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# Higher songs of city birds may not be an individual response to noise

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It has been observed in many songbird species that populations in noisy urban areas sing with a higher minimum frequency than do matched populations in quieter, less developed areas. However, why and how this divergence occurs is not yet understood. We experimentally tested whether chronic noise exposure during vocal learning results in songs with higher minimum frequencies in great tits (*Parus major*), the first species for which a correlation between anthropogenic noise and song frequency was observed. We also tested vocal plasticity of adult great tits in response to changing background noise levels by measuring song frequency and amplitude as we changed noise conditions. We show that noise exposure during ontogeny did not result in songs with higher minimum frequencies. In addition, we found that adult birds did not make any frequency or song usage adjustments when their background noise conditions were changed after song crystallization. These results challenge the common view of vocal adjustments by city birds, as they suggest that either noise itself is not the causal force driving the divergence of song frequency between urban and forest populations, or that noise induces population-wide changes over a time scale of several generations rather than causing changes in individual behaviour.

## 1. Introduction

The past decade has seen a dramatic increase in research into the effects of anthropogenic noise on the lives of animals, and much of that research has focused on vocal behaviour and communication. Anthropogenic noise from street, air and boat traffic is dominated by low-frequency sounds, and associated upward changes in the frequency components of acoustic signals have been reported in half of the songbird species tested on five continents [1], some whale species [2], frogs [3] and even insects [4], suggesting that this is a global phenomenon. However, there are few studies examining the mechanisms underlying the observed changes.

Vocal differences have been found between individuals in noisy urban centres and conspecifics in quieter areas in more than 25 species of songbirds [1]. One trait that has been the focus of most studies is minimum song frequency. The first study to describe an increase in song frequency was a study of great tits (*Parus major*), one of the commonest songbirds across Europe and Asia, and a successful urban colonizer [5]. Urban great tits have been found to sing with a higher minimum frequency than those in nearby forest populations in over 30 city-forest paired locations in Great Britain, Continental Europe and Japan [6–8]. Some studies found that even within a city, birds in noisier areas sang with higher minimum frequencies than those in quieter areas [5,9]. Three mechanisms have been proposed as to how noise may lead to the observed changes: ontogenetic effects including vocal learning, adult song plasticity and microevolutionary changes [10].

### (a) Noise impacts on vocal development

There are several processes by which low-frequency noise exposure during vocal learning might lead to upward changes in song frequency. In continuous noise at levels above 93 dB (re. 20  $\mu$ Pa) songbirds are likely to experience a temporary

hearing threshold shift, and above 110 dB to experience permanent ear damage [11]. Although noise levels in cities or near motorways may have transient peaks above 90 dB, it is rare that such events are sustained long enough to induce hearing damage in birds [11]. In juvenile songbirds, noise loud enough to cause temporary threshold shifts, resulted in severe impairment of song development, and in the formation of the underlying song control circuits in the brain [12,13]. That noise disrupting hearing impairs vocal learning or song production is not surprising, however the effects of intermediate noise levels, like those commonly experienced in urban habitats, on song development are less known.

Noise at more moderate levels is unlikely to result in physical damage to the ears, but still can impact the process of song learning in multiple ways. In cities and along motorways, anthropogenic noise is predominantly low frequency, with most of the sound energy concentrated in the frequency range below 3 kHz. It is possible that such low-frequency noise masks the lower frequency parts of songs. Young birds listening to adult tutors may therefore hear the higher frequency components of songs better, and hence be more likely to learn those aspects of the tutor songs [14,15]. Young songbirds go through periods of sensory and sensorimotor plasticity during the song learning process. During the sensorimotor phase, vocal output is compared with memorized 'templates' based on tutor songs [16]. Noise may also interfere with this self-assessment of a juvenile's own song, and thus bias song output towards higher pitches [17].

Alternatively, noise could disrupt vocal learning by a more indirect effect, such as by inducing physiological stress responses that interfere with learning behaviour or cortical development. In rats, it has been shown that chronic exposure to intermediate (approx. 70 dB sound pressure level (SPL)) levels of noise causes a delay in the development of the auditory cortex [18]. Rats exposed to higher levels of noise (90 dB SPL) during development grew more slowly and performed poorly in learning tasks [19]. In human children, noise has been found to disrupt learning and memory, especially language comprehension tasks (e.g. [20,21]), as well as accuracy of vocal production [22]. Whether traffic noise leads to learning or developmental delays in birds is not known, but experimental exposure to chronic noise has been shown to increase embryo mortality and delay somatic growth in zebra finches [23], which suggests that birds may suffer some of the same physiological consequences of noise pollution that have been found in mammals.

## (b) Noise, adult plasticity and microevolutionary changes

Traffic noise might not influence song learning or song features during ontogeny, but rather may trigger changes in the songs of individual adults, or influence song features on longer, microevolutionary time scales. Adult birds across a broad taxonomic range have been shown to have a degree of vocal plasticity in response to changes in background noise levels. Even phylogenetically basal birds exhibit the Lombard effect [24], a vocal phenomenon whereby vocal amplitude increases as background noise increases [25]. The Lombard effect in humans is often, but not always, associated with a concurrent increase in vocal frequency or a shift in spectral tilt (reviewed in [25]). One hypothesis, therefore, is that birds in noisy urban areas sing louder owing to the Lombard effect, and this, in turn, results in a rise in pitch [26].

While it is generally assumed that the primary driving force behind the upward shift in song frequency is the high level of low-frequency noise, experimental evidence in support of this assumption is limited. In the house finch *Haemorrhous mexicanus*, individuals sang with a higher minimum frequency when exposed temporarily to noise [27]. Silvereyes (*Zosterops lateralis*), immediately lowered the minimum frequency of their calls in response to high-frequency noise playback, but did not raise minimum frequency in response to low-frequency noise [28]. In another study, long-term experimental traffic noise exposure in zebra finches *Taeniopygia guttata*, led adult males to sing with lower minimum frequencies after a five month long period of noise exposure [29], an adjustment opposite of what would be predicted by the hypothesis that birds shift songs up to gain a release from masking noise, and opposite of what would be predicted by the Lombard hypothesis.

In great tits, the response at the individual level is unclear. When exposed to experimental noise playback in the field, fewer than half (41%) of the individuals switched to new song types, while the rest (59%) did not [30]. Of those individuals that did switch song types, the switch was made to a song type with a higher minimum frequency than the song type they were singing when low-frequency noise presentation began, or to a lower song type when high-frequency noise began. Additionally, many of the birds switched song types only after the noise exposure ended [30], calling into question whether the noise exposure induced the song change.

Finally, it may be that the differences in frequency between the vocalizations of urban and rural populations are not the result of individual plasticity, but of gradual changes over many generations. As in birds, upwards trends in frequency have been reported in grasshoppers living near noisy motorways [4]. Lampe and colleagues [31] demonstrated that this signal divergence is the outcome of both cross-generational effects of environmental noise and developmental plasticity. In songbirds, such cross-generational effects could be mediated by genetic or cultural changes. Although birds have much longer generation times than grasshoppers, precluding similar experimental investigations, Luther & Derryberry [32] used historical records to reconstruct changes in song over a 36 year period in a city population of songbirds in San Francisco, and found that song frequencies have changed over time as noise levels have risen. Since the songs of most oscine songbirds are learned [33], it is hypothesized that their vocalizations could adapt more quickly to environmental changes, through cultural evolution [34]. In line with this notion, Rios-Chelen *et al.* [35] found that oscines diverged more in minimum song frequency between noisy and quiet habitats than suboscines, which are not thought to acquire their song through vocal production learning. Thus, although urban noise pollution is a relatively recent phenomenon, it is possible that the trends of vocal divergence of urban birds across the globe are indicators not of many individuals responding individually to fluctuating noise conditions, but rather the result of cultural or microevolutionary shifts at the population level.

We present here data from two behavioural domains: vocal learning and adult song plasticity. In particular, we present: (i) the first experimental study, to our knowledge, of the effects of chronic exposure on song frequency in a songbird that is a frequent inhabitant of noise-polluted habitats, and (ii) tests of adult vocal plasticity in response to changing background noise conditions.

## 2. Material and methods

### (a) Vocal Ontogeny study

#### (i) Birds and rearing conditions

We collected 20 male nestling great tits 8–12 dph (days post hatch) in forests around Starnberg, Germany. We used molecular markers to sex nestlings before collection. Nestlings were then hand-raised in the laboratory in two acoustically isolated groups and exposed to one of two noise treatments during their entire first year. Siblings were divided between the two treatment groups (1–2 siblings per treatment group, depending on the number of males in a nest box). After birds were fledged and feeding independently (approx. 50 dph) they were housed in single cages (125 × 44 × 40 cm) visually, but not acoustically, isolated from other birds in their group.

#### (ii) Noise exposure and song tutoring

The two noise treatments were city-like ('CITY') noise, consisting of filtered white noise 0–3 kHz with a 500 Hz linear roll-off. The CITY noise was designed to simulate the average noise profile found in a busy urban area, while at the same time being more extreme both in spectral shape and temporal consistency (see the electronic supplementary material, figure S1). Further details describing the degree of masking of the minimum frequency of each tutor song in each noise treatment is provided in the electronic supplementary material, table S1. The control noise group was exposed to noise with the same bandwidth as the CITY group, but covering high, rather than low frequencies (3–6 kHz band-pass filtered white noise, 500 Hz linear fade-in, 100 Hz linear roll-off). The CONTROL noise treatment was designed to control for non-auditory effects of noise exposure, but with noise in a different bandwidth than that of most traffic or urban noise sources. The SPL of the noise was 60–63 dB SPL (re. 20  $\mu$ Pa) at the position of the middle perch in each cage. In addition to the noise playback, both groups of birds were tutored with nine great tit songs (electronic supplementary material, figure S2) that were recorded the previous year from nine adult males in the quiet forested area where our nestlings were collected. Typical great tit song consists of several repetitions of identical song phrases (motifs). Phrases or motifs often contain two to four different notes or 'syllables', and the bird alternates between high and low pitched syllables to produce the characteristic great tit 'teacher teacher' song (electronic supplementary material, figure S2). Tutor songs were selected to include both the lowest and highest minimum frequency song types recorded in the population. As great tits probably learn songs from tutors during both their pre-dispersal time near their natal area and post-dispersal from neighbours during territory formation in the late winter/early spring the following year, we continued tutor playback until the birds were a full year old, when song is thought to crystallize and then remain constant throughout life [36]. Each tutor file was 30 s long and included 10–24 motifs in total. Playback consisted of 1 h blocks of the nine tutor files in randomized order. These 1 h tutor playback blocks occurred one to four times per day during the first year post-hatch.

#### (iii) Song recording

When the birds were 1 year old (340–370 dph), they were moved individually into wire cages inside custom built sound-isolating recording chambers that measured 70 × 50 × 50 cm inside. Cages were equipped with two wooden perches approximately 35 cm below a microphone (Behringer C-2), mounted above the centre of the cage. Song was recorded using SOUND ANALYSIS PRO v. 2.063 [37], to a computer hard drive through an M-Audio Delta 44 external sound card (44.1 kHz, 16 bit). Birds were first recorded for 1–2 weeks in the same background noise condition in which they were reared. Noise playback in the recording

chambers was through a Pioneer A-109 stereo amplifier and Kenwood JVC Pro-III loudspeakers.

#### (iv) Song analyses

From the 20 nestling birds, nine CITY and 10 CONTROL birds survived to adulthood. Some birds did not sing enough for statistical analyses in the recording chambers, so were excluded from the analysis, leaving seven birds in the CITY group and six birds in the CONTROL group.

To determine whether mean minimum frequency of our noise-exposed birds differed from the minimum frequency of the tutor songs, we measured a mean minimum for each song type in each bird's repertoire. When possible we took these measurements from 50 or more repetitions of the song type, but included songs only if there were at least 12 repetitions.

Minimum frequency was measured in all three studies (this and the two below) at a set threshold below the peak frequency [38]. To this end, we used a script written in IGOR PRO v. 5 (Wave-metrics Inc.) that determined the peak frequency, and the frequency –22 dB below that peak in a power spectrum ( $f_s$  22 kHz, 1024-point FFT, Hamming window, 22 Hz frequency resolution) for each syllable. We used the –22 dB cut-off because this was the lowest threshold at which the minimum frequency could be measured given the signal-to-noise ratio of the quietest syllable type in noise for our set of recordings.

#### (v) Statistical analyses

For each bird we took the mean minimum frequency of each song type in the bird's repertoire. The average number of syllables measured per song type was 41.7 (range 17–61) in the CITY noise condition, and 41.8 (range 14–60) in the CONTROL condition. For each bird, an overall mean minimum frequency was calculated by taking the average of all these mean minimum frequencies for all the song types in their recorded repertoire. We then tested whether the mean of the minimum frequencies of all the songs in each bird's repertoire were different from those of tutor songs with a Kruskal–Wallis test.

### (b) Adult Plasticity study 1

#### (i) Syllable frequency plasticity—birds and noise treatments, song analysis

We tested whether the hand-raised birds from our Vocal Ontogeny study would modify the frequency content of their songs when background noise conditions changed after song crystallization (more than 365 days post hatch). We compared the minimum frequency of songs of birds in different noise conditions with the same song types sung in the noise condition in which they were reared. After birds were recorded in their 'home' noise condition, we changed the noise playback to either the opposite noise treatment (i.e. CITY birds were exposed to CONTROL noise, and CONTROL birds were exposed to CITY noise), or to a no-noise treatment. Birds were housed in the second noise treatment for 1–2 weeks, with noise playback at the same intensity levels as the previous noise condition (60–63 dB SPL) or, in the no-noise treatment, with no playback during the 1–2 week period (ambient noise levels in the sound recording chambers was 35–40 dB SPL).

#### (ii) Syllable frequency plasticity—statistical analyses

We compared the mean minimum frequency of each syllable type that each individual sang in more than one noise condition. We only included a syllable if the individual sang it at least 10 times in each noise treatment. Syllables were given unique identifiers, so that even if more than one individual sang the same syllable type, these were only compared within a bird, not between individuals. We compared differences in minimum frequency between



noise treatments (CITY, CONTROL and NO-NOISE) using a linear mixed model (LMM) with syllable nested inside bird ID as a random factor, the difference in min frequency as predicted value, and pairs of background noise treatments as a fixed factor (CITY versus CONTROL, CITY versus NO-NOISE, and CONTROL versus NO-NOISE). The test was performed independent of noise presentation order. Because of low sample sizes, we have included effect sizes for each test in the three Adult Plasticity studies (electronic supplementary material, figures S3 and S4). In significant models we calculated adjusted  $r^2$  for generalized linear models according to [39]. In non-significant models we plotted effect sizes with confidence intervals. We calculated significance using an analysis of deviance test with 2 degrees of freedom. Statistical analyses were done with R v. 3.2.1 and the packages lme4 and MuMIn.

### (c) Adult Plasticity study 2

#### (i) Syllable type usage—birds, treatment and song analysis

To test whether birds might selectively use certain song types from within their repertoires to minimize overlap with current noise profiles, we recorded adult birds from our Vocal Ontogeny study (see above) in both their 'home noise' and in the 'opposite noise' (i.e. CITY noise for CONTROL birds, and CONTROL noise for CITY birds). We then compared the average minimum frequencies of all song types sung during three randomly selected 5 min intervals in each background noise condition. If birds selectively sing song types with higher minimum frequencies in low-frequency noise than they do in high noise or no noise, then these differences should be detected in the mean minimum frequencies sung during these randomly chosen intervals in each noise condition.

#### (ii) Syllable type usage—statistical analyses

We tested the influence of rearing conditions on the minimum frequency of songs during three 5 min intervals, and whether the minimum song frequency changed when noise conditions changed, with LMMs. Noise treatment during rearing was a fixed effect, and individual was a random effect. Songs were taken as repeated measurements of one individual without distinguishing different syllables. First we compared the minimum frequency of song of seven birds raised in the CITY-noise condition with four birds raised in CONTROL-noise (1553 songs total, 18 to 296 songs per bird). We tested with a log-likelihood test whether the model was better than a null-model without treatment.

Second, we investigated whether birds switched to using song types with different minimum frequencies in a changed noise condition (five CITY birds exposed to CONTROL noise, and four CONTROL birds first exposed to CITY noise; in total 1538 differences in minimum frequency, with 43 to 271 measured songs per individual). Rearing condition was a fixed effect, and individuals were random effects. Here the tested value was the difference of the minimum frequency in the changed condition to the average minimum frequency of the same individual in the condition it had been raised in (means calculated from 36 to 296 songs per individual). Again the model was compared to a null-model with no influence of the raising and corresponding testing condition.

### (d) Adult Plasticity study 3

#### (i) Lombard effect—animals, housing, noise playback and song recording

Finally, we tested whether noise-induced increases in song amplitude correspond with increases in song frequency in adult birds. We caught six adult male great tits from quiet forest areas around Starnberg, Germany. Birds were housed singly in cages  $125 \times 44 \times 40$  cm, which were placed on tables in a sound-shielded room and provided with food and water ad libitum.

Birds were visually, but not acoustically isolated from each other, although the sounds of neighbouring birds were dampened by panels of sound-absorbing foam.

We exposed singing birds to filtered white noise (1–10 kHz band pass Butterworth filter) at levels between 65–70 dB(A) SPL, measured at the position of the perches. Noise was played from a computer with an external digital sound card (Edirol UA-25EX), fed through a Pioneer A-109 stereo amplifier to two JBL Control 1 Pro loudspeakers. During recording sessions, all but two perches were removed from the cages, with the two remaining perches placed at the same height and 15 cm apart. Sennheiser ME62 microphones were positioned 50 cm above the cage, equidistant between the two perches. We calibrated the recordings using a calibration microphone (Behringer ECM8000) and acoustic calibrator (Casella CEL-184). When birds were habituated to the room and singing regularly, we recorded their song in the quiet room (average ambient room noise 35 dB(A) SPL) and then again during noise playback. Recordings were made using Sound Analysis PRO v. 2.063 at a sampling rate of 44.1 kHz and 16 bit resolution.

#### (ii) Lombard effect—song analysis

Using calibrated song recordings in Avisoft SASLAB PRO v. 5.2, we measured the root mean square (RMS) amplitude of each individual syllable in each motif in each song type that birds sang in both no-noise and noise conditions. We then subtracted the noise using logarithmic computational rules [25] to determine the RMS values for the syllables themselves in both noise treatments.

For measuring the peak frequency of each syllable, we resampled the song recordings at 22.05 kHz, and created power spectra for each syllable (1024-pt FFT, Hamming window, 22 Hz frequency resolution). Because of the signal-to-noise ratios in these recordings and because of the wideband spectrum of the noise used to elicit the Lombard effect, minimum frequency would be difficult to measure reliably.

#### (iii) Lombard effect—statistical analyses

Amplitude and frequency of the same syllables between the noise-exposed and control treatments were analysed with LMMs, with noise or no-noise as fixed effects, and individual and syllable type nested in individuals as random factors. We tested for differences in the mean values between conditions of 55 syllables of six individuals (3 to 23 syllables each), which were sung at least 20 times in each treatment. The significance of the models was tested by comparing them to null models with a log-likelihood test.

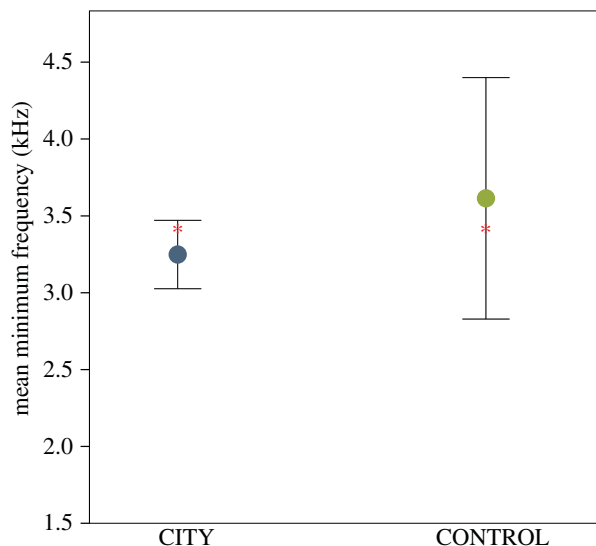
The observed change in amplitude was investigated in additional LMMs with birds as random effects. Here we used amplitude in the no-noise condition, bandwidth of the syllable and the distance of the peak frequency to the median peak frequency of all syllables in silent conditions as predictor variables for the rise in amplitude. We used the Akaike Information Criterion (AIC) to include or exclude variables in a model [40,41] and calculated the explained variance  $R^2$  for fixed effects in linear mixed models [39].

## 3. Results

### (a) Vocal Ontogeny study

Birds exposed to the CITY-noise did not sing with higher minimum frequencies than the tutors or than the CONTROL-noise birds, when singing in their 'home' noise condition (figure 1) (Kruskal–Wallis  $\chi^2_2 = 2.57$ ,  $p$ -value = 0.28).

We further examined the specific songs that birds in each group learned from tutors and found that city-noise birds did not preferentially learn or sing song types with the highest minimum frequencies. In fact, three of the four birds that



**Figure 1.** Mean minimum frequencies of songs produced by great tits reared in either low-frequency (CITY) noise or high-frequency (CONTROL) noise did not differ from the mean minimum frequency of the tutors (stars), which were recorded in the quiet forested areas where the experimental birds were collected as nestlings. Error bars indicate 95% CIs of the means.

copied tutor songs in the city-noise group learned and sang the tutor song with the lowest minimum frequency, even though this song type had the greatest degree of overlap with the background noise (electronic supplementary material, tables S1 and S2). The control-noise birds also did not selectively copy tutor song types that would have minimized overlap between song and noise.

Not all birds copied the tutor songs. Songs that could not be identified as imitations of tutor songs were also included in the minimum frequency analysis. That birds in both noise groups ‘invented’ songs rather than only copying tutors provided an interesting perspective on noise avoidance in song frequency. Even when birds invented new song types, they did not produce songs with higher or lower minimum frequencies in different noise conditions (Mann–Whitney  $U$  test,  $n_{\text{city}}=6$  and  $n_{\text{control}}=5$ ,  $W=10$ ,  $p=0.42$ ).

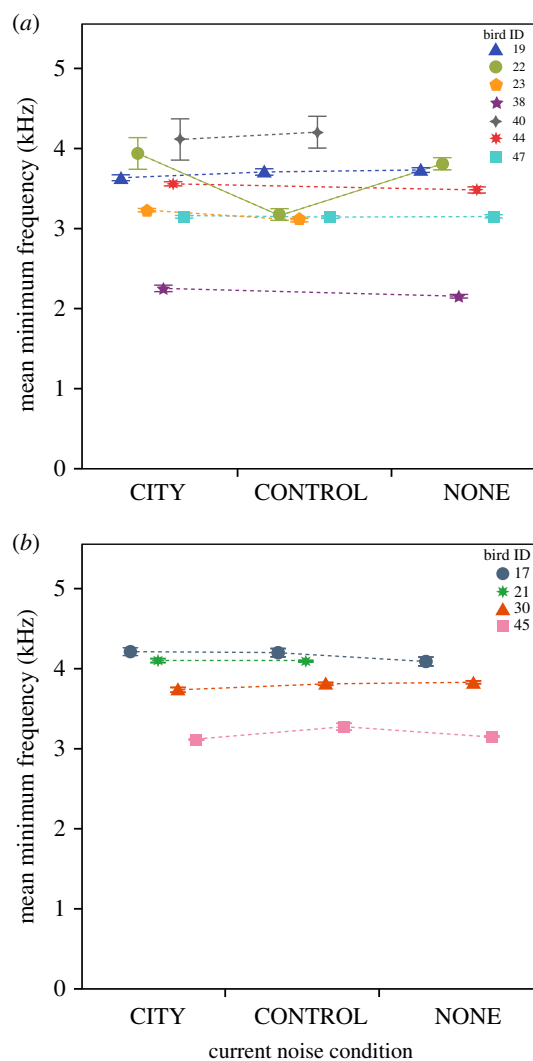
### (b) Adult Plasticity study 1—syllable frequency plasticity

When background noise was turned off, the mean minimum frequency did not differ from that produced during noise playback. In this comparison we again took the average minimum frequency of all the different song types in the bird’s repertoire.

Birds sang less often in the second noise condition (either no-noise or ‘opposite noise’) than they did in their ‘home’ noise condition. We presume that this decline in song output had to do with the advancing date in the season, as great tits sing most early in the spring and song output decreased overall with each successive week. Despite the smaller sample size of song types, we did not find a shift in frequency of individual syllable types as background noise conditions changed ( $\chi^2_2=0.98$ ,  $p=0.6125$ ).

### (c) Adult Plasticity study 2—syllable type usage

For each bird that sang in both noise conditions, we compared minimum frequency in three 5 min intervals during which the bird was actively singing.

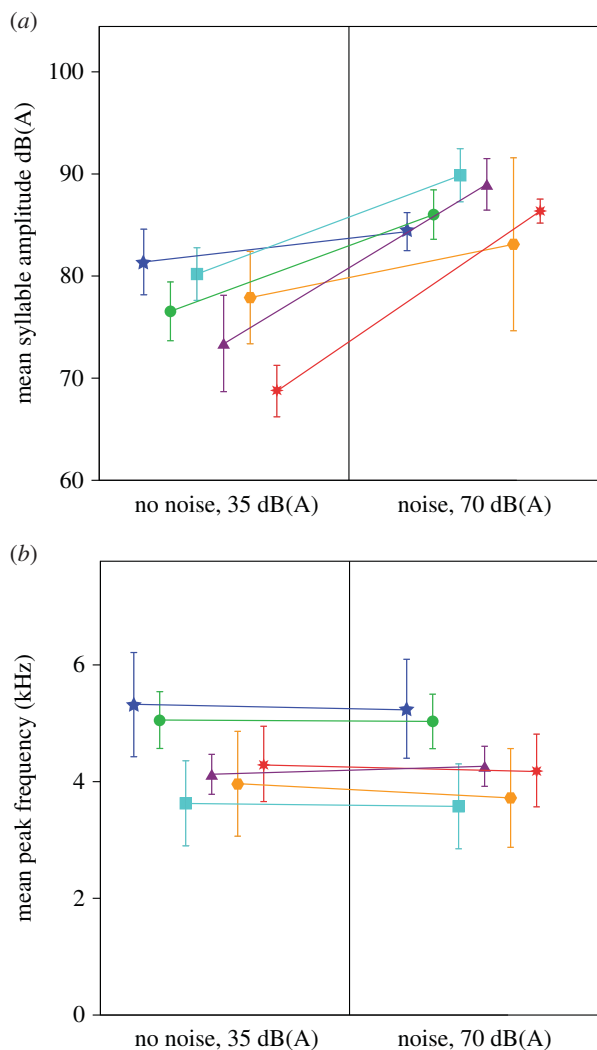


**Figure 2.** Neither the birds reared in low-frequency (CITY) noise (a), nor those reared in high-frequency (CONTROL) noise (b) switched to song types that differed in minimum frequency when the background noise conditions changed. Mean minimum frequencies for three, 5-min long randomly selected song bouts for each bird are shown. Means for each bird in each background noise condition are shown; error bars indicate 95% CIs for the data. Colour denotes individuals.

We found no significant differences in the minimum frequency between birds raised in CITY noise and birds raised in CONTROL noise when they were singing in their ‘home’ noise conditions (figure 2) (maximum-likelihood test, model with treatment versus model without,  $\log\text{LikNull-Model} = -598.56$ ,  $\chi^2_1 = 1.76$ ,  $p < 0.19$ ). In the model, 71% of the variance can be attributed to the individuals (here we did not further distinguish between individual and syllable variation). When we tested whether the average minimum frequency of songs used changed in a new noise condition, we again found no significant difference for either CITY or CONTROL birds singing in the opposite noise condition (figure 2b) (maximum-likelihood test, model with treatment versus model without,  $\log\text{LikNull-Model} = -275.98$ ,  $\log\text{Lik-Model} = -275.81$ ,  $\chi^2_1 = 1.76$ ,  $p < 0.58$ ) (figure 2). Forty-two per cent of the variation in the differences in minimum frequency could be attributed to individual.

### (d) Adult Plasticity study 3—Lombard effect

All birds exhibited the Lombard effect when exposed to increased background noise levels (figure 3a). Song



**Figure 3.** Wild-caught adult male great tits sang with higher amplitudes when background noise levels increased (a), but did not increase song frequency as vocal amplitudes increased (b). Means, plus 95% CIs for each individual are shown. Colour denotes individuals.

amplitude was significantly higher in noise (maximum-likelihood test, model with treatment versus model without,  $\log\text{Lik}_{\text{Null-Model}} = -381.03$ ,  $\log\text{Lik}_{\text{Model}} = -344.12$ ,  $\chi^2_1 = 73.82$ ,  $p < 0.0001$ ), rising, on average, by 10 dB (LMM, random factors 6 birds, 55 syllables; fixed effect, estimate for noise treatment is  $9.9 \pm 0.8$  dB,  $t\text{-value} = 12.20$ ,  $r^2$  for fixed factors,  $r^2_{\text{GLIMM}} = 0.41$ ). In contrast, peak frequencies did not change significantly in noise (LMM, 6 birds, 55 syllables; estimated noise effect:  $-43 \pm 27$  Hz,  $t\text{-value} = -1.575$ ), and the model was not significantly different from the null hypothesis (maximum-likelihood test, model with treatment versus model without,  $\log\text{Lik}_{\text{Null-Model}} = -837.65$ ,  $\log\text{Lik}_{\text{Model}} = -836.42$ ,  $\chi^2_1 = 2.47$ ,  $p = 0.12$ ).

## 4. Discussion

We found that birds collected as nestlings from quiet forested areas, and reared in the laboratory with chronic exposure to noise did not sing songs that differed in frequency from those of the tutors from quiet forested areas. The minimum song frequencies were the same as those of the tutors both when we considered all the song types within their tutors' repertoires, and if we considered which song types they used in different noise conditions. While the commonest suggested reason for

the higher frequency songs observed in urban birds has been that these pitch shifts are a response to low-frequency noise, our experiments indicate that chronic exposure to city-like noise did not induce frequency shifts within individual birds. Although there have only been a few studies that have examined the effects of noise exposure on individual song frequency shifts [27,29,30], our findings are contrary to those previous studies, but support the prediction of Slabbekoorn and den Boer-Visser that urban-forest population differences are likely to result from differences in song repertoire composition rather than individuals modifying the same song types [8].

The birds in our ontogeny study were exposed to tutor songs that varied in minimum frequency as well as overall bandwidth and song structure. While not all birds copied tutors from the playback, some birds in both the CITY and CONTROL noise groups sang both the lowest and highest tutor song types, even though the lowest song was most heavily masked by the CITY noise, and the highest was most heavily masked by the CONTROL noise. In addition, we did not find that exposure to higher frequency noise resulted in songs with lower frequencies than those of tutors, or of CITY-noise birds, as might be predicted from the 'masking release' hypothesis.

A recent study found that young zebra finches which were exposed to low-frequency noise during early vocal ontogeny did not develop adult songs with higher minimum frequencies [42]. While this finding is important for a general understanding of the auditory feedback mechanisms during vocal production learning, it is not clear whether wild zebra finch populations differ at all in song pitch. Our data show that even in a species in which the urban upward shift of song frequencies is well documented, this pattern cannot be explained by developmental plasticity during vocal learning. Moreover, unlike zebra finches, great tits typically have a repertoire of several different song types and our results show that males not only stayed on pitch when they copied songs from their tutors in noise, but also did not selectively acquire those song types into their repertoires that are particularly well-suited to the noise conditions under which they are memorized.

We also tested whether adult birds would flexibly adjust either the minimum frequency of their songs or the song types they chose to sing when background noise conditions were changed. Neither our hand-reared birds, nor wild-caught adult birds, changed the frequency of their songs when background noise conditions were changed, either to a different noise profile or to a no-noise condition.

Previous studies reported individual frequency shifts related to noise exposure in several songbird species (e.g. [27–30]). Based on an experiment on free-ranging great tits, Halfwerk and Slabbekoorn suggested that males selected those song types from their repertoire that are particularly well suited for certain noise conditions [30]. However, the birds in our experiment did not show any tendency towards differential song type usage dependent on current noise conditions, as they neither sang higher-frequency song types in low-frequency noise nor lower-frequency song types in high-frequency noise. Can this conflicting evidence be explained by different methodology? Our noise treatment was different from that of Halfwerk & Slabbekoorn [30] and similar studies in other songbird species [27,28], in that the period of noise exposure was of a much longer duration (2–3 weeks versus several minutes). It may be that song type switching is a viable strategy only when



dealing with transient increases in background noise level, but when faced with chronic noise, the need to display a large repertoire size, or to use song-type matching in territorial encounters with neighbours, overrides any potential benefits that might be gained by using only the higher frequency subset of the song repertoire. However, if a switch in song types only occurs in response to short-term fluctuations in noise, this cannot account for the observed divergence in minimum frequency between birds in noisy and quiet habitats.

Great tits in our study responded to increased background noise levels with a rise in amplitude, as predicted by the Lombard effect [25]; however, this increase in amplitude was not accompanied by an increase in frequency. In humans, vocal frequency often increases along with amplitude in Lombard-induced speech, independent of a potential release from masking [43]. However, the frequency rise and spectral tilt often observed during Lombard speech may be controlled independently [44,45]. That the great tits in our study did not show an upward shift in frequency when their songs got louder suggests that frequency and amplitude are not strictly coupled in song production, just as in call production in this species [46]. In contrast, call frequency varies with amplitude in elegant-crested tinamous (*Eudromia elegans*) [24] and budgerigars (*Melopsittacus undulatus*) [47]. This diversity may reflect phylogenetic differences in vocal control, with songbirds having greater flexibility in their vocalizations because of independent regulation of frequency and amplitude.

The change in song frequency observed in urban populations is often assumed to be an adaptive response to the low-frequency noise typical of these areas (but see [48]). However, environmental acoustics of cities differ from the original habitats of birds in more than just noise profile [49], and the combined effects of noise and altered habitat acoustics may impose a stronger selective force on vocal signalling than does traffic noise alone [50]. While several studies reported positive correlations between breeding bird species richness/abundance and proximity to noisy roads (reviewed in [1,51]), a recent study attempted to disentangle the effects of traffic noise from the traffic itself and found that roads and vehicles on them explained the negative effects better than the noise per se [51]. Male density may also contribute to changes in song [52] as has been found in urban Japanese great tits [6] and northern cardinals (*Cardinalis cardinalis*) [53]. However,

no correlation between male density and minimum frequency was found in cardinals [53] or Eurasian blackbirds (*Turdus merula*) [54]. Little is known about the impact of air or water pollution on the behaviour of wild animals, but as air pollutants such as ozone and nitrogen oxides irreversibly damage birds' lungs [55], and chronic exposure to hydrocarbons emitted by traffic is correlated with reduced growth rates [56] and body weight [57] in birds, it is not hard to imagine that air pollution could indirectly lead to changes in vocalizations.

Our data suggest that, while higher minimum frequencies of songs may be found in areas of higher noise levels, a causal link between noise and pitch shifts is not clear. Changes in minimum or peak song frequency did not occur in great tits that were exposed to chronic traffic-like noise during song ontogeny, nor did it occur as a result of individual adult plasticity. Our birds consistently sang on pitch and with the same mean minimum frequencies in all noise conditions, which suggests that observed changes between populations of rural and forest birds are not the result of individual responses, but may instead be the outcome of slower, population-wide changes. Such changes may be mediated by selection for songs that transmit particularly well in noisy habitats, and the subsequent spread of these songs through cultural transmission. This scenario would favour loud songs, which at the same time are often higher pitched owing to vocal production dynamics [58].

**Ethics.** All procedures described in this manuscript were conducted in accordance with appropriate German regulations (licence 55.2-1-54-2532.3-13-09 by the government of Upper Bavaria) and the guidelines for the treatment of animals in behavioural research and teaching of the Association for the Study of Animal Behaviour (ASAB).

**Data accessibility.** Data are archived at the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.fc10f>) [59].

**Authors' contributions.** H.B., P.J.B.S. and S.A.Z. designed the study. S.A.Z. carried out the experiments. S.A.Z. and E.N. analysed the data. S.A.Z. led the writing of the manuscript with critical input from all authors.

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## References

1. Brumm H, Zollinger SA. 2013 Avian vocal production in noise. In *Animal communication and noise* (ed. H Brumm), pp. 187–227. Berlin, Germany: Springer.
2. Tyack P, Janik VM. 2013 Effects of noise on acoustic signal production in marine mammals. In *Animal communication and noise* (ed. H Brumm), pp. 251–271. Berlin, Germany: Springer.
3. Schwartz JJ, Bee MA. 2013 Anuran acoustic signal production in noisy environments. In *Animal communication and noise* (ed. H Brumm), pp. 91–132. Berlin, Germany: Springer.
4. Lampe U, Schmoll T, Franzke A, Reinhold K. 2012 Staying tuned: grasshoppers from noisy roadside habitats produce courtship signals with elevated frequency components. *Funct. Ecol.* **26**, 1348–1354. (doi:10.1111/1365-2435.12000)
5. Slabbekoorn H, Peet M. 2003 Birds sing at a higher pitch in urban noise. *Nature* **424**, 267. (doi:10.1038/424267a)
6. Hamao S, Watanabe M, Mori Y. 2011 Urban noise and male density affect songs in the great tit *Parus major*. *Ethol. Ecol. Evol.* **23**, 111–119. (doi:10.1080/03949370.2011.554881)
7. Mockford EJ, Marshall RC. 2009 Effects of urban noise on song and response behaviour in great tits. *Proc. R. Soc. B* **276**, 2979–2985. (doi:10.1098/rspb.2009.0586)
8. Slabbekoorn H, den Boer-Visser A. 2006 Cities change the songs of birds. *Curr. Biol.* **16**, 2326–2331. (doi:10.1016/j.cub.2006.10.008)
9. Salaberria C, Gil D. 2010 Increase in song frequency in response to urban noise in the great tit *Parus major* as shown by data from the Madrid (Spain) city noise map. *Ardeola* **57**, 3–11.
10. Brumm H, Slabbekoorn H. 2005 Acoustic communication in noise. In *Advances in the study of behavior*, vol. 35 (eds PJB Slater, CT Snowdon, TJ Roper, HJ Brockmann, M Naguib), pp. 151–209. San Diego, CA: Elsevier Academic Press Inc.
11. Dooling RJ, Blumenrath SH. 2013 Avian sound perception in noise. In *Animal communication and noise* (ed. H Brumm), pp. 229–250. Berlin, Germany: Springer.
12. Marler P, Konishi M, Lutjen A, Waser MS. 1973 Effects of continuous noise on avian hearing and



- vocal development. *Proc. Natl Acad. Sci. USA* **70**, 1393–1396. (doi:10.1073/pnas.70.5.1393)
13. Iyengar S, Bottjer SW. 2002 The role of auditory experience in the formation of neural circuits underlying vocal learning in zebra finches. *J. Neurosci.* **22**, 946–958.
  14. Brumm H. 2006 Animal communication: city birds have changed their tune. *Curr. Biol.* **16**, R1003–R1004. (doi:10.1016/j.cub.2006.10.043)
  15. Hansen P. 1979 Vocal learning: its role in adapting sound structures to long-distance propagation and a hypothesis on its evolution. *Anim. Behav.* **27**, 1270–1271. (doi:10.1016/0003-3472(79)90073-3)
  16. Brainard MS, Doupe AJ. 2000 Auditory feedback in learning and maintenance of vocal behaviour. *Nat. Rev. Neurosci.* **1**, 31–40. (doi:10.1038/35036205)
  17. Kuebrich BD, Sober SJ. 2015 Variations on a theme: songbirds, variability, and sensorimotor error correction. *Neuroscience* **296**, 48–54. (doi:10.1016/j.neuroscience.2014.09.068)
  18. Chang EF, Merzenich MM. 2003 Environmental noise retards auditory cortical development. *Science* **300**, 498–502. (doi:10.1126/science.1082163)
  19. Zheng Y, Meng M, Zhao C, Liao W, Zhang Y, Wang L, Wen E. 2014 Impact of environmental noise on growth and neuropsychological development of newborn rats. *Anat. Rec.* **297**, 949–954. (doi:10.1002/ar.22872)
  20. Cohen S, Evans GW, Stokols D, Krantz DS. 1986 Environmental stress and cognitive performance. In *Behaviour, health, and environmental stress* (eds S Cohen, GW Evans, D Stokols, DS Krantz), pp. 143–183. Berlin, Germany: Springer.
  21. Evans GW, Lepore SJ. 1993 Nonauditory effects of noise on children. *Child. Environ.* **10**, 31–51.
  22. Riley KG, McGregor KK. 2012 Noise hampers children's expressive word learning. *Lang. Speech Hear. Serv. Sch.* **43**, 325–337. (doi:10.1044/0161-1461(2012/11-0053))
  23. Potvin DA, Macdougall-Shackleton SA. 2015 Traffic noise affects embryo mortality and nestling growth rates in captive zebra finches. *J. Exp. Zool. A Ecol. Genet. Physiol.* **323**, 722–730. (doi:10.1002/jez.1965)
  24. Schuster S, Zollinger SA, Lesku JA, Brumm H. 2012 On the evolution of noise-dependent vocal plasticity in birds. *Biol. Lett.* **8**, 913–916. (doi:10.1098/rsbl.2012.0676)
  25. Brumm H, Zollinger SA. 2011 The evolution of the Lombard effect: 100 years of psychoacoustic research. *Behaviour* **148**, 1173–1198. (doi:10.1163/000579511X605759)
  26. Brumm H, Naguib M. 2009 Environmental acoustics and the evolution of bird song. In *Advances in the study of behavior*, vol. 40 (eds M Naguib, K Zuberbühler, NS Clayton, VM Janik), pp. 1–33. Cambridge, MA: Academic Press.
  27. Bermudez-Cuamatzin E, Rios-Chelen AA, Gil D, Garcia CM. 2011 Experimental evidence for real-time song frequency shift in response to urban noise in a passerine bird. *Biol. Lett.* **7**, 36–38. (doi:10.1098/rsbl.2010.0437)
  28. Potvin DA, Mulder RA. 2013 Immediate, independent adjustment of call pitch and amplitude in response to varying background noise by silvereyes (*Zosterops lateralis*). *Behav. Ecol.* **24**, 1363–1368. (doi:10.1093/beheco/art075)
  29. Potvin DA, MacDougall-Shackleton SA. 2015 Experimental chronic noise exposure affects adult song in zebra finches. *Anim. Behav.* **107**, 201–207. (doi:10.1016/j.anbehav.2015.06.021)
  30. Halfwerk W, Slabbekoorn H. 2009 A behavioural mechanism explaining noise-dependent frequency use in urban birdsong. *Anim. Behav.* **78**, 1301–1307. (doi:10.1016/j.anbehav.2009.09.015)
  31. Lampe U, Reinhold K, Schmoll T. 2014 How grasshoppers respond to road noise: developmental plasticity and population differentiation in acoustic signalling. *Funct. Ecol.* **28**, 660–668. (doi:10.1111/1365-2435.12215)
  32. Luther DA, Deryberry EP. 2012 Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *Anim. Behav.* **83**, 1059–1066. (doi:10.1016/j.anbehav.2012.01.034)
  33. Catchpole CK, Slater PJB. 2008 *Bird song*, 2 edn. Cambridge, UK: Cambridge University Press.
  34. Lachlan RF, Servideo MR. 2004 Song learning accelerates allopatric speciation. *Evolution* **58**, 2049–2063. (doi:10.1111/j.0014-3820.2004.tb00489.x)
  35. Rios-Chelen AA, Salaberria C, Barbosa I, Macias Garcia C, Gil D. 2012 The learning advantage: bird species that learn their song show a tighter adjustment of song to noisy environments than those that do not learn. *J. Evol. Biol.* **25**, 2171–2180. (doi:10.1111/j.1420-9101.2012.02597.x)
  36. McGregor PK, Krebs JR. 1982 Song types in a population of great tits (*Parus major*): their distribution, abundance and acquisition by individuals. *Behaviour* **79**, 126–152. (doi:10.1163/156853982X00210)
  37. Tchernichovski O, Lints TJ, Deregnacourt S, Cimenser A, Mitra PP. 2004 Studying the song development process rationale and methods. In *Behavioral neurobiology of birdsong* (eds HP Zeigler, P Marler), pp. 348–363. New York, NY: New York Academy of Sciences.
  38. Zollinger SA, Podos J, Nemeth E, Goller F, Brumm H. 2012 On the relationship between, and measurement of, amplitude and frequency in birdsong. *Anim. Behav.* **84**, e1–e9. (doi:10.1016/j.anbehav.2012.04.026)
  39. Nakagawa S, Schielzeth H. 2013 A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142. (doi:10.1111/j.2041-210x.2012.00261.x)
  40. Burnham KP, Anderson DR. 2002 *Model selection and multimodel inference: a practical information-theoretic approach*. New York, NY: Springer.
  41. Crawley MJ. 2007 *The R Book*. New York, NY: John Wiley & Sons.
  42. Potvin DA, Curcio MT, Swaddle JP, MacDougall-Shackleton SA. 2016 Experimental exposure to urban and pink noise affects brain development and song learning in zebra finches (*Taenopygia guttata*). *PeerJ* **4**, e2287. (doi:10.7717/peerj.2287)
  43. Lu Y, Cooke M. 2008 Lombard speech: effects of task and noise type. *J. Acoust. Soc. Am.* **123**, 3072. (doi:10.1121/1.2932849)
  44. Eliades SJ, Wang XQ. 2012 Neural correlates of the Lombard effect in primate auditory cortex. *J. Neurosci.* **32**, 10 737–10 748. (doi:10.1523/JNEUROSCI.3448-11.2012)
  45. Eliades SJ, Wang X. 2008 Neural substrates of vocalization feedback monitoring in primate auditory cortex. *Nature* **453**, 1102–1106. (doi:10.1038/nature06910)
  46. Templeton CN, Zollinger SA, Brumm H. 2016 Traffic noise drowns out great tit alarm calls. *Curr. Biol.* **26**, R1173–R1174. (doi:10.1016/j.cub.2016.09.058)
  47. Osmanski MS, Dooling RJ. 2009 The effect of altered auditory feedback on control of vocal production in budgerigars (*Melopsittacus undulatus*). *J. Acoust. Soc. Am.* **126**, 911–919. (doi:10.1121/1.3158928)
  48. Nemeth E, Zollinger SA, Brumm H. 2012 Effect sizes and the integrative understanding of urban bird song. *Am. Nat.* **180**, 146–152. (doi:10.1086/665994)
  49. Mockford EJ, Marshall RC, Dabelsteen T. 2011 Degradation of rural and urban great tit song: testing transmission efficiency. *PLoS ONE* **6**, e28242. (doi:10.1371/journal.pone.0028242)
  50. Nemeth E, Brumm H. 2010 Birds and anthropogenic noise: are urban songs adaptive? *Am. Nat.* **176**, 465–475. (doi:10.1086/656275)
  51. Summers PD, Cunnington GM, Fahrig L. 2011 Are the negative effects of roads on breeding birds caused by traffic noise? *J. Appl. Ecol.* **48**, 1527–1534. (doi:10.1111/j.1365-2664.2011.02041.x)
  52. Nemeth E, Brumm H. 2009 Blackbirds sing higher-pitched songs in cities: adaptation to habitat acoustics or side-effect of urbanization? *Anim. Behav.* **78**, 637–641. (doi:10.1016/j.anbehav.2009.06.016)
  53. Narango DL, Rodewald AD. 2016 Urban-associated drivers of song variation along a rural-urban gradient. *Behav. Ecol.* **27**, 608–616. (doi:10.1093/beheco/arv197)
  54. Ripmeester EAP, Mulder M, Slabbekoorn H. 2010 Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. *Behav. Ecol.* **21**, 876–883. (doi:10.1093/beheco/arq075)
  55. Rombout PJ, Dormans JA, van Bree L, Marra M. 1991 Structural and biochemical effects in lungs of Japanese quail following a 1 week exposure to ozone. *Environ. Res.* **54**, 39–51. (doi:10.1016/S0013-9351(05)80193-8)
  56. Albers PH. 2006 Birds and polycyclic aromatic hydrocarbons. *Avian Poult. Biol. Rev.* **17**, 125–140. (doi:10.3184/147020606783438740)
  57. Llacuna S, Gorriç A, Riera M, Nadal J. 1996 Effects of air pollution on hematological parameters in passerine birds. *Arch. Environ. Contam. Toxicol.* **31**, 148–152. (doi:10.1007/BF00203919)
  58. Zollinger SA, Brumm H. 2015 Why birds sing loud songs and why they sometimes don't. *Anim. Behav.* **105**, 289–295. (doi:10.1016/j.anbehav.2015.03.030)
  59. Zollinger SA, Slater PJB, Nemeth E, Brumm H. 2017 Data from: Higher songs of city birds may not be an individual response to noise. Dryad Digital Repository. (<http://dx.doi.org/10.5061/dryad.fc10f>)