dB at 1 m from the speaker (measured with a CEL 231 sound level meter). This volume is of a biologically realistic level.

A single repeated strophe was used to generate song type loops in Avisoft Pro Software, Version 4.39 (Avisoft Bioacoustics, Berlin, Germany). We trimmed selected strophes to a duration of approximately 3 s, always keeping phrases complete. Subsequently, we filtered them with a high-pass filter (1500 Hz), and normalized them to an amplitude of 75% of a volt. Finally, a silent gap of the same duration as the trimmed strophe was inserted after the trimmed strophe, and both were copied about 20 times one after another, until the 2 min loop was completed. Although this leads to a relatively stereotypic singing style, the playback stimuli do reflect naturally occurring singing behaviour. We selected nine unique song types for the treatment Own and nine unique song types for the treatment Novel to generate our 18 unique playback stimuli.

The strophes we used for treatment Novel were selected specifically for each individual from a large database with recordings from 20 sites across Europe (Slabbekoorn & den Boer-Visser 2006). Each novel song type consisted of two or three notes and was very unlikely to be familiar to the focal individuals as each was from a different individual originating from a distant site in Belgium, England, France, Luxembourg or the Czech Republic. Song types were selected to be distinct from any of the song types sung by the focal individual during phase 1 and we aimed to use song types that were unlike any from the local population. The selection process was based on sonagraphic inspection without more elaborate quantification. In treatment Own, the loop consisted of a strophe from the second least-often sung song type by the focal individual during phase 1. If the individual sung only two song types during phase 1 the less common song type was chosen. We chose this set-up to allow for a considerable change in repertoire use, as we expected great tits to match song types and therefore to increase the use of the played-back song type. Note that our set-up was such that our two playback stimulus sets differed in multiple ways: Own comprised a familiar song type sung by the focal males themselves and Novel comprised an unfamiliar song type sung by an unfamiliar individual. We aimed to expose our experimental birds to two diverse but consistent stimulus groups to test flexibility in repertoire use. Our set-up does not allow us to attribute behavioural changes to more specific stimulus aspects of the song types or the individual.

Repertoire and Song Measurements

We compared the number of song types sung per individual between phases for each treatment and we counted the 'new' song types sung in phases 2 or 3 that were not present in phase 1. We also tested whether there was a correlation between the number of 'new' song types added or old song types dropped and the time in the season.

We counted the phrases of each song type sung for each individual per phase. From each series of consecutive strophes of the same song type, five phrases were selected based on quality (i.e. low noise levels), and measured using Luscinia software (http://luscinia.sourceforge.net/). From the measurements obtained, we calculated average values for the following parameters: (1) duration of the first note; (2) total duration of the phrase (until start of the next phrase); (3) sound percentage (sum of the duration of notes/total duration × 100); (4) number of notes; (5) maximum frequency of the phrase; (6) minimum frequency of the phrase; (7) mean peak frequency (averaged over all notes); and (8) mean frequency bandwidth (averaged over all notes).

Song Type Similarity and Use

We assessed the overall acoustic similarity between each of the different song types sung and the song type played back for individuals in treatment Novel. First we visually compared sonagrams of the different song types using Avisoft Pro Software, Version 4.39. We further explored the similarity with a discriminant function analysis using six of the eight acoustic parameters calculated for each song type. The number of notes and the average bandwidth were not tested because of a lack of variation or a large deviation from normality.

The Euclidean distance between each song type and the song type played back, based on all eight parameters calculated, was used as a dissimilarity index. We tested whether the dissimilarity was correlated with a change in song type use between phase 1 and phase 3. Song type use was estimated as the percentage of phrases from each song type out of the total number of phrases sung during each phase.

Furthermore, we explored whether 'new' song types sung in phase 2 in immediate response to playback were special in the sense that they were more variable or, in other words, were performed with less stereotypy. We tested for differences between phases in the average coefficient of variation (CV = standard deviation divided by the mean) per song type in six of the eight acoustic parameters (the number of notes and the average bandwidth were left out again).

We tested all data for normality before performing any statistical test. We tested for outliers and carried out transformations when the data were not normally distributed. We restricted ourselves to nonparametric tests in those cases for which we were not successful in generating a data set satisfying the assumption of normality. All tests were two tailed.

RESULTS

Repertoire Size

We were able to get a sufficient set of recordings in each phase for 25 individuals in nine triplets (two individuals were omitted). The mean number of song types sung per phase ± SD was 4.25 ± 1.32 (range 2–7; Fig. 1). A song type accumulation curve described the pattern of repertoire saturation with the accumulation of recorded phrases per individual. A logarithmic regression ($r^2 = 0.59$) on these data indicated that, with a mean ± SD of 1102 ± 602 phrases recorded per phase per individual, we detected on average 90% of the song types in the individual repertoires. This suggests that the 'real' number of song types in the repertoire (for our assessment method of collecting at least 15 min of dawn chorus singing on 2–5 consecutive or semiconsecutive days) may have ranged from two to eight. We still detected at least 80% of the song types for the individual for which we recorded the minimum of 420 phrases in a single phase.

There were no significant differences between phases (Fig. 2a) in the average number of song types sung per individual in each of the three treatments (Wilcoxon signed-ranks test: all $P > 0.2$). However, a comparison between the repertoire assessment in phase 1 and the accumulated repertoire size over all two (Control) or three phases (for Novel and Own) led to a nonsignificant tendency for an increase for the first group (Wilcoxon matched-sets test: $Z = -1.89$, $N = 8$, $P < 0.1$) and a significant increase for the latter two groups (Wilcoxon signed-ranks test: $Z = -2.53$ and -2.46, $N = 8$ and $N = 9$, respectively, both $P < 0.02$). A Spearman correlation ($r_S = 0.66$, $N = 25$, $P < 0.001$) indicated that the assessment in phase 1 and the accumulated repertoire assessment across phases (which we refer to as the 'accumulated repertoire' from here onwards) show a relatively consistent rise in repertoire size for all individuals, although the relative ranking among individuals changed in several cases. The accumulated repertoire was significantly higher for the Novel group than for the Control (Mann–Whitney U test: $Z = -2.08$, $N_1 = N_2 = 8$, $P < 0.05$) and
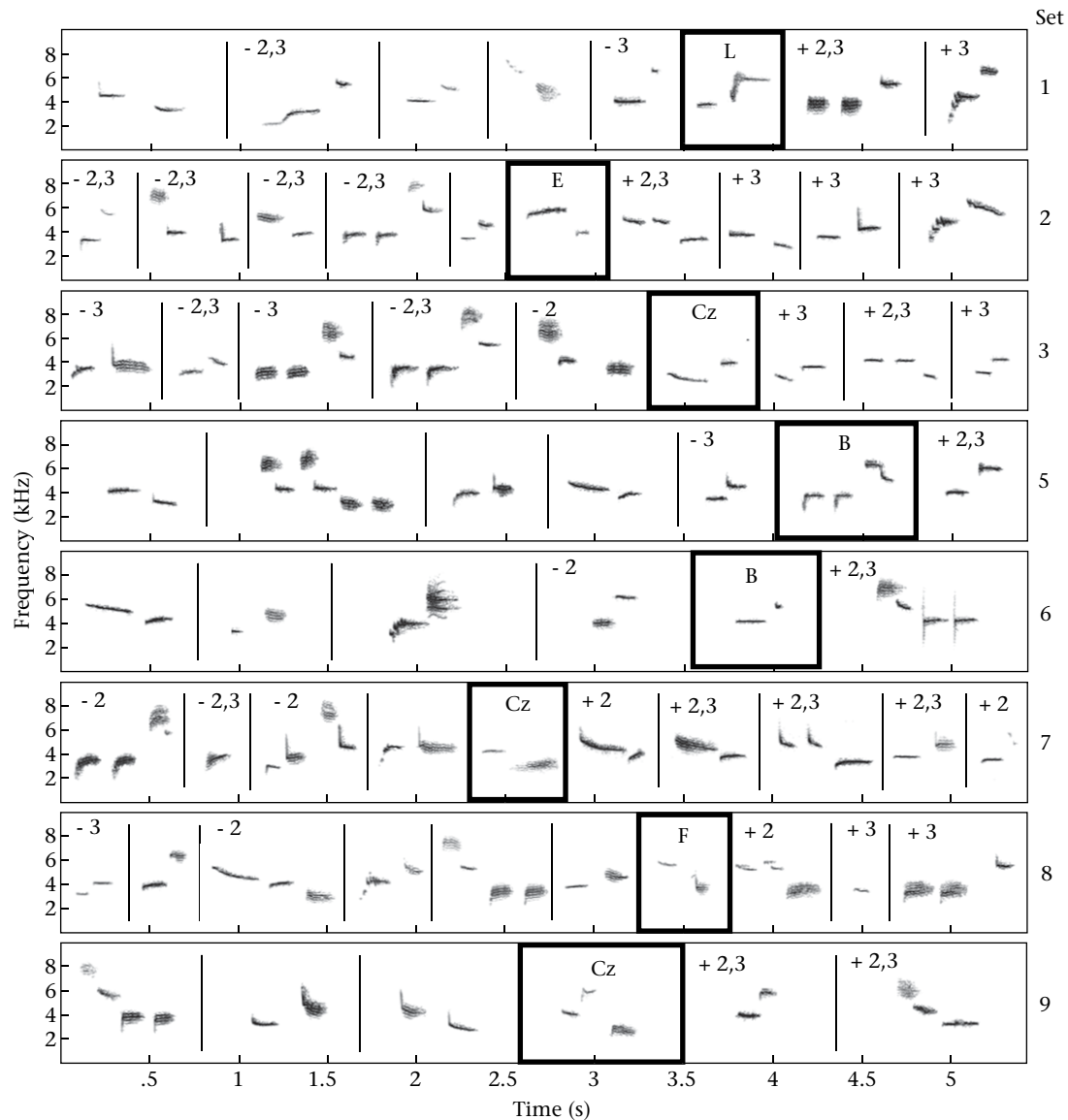
Figure 1. Sonagrams of all song types sung by eight individuals (sets 1–9, number 4 was omitted because insufficient recordings were obtained) from treatment Novel. Song types in the bold square were not sung by the individual, but are the novel song types as used during playback. These song types originate from: L: Luxembourg; E: England; Cz: Czech Republic; B: Belgium; F: France (for more details on locations, see Slabbekoorn & den Boer-Visser 2006). Sonagrams on the left of the bold squares are song types sung in phase 1, and also in phases 2 and 3, except for when their absence in these phases is indicated by −2, −3 or −2, 3. Sonagrams on the right of the bold squares are song types that were not present in phase 1, but that appeared in phase 2 (+2), phase 3 (+3) or both (+2, 3).

showed a tendency to be higher than for the Own group ($Z = -1.95$, $N_1 = 8$, $N_2 = 9$, $P = 0.05$). The accumulated repertoire of the Own group was not different from that of the Control group ($Z = -0.39$, $N_1 = 9$, $N_2 = 8$, $P > 0.7$).

Repertoire Turnover

'New' song types appeared in, and other song types dropped out of, the repertoire in all treatments, but especially among individuals in treatment Novel (and here we refer to the 'active repertoire' assessed in the classical way in a restricted time period and across a limited set of social conditions). In many of the 25 individuals at least one 'new' song type was recorded in phase 2 or 3 that was not present in our recordings during phase 1: three of eight Control individuals; all eight Novel individuals; and seven of nine Own individuals. This partly reflects our mean sampling accuracy of 90% of the active repertoire. However, the average number of 'new' song types sung by individuals during phase 3, in relation to phase 1, was significantly different between treatments (Kruskal–Wallis test: $\chi^2_2 = 8.13$, $P = 0.02$; Fig. 2b). This difference was due to a four-fold increase in the group of individuals treated with a Novel song type compared to birds from the Control group (Mann–Whitney U test: $Z = -2.91$, $N_1 = N_2 = 8$, $P < 0.01$). The group treated with Own song types did not have significantly more new song types than the Control group ($Z = -1.56$, $N_1 = 9$, $N_2 = 8$, $P > 0.1$) and showed a nonsignificant tendency to have fewer than the Novel group ($Z = -1.80$, $N_1 = 9$, $N_2 = 8$, $P = 0.07$). The increased number of 'new' song types in response to playback of novel song types cannot be attributed to sampling issues and suggests that playback in general, but especially novel song types, stimulated birds to sing song types that were not part of the active repertoire on previous days.

There was no correlation between the average (all treatments combined) number of 'new' song types added to the active repertoire and the time of the season (Spearman correlation: $r_S = 0.01$,
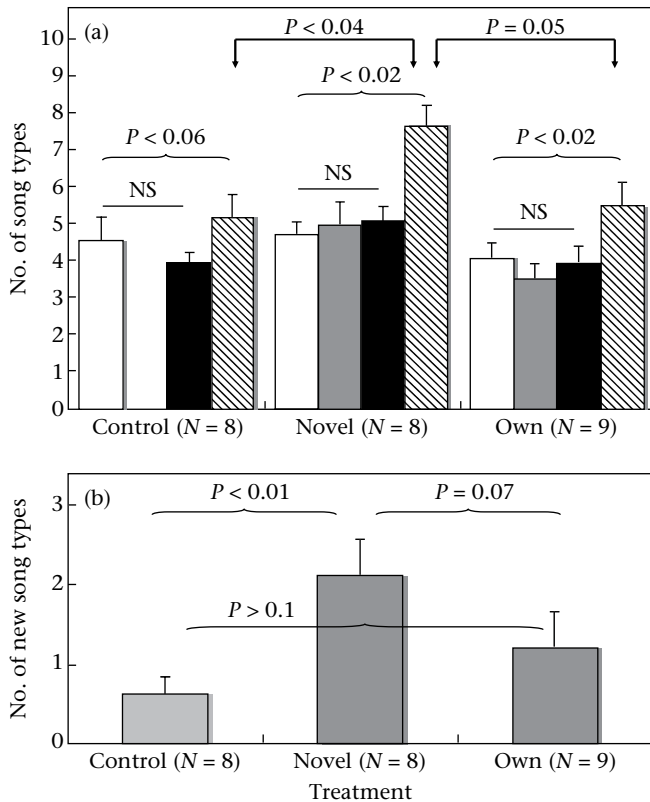
Figure 2. (a) The number of song types sung by individuals of each treatment ($\overline{X} \pm$ SE) in each phase: the 'active repertoire' (1 = white bars; 2 = grey bars; 3 = black bars). Striped bars indicate the 'accumulated repertoire'. (b) The number of 'new' song types, sung during phase 3 but not during phase 1 by individuals of each treatment ($\overline{X} \pm$ SE).

$N = 9$, $P = 0.98$). In contrast there was a significant negative correlation between the average number of song types dropped and the time of the season ($r_S = -0.88$, $N = 9$, $P = 0.002$). Individuals recorded early in the season dropped more song types from their active repertoire between the first and later phases of our experiments than individuals that were recorded later in the season (Fig. 3).

Lack of Acoustic Similarity

In individuals treated with a novel song type, none of the 'new' song types sung during phases 2 or 3 were considered accurate copies of the novel song type used for playbacks. However, some of these song types contained notes that resembled shape or frequency characteristics of the notes in the novel song type played back (Fig. 1). 'New' song types were typically unique, not resembling any of the song types in the initial active repertoire. The majority were also not known from the local population, but several 'new' song types were found in repertoires of other birds in the Leiden population.

The first two canonical functions of the discriminant function analysis explained a high percentage of the variance (cumulative average $\pm$ SD for both functions = 95.3 $\pm$ 3.74). This resulted in plots in which the different song types form distinct clusters (Fig. 4). In three of the eight individuals from treatment Novel, at least one of the 'new' song types was plotted nearer to the treatment than any of the other song types in the accumulated repertoire (Fig. 4a). However, in the remaining five individuals one of the song types in the initial active repertoire was plotted closer (Fig. 4b). This means that there is no evidence that 'new' song types matched the acoustic structure of the novel song type stimulus.
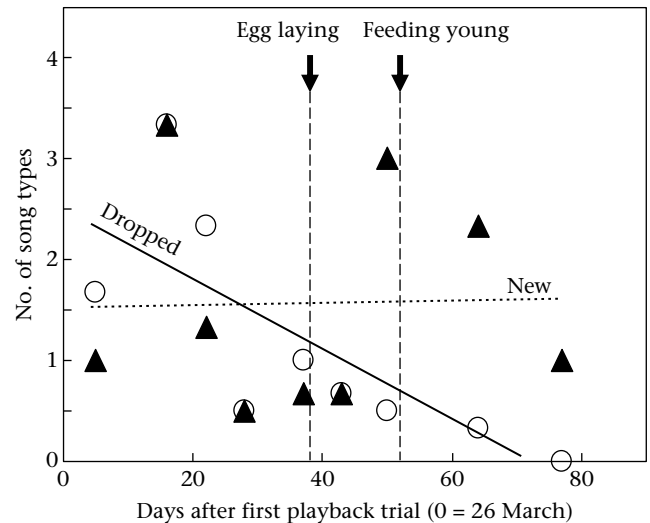

Figure 3. The relationship between repertoire turnover and the progression of the season. Triangles indicate the mean number of 'new' song types that appeared in phase 2 or 3 and that were not present in phase 1. Circles indicate the mean number of song types present in phase 1, but dropped in phase 3. Each data point is the mean of three individuals, one from each treatment group, except for the samples on days 28 and 50, which are based on only two individuals.

Shifts in Song Type Use

The relative rate at which the different song types of a repertoire were sung varied between phases. The many new and dropped song types, and large changes in the use of song types that were retained throughout the three phases, led to large shifts in the percentage at which song types were sung. Typically the great tits had one song type that was sung most often, comprising a mean $\pm$ SD of 49 $\pm$ 13% of all phrases sung. However, the use of this song type also varied dramatically: the percentage of the total number of phrases dropped on average 28 $\pm$ 21%. For five individuals, recorded early in the season, this most frequently used song type was even one of the song types that dropped completely in phase 3. The maximum change in use of a specific song type between phases 1 and 3 concerned a 29% increase on average (range 8–68%) and a 29% decrease (range 8–84%). New song types appeared at an average percentage of 21% (range 3–78%) among the total number of phrases sung.

The playback of Own song types led to a significant rise (Wilcoxon signed-ranks test: $Z = -0.88$, $N = 9$, $P = 0.002$) in the use of the played-back song type from 16 $\pm$ 10% to 41 $\pm$ 26%: eight of nine song types matched; only one individual refrained from singing the specific song type. The use of the played-back song type did not lead to a lasting effect, as the use was down again to 25 $\pm$ 17% in phase 3, which was no longer different from the use in phase 1 (Wilcoxon signed-ranks test: $Z = -0.008$; $N = 9$, $P = 0.98$).

There were also considerable shifts in repertoire use in the group treated with Novel song types, but the changes in specific song types were not related to how similar they were to the played-back song type (Fig. 4c, d). Overall there was no correlation between the dissimilarity index and the change in the percentage at which phrases occurred in phases 1–3; in seven of eight individuals the correlation between similarity and change in use (increase or decrease) was not significant ($P > 0.5$). A significant correlation between the dissimilarity index and the change in phrase percentage from phases 1 to 3 was found in only one individual (Fig. 4c). This analysis combined song types that were already present in phase 1 and sung later at a different rate relative to the other song types in the active repertoire, as well as the 'new'
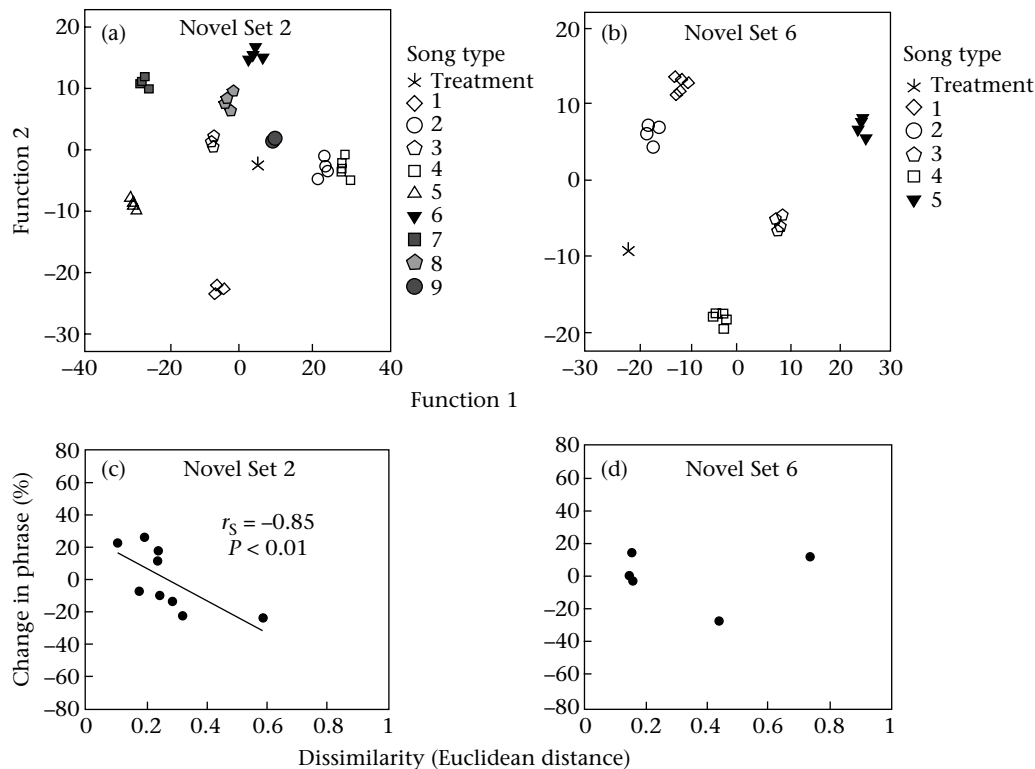
Figure 4. (a–b) Plots of the first two canonical functions from a discriminant function analysis for all the song types sung by two individuals (Set 2 and Set 6) treated with a novel song type, including the novel song type that was used for playback. Open symbols are labels for song types in the original active repertoire (phase 1) and closed symbols are labels for 'new' song types (phases 2 and/or 3). There were five replicate measurements per song type, forming distinct clusters. (c–d) Correlation between the change in use of each song type from phase 1 to phase 3 and the dissimilarity index (Euclidean distances) of each song type in relation to the novel song type that was used for playback. (c) The only case in which the similarity between song type sung and song type heard through playback was related to a change in use: more similar song types became relatively more frequent in phase 3. The other seven individuals in this treatment did not show such a pattern (e.g. Novel Set 6 shown in (d)).

song types that were not yet present in phase 1, but turned up as part of the accumulated repertoire.

Lack of Stereotypy in 'New' Song Types

The duration of the first note was distinct from all other parameters that we measured in terms of variability (Fig. 5). We calculated the coefficient of variation over 5–15 renditions (mean 8.7) of the same song type in the treatment Novel group. In the already most variable parameter, the duration of the first note, the coefficient of variation of the 'new' song types sung in phase 2 ($N = 8$ individuals; 15 song types) was higher than the coefficient of variation of song types sung in phase 1 ($N = 8$ individuals; 37 song types), and higher than the same 'new' song types sung in phase 3 ($N = 7$ individuals; 9 song types; ANOVA, Tukey's *b* post hoc test: $F_2 = 7.52$, $P = 0.02$, significant after Bonferroni correction for multiple testing). The coefficient of variation of this same parameter, duration of the first note, was equally variable across all three phases for the song types already present in phase 1 ($N = 7$ individuals; 19 song types). These results suggest that song types sung in immediate response to playback, and that were not recorded before, were distinct in lack of stereotypy from all other song types. This distinction was gone in the subsequent days after which the focal individuals had had ample opportunity to sing and practise these 'new' song types repeatedly.

DISCUSSION

We have shown that great tits make use of their song type repertoire in a very flexible way. All birds recorded across two or three phases revealed dramatic changes in how often they sang the

different song types in their repertoire. Several song types were missing in one or two of the recording phases, which suggests that they were not always actively used and we therefore regard it useful to refer to the 'active repertoire' when discussing the within-phase repertoire assessments. The size of this active repertoire remained relatively constant between phases, but the three phases accumulated into a significantly larger combined repertoire, which we refer to as the 'accumulated repertoire'. Turnover occurred in individuals of all treatments, whereas 'new' song types appeared more often among birds treated with a novel song type. Across the breeding season there was no change in the likelihood of acquiring 'new' song types, but the dropping of song types decreased towards the end of the season. None of the 'new' song types added to the repertoire were considered accurate copies of the novel song type used during playbacks. Only some of the song types suggested partial matching. Moreover, there was no correlation between the change in use of the different song types (before and after playbacks) and their similarity with the novel song type played back.

How Big is a Great Tit Repertoire?

The considerable and rapid turnover in active repertoire composition found in our study warrants the question whether our repertoire assessment within a single phase is still a relevant measure and useful in comparing studies. The recording effort and timing are not likely to be very different from those in other studies, and also the outcome has led to similar and, if different, larger instead of smaller repertoires. The average ± SD for the active song type repertoire size in our study area was 4.25 ± 1.32, and this is very comparable to a population in Great Britain (3.07 ± 0.60; McGregor et al. 1981) and a population in Belgium (4.16 ± 1.27;
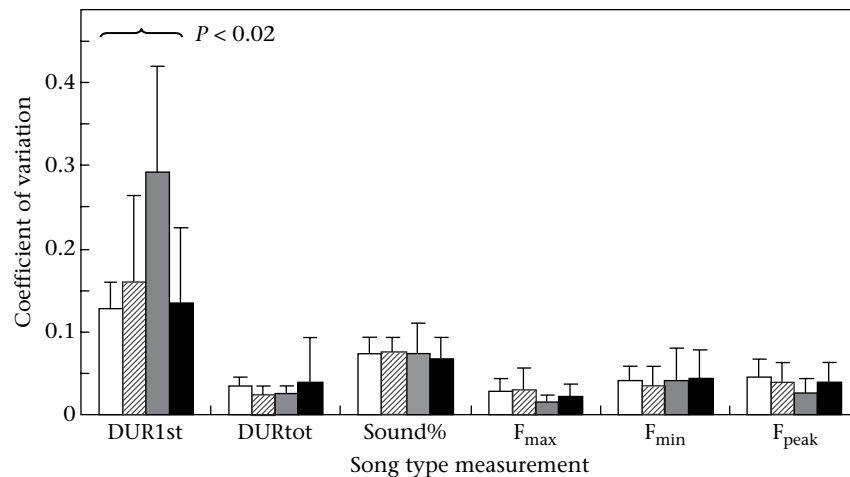
Figure 5. The coefficient of variation for six of the eight acoustic parameters for song types sung by individuals in treatment Novel: the duration of the first element (DUR1st); the total duration of the phrase (DURtot); the sound percentage (Sound%); the maximum frequency (F_{max}); the minimum frequency (F_{min}); and the peak frequency (F_{peak}). We calculated this coefficient for a set of renditions of the same song type and the error bars reflect the standard deviation among individual song types of different individuals (see text). White bars: old set of song types sung in phase 1; striped bars: the accumulated repertoire; grey bars: 'new' song types sung during phase 2; black bars: the same 'new' song types sung during phase 3.

Lambrechts & Dhondt 1986). When we consider that we recorded only 90% of the song type repertoire on average, the actual number of song types sung on the recording days within a single phase may have been 4.72, and, similarly, the other studies may also reflect an underestimation.

However, when we consider the accumulated repertoire size as recorded over three phases, both groups of experimental birds that received a playback treatment had a significantly larger repertoire. The eight birds that were confronted with a novel song type they had never heard before also had significantly more new song types after playback. Their average accumulated repertoire size was 7.25 and ranged from 5 to 9, all well above the corrected average. Would these birds have come up with other song types again if we had played another novel song type on subsequent days? Our results clearly suggest that recording effort and the time span and social conditions, such as number of new neighbours (McGregor & Krebs 1989), will have a big impact on repertoire assessments in great tits. We do not know whether this is also true for other species (but see Nicholson et al. 2007). The well-studied song sparrow, *Melóspiza melodia*, seems not to have such flexibility (Marler & Peters 1987; Nordby et al. 2002): changes in repertoire composition in very similar playback experiments only concern how often song types are sung, not the emergence of 'new' song types (Burt et al. 2002; Anderson et al. 2005).

Despite a high turnover in active repertoire composition and many new song types in our great tit study, there was no significant increase in repertoire size when we compared phases before and after playback. Birds adding previously unrecorded song types almost always also dropped song types that were part of the earlier active repertoire assessment. Therefore, our results suggest that great tit males are able to produce more different song types than they are actually singing within a particular timeframe. A recent study on sedge warblers, *Acrocephalus schoenobaenus*, also revealed the sudden appearance of previously unrecorded syllables without an increase in repertoire size (Nicholson et al. 2007). Male sedge warblers stop singing a few days after a female has been attracted, and female preferences have always been assumed to be the main driving force of their relatively large repertoire (Catchpole 2000). However, syllable sharing among male competitors also seems to be behind the observed changes in repertoire composition in this species as sharing within pairs of new neighbours increased over a period of several days.

If it is true that singing a large repertoire has an advantage in inter- and intrasexual signalling (Krebs et al. 1981; Baker et al. 1986; Gil & Gahr 2002), why then are great tits and sedge warblers not making use of their full potential at all times? Are there cognitive limitations related to some sort of working memory? In that case, the traditional repertoire assessments may still carry a biologically more relevant signal about male quality than more extensive effort assessments. However, these new insights warrant new studies testing the signalling function of active and accumulated song type repertoire size (including silent or passive song types). In this context, it would also be interesting to explore seasonal changes in flexibility further: although there was a lack of a seasonal pattern in appearance of new song types in our great tit study, dropping of song types was especially high early in the season after which neighbour changes would become less common.

Functional Implications of Repertoire Turnover

The advantage of having a large repertoire in the context of male–male territorial interactions has mostly been explained as an increased chance of matching song types sung by neighbours (Krebs et al. 1981; Falls et al. 1982; Beecher et al. 2000). Matching or partial matching may increase signalling strength or may be used to address signals at particular individuals (Krebs et al. 1981; McGregor et al. 1992; Burt et al. 2001), both of which are thought to allow neighbours to settle disputes vocally, before one of them gets hurt or loses energy or time unnecessarily. In some song sparrow populations, song type sharing can be positively correlated with territory tenure (Beecher et al. 2000) and across-season survival rates (Wilson et al. 2000), providing evidence for direct fitness consequences. If matching neighbours is the critical feature, it would make no difference whether territorial males had a small or a large active repertoire as long as they had, for example, two matching song types in common with their direct neighbour. However, small repertoires with two matching song types are only likely to occur if birds are able to exchange song types to adjust to new neighbours. This may be impossible for song sparrows, but our results suggest this is exactly what great tits are able to do.

The advantage of having a large repertoire in the context of mate attraction is not well understood (Kroodsma 2004). Although repertoire size is correlated with mating success in several species (Yasukawa et al. 1980; Catchpole 1986; Hasselquist et al. 1996),

including the great tit (Baker et al. 1986), it is not clear whether females actually assess repertoire size, or some other correlated vocal or ecological parameter. Nevertheless, active repertoire size in great tits is correlated with a higher lifetime reproductive success (McGregor et al. 1981), which can be seen as the outcome of the signal's impact on both males and females, but also as the result of a vocal relationship with some male quality (see e.g. Doutrelant et al. 2000; Parker et al. 2006). Learning a large repertoire may have a neurobiological cost (Nottebohm et al. 1981; Canady et al. 1984), and therefore may carry an honest signal of male quality. Similarly, with our current insight into the flexible repertoire of great tits, we may now argue that singing a particular set of song types within a certain time period may require some sort of cognitive capacity. If this capacity also positively affects survival and reproductive success, active repertoire size may indeed carry valuable information for females looking for the best father for their offspring. However, more critical studies are needed to show whether and how repertoire size is used as a cue for mate choice (Kroodsma 2004), and whether repertoire size (active and/or accumulated) is correlated with some male quality.

Recall From Memory Versus Open-ended Learning

It is now clear from our results that adult great tit males are able to come up with more song types than those that they have been singing for a day or two (the set traditionally used for repertoire assessments). However, it remains unclear whether the 'new' song types were really newly generated or just recalled from memory. The 'new' song types sung in response to playbacks of a novel song type were more variable in the duration of the first note than other song types in the active repertoire. A larger variation in song components is usually observed in plastic song of the sensorimotor phase for song learning (Hultsch & Todt 2004). This suggests they form, at least temporarily, a different category of song types, which may be because they have not been used recently or because they are being practised for the very first time.

McGregor & Krebs (1989) suggested that adult great tits may be able to learn song types from new neighbours in successive breeding seasons (i.e. they may be open-ended learners). Adult song learning has been demonstrated in other songbird species, such as in canaries and European starlings (Nottebohm & Notte-bohm 1978; Eens et al. 1992). However, the details of the sensitive periods for song memorization and song production have not been studied in the great tit. Our results do not exclude, but also do not provide evidence for, the ability to memorize songs in adults. None of the individuals treated with a novel song type copied the detailed structure of the song type used in the playbacks, suggesting they were not able to learn or improvise them. However, our playback protocol provided only a very brief exposure, and it is still possible that longer treatments could enable birds to converge more towards the novel song type. Furthermore, other types of behavioural interactions may be needed to learn new song types (Hultsch & Todt 2004), and thus the possibility of more accurate imitation as an adult cannot yet be ruled out.

Nevertheless, recalling song types from memory may be the most plausible explanation for the observed changes in repertoire composition in great tits. The phenomenon has been reported in several other songbird species. In white-crowned sparrows, *Zonotrichia leucophrys*, for example, young birds may start singing multiple song types, but later in life, after interactions with conspecifics in the breeding territory, they restrict themselves to a single song type (Nelson 2000b). It has even been shown with hand-reared individuals, of this so-called 'nonrepertoire species', that they are able to recall the previously sung but deleted song types from their memory (Hough et al. 2000). Similarly, hand-reared European starlings and nightingales are able to store song

types (not imitated early in life) silently, and these can be activated later in life through interactive playbacks (Chaiken et al. 1994; Geberzahn et al. 2002; Geberzahn & Hultsch 2003).

Great tits are able to memorize and discriminate song types they do not sing (McGregor & Avery 1986), and young males have plenty of opportunity to get exposed to a wide variety of song types sung by nearby territory holders before they settle in a territory of their own. The 'new' song types in our study did not match the unfamiliar Novel song types from very distant populations, but several of them were also present in the repertoires of other birds in our study population. At the moment, therefore, the most likely explanation for the emergence of 'new' song types in adult great tits seems to be that they recall song types from memory. Previously heard and memorized song types, irrespective of whether they have been sung before or not, appear to be activated by our playback simulation of the arrival of a new neighbour with an unfamiliar song type.

Conclusions

Our results show that the composition and use of the song type repertoire of individual great tits is more flexible than previously thought. Repertoire turnover, probably mediated by social interactions, may be explained by a mechanism of recalling unsung song types from memory, although adult learning abilities can still not be excluded for great tits. The relatively constant repertoire size per individual despite considerable song type turnover, and a significantly larger repertoire when all phases are accumulated, warrants a conceptual reconsideration of the role of repertoire size as a signal of male quality. Future studies on any species will probably benefit from considering the potential of an existing active and passive song type or syllable repertoire, which together form an accumulated repertoire. The hypothesis of a limited working memory constraining the exploitation of a larger song type library memorized earlier in life needs further exploration. However, we do know now that, relatively independent of the actively used repertoire size, great tits may apply their repertoire flexibility to adjust to new neighbours as well as to changes in environmental conditions, such as a rise in anthropogenic noise (Slabbekoorn & den Boer-Visser 2006; Slabbekoorn & Ripmeester 2008).

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