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

2

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9 **Abstract**

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

## 25 **Introduction**

26 The past decade has seen a dramatic increase in research into the effects of anthropogenic  
27 noise on the lives of animals, and much of that research has focused on vocal behaviour and  
28 communication. Anthropogenic noise from street, air and boat traffic is dominated by low  
29 frequency sounds, and associated upward changes in the frequency components of acoustic  
30 signals have been reported in half of the songbird species tested on 5 continents [1], some  
31 whale species [2], frogs [3] and even insects [4], suggesting that this is a global  
32 phenomenon. However, there are few studies examining the mechanisms underlying the  
33 observed changes.

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

45 *Noise impacts on vocal development*

46 There are several processes by which low-frequency noise exposure during vocal learning  
47 might lead to upward changes in song frequency. In continuous noise at levels above 93 dB  
48 (re. 20 $\mu$ Pa) songbirds are likely to experience a temporary hearing threshold shift, and  
49 above 110 dB to experience permanent ear damage [11]. Although noise levels in cities or  
50 near motorways may have transient peaks above 90 dB, it is rare that such events are  
51 sustained long enough to induce hearing damage in birds [11]. In juvenile songbirds, noise  
52 loud enough to cause temporary threshold shifts, resulted in severe impairment of song  
53 development, and in the formation of the underlying song control circuits in the brain [12,  
54 13]. That noise disrupting hearing impairs vocal learning or song production is not  
55 surprising, however the effects of intermediate noise levels, like those commonly  
56 experienced in urban habitats, on song development are less known.

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

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

#### 80 *Noise, adult plasticity and microevolutionary changes*

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

91 While it is generally assumed that the primary driving force behind the upward shift in song  
92 frequency is the high level of low-frequency noise, experimental evidence in support of this  
93 assumption is limited. In the house finch *Haemorhous mexicanus*, individuals sang with a  
94 higher minimum frequency when exposed temporarily to noise [27]. Silvereyes (*Zosterops*  
95 *lateralis*), immediately lowered the minimum frequency of their calls in response to high-  
96 frequency noise playback, but did not raise minimum frequency in response to low-  
97 frequency noise [28]. In another study, long-term experimental traffic noise exposure in  
98 zebra finches *Taeniopygia guttata*, led adult males to sing with *lower* minimum frequencies  
99 after a 5 month long period of noise exposure [29], an adjustment opposite of what would  
100 be predicted by the hypothesis that birds shift songs up to gain a release from masking  
101 noise, and opposite of what would be predicted by the Lombard hypothesis.

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

110 Finally, it may be that the differences in frequency between the vocalizations of urban and  
111 rural populations are not the result of individual plasticity, but of gradual changes over  
112 many generations. As in birds, upwards trends in frequency have been reported in  
113 grasshoppers living near noisy motorways [4]. Lampe and colleagues [31] demonstrated that

114 this signal divergence is the outcome of both cross-generational effects of environmental  
115 noise and developmental plasticity. In songbirds, such cross-generational effects could be  
116 mediated by genetic or cultural changes. Although birds have much longer generation times  
117 than grasshoppers, precluding similar experimental investigations, Luther and Derryberry  
118 [32] used historical records to reconstruct changes in song over a 36 year period in a city  
119 population of songbirds in San Francisco, and found that song frequencies have changed  
120 over time as noise levels have risen. Since the songs of most oscine songbirds are learned  
121 [33], it is hypothesized that their vocalizations could adapt more quickly to environmental  
122 changes, through cultural evolution [34]. In line with this notion, Rios-Chelen et al. [35]  
123 found that oscines diverged more in minimum song frequency between noisy and quiet  
124 habitats than suboscines, which are not thought to acquire their song through vocal  
125 production learning. Thus, although urban noise pollution is a relatively recent  
126 phenomenon, it is possible that the trends of vocal divergence of urban birds across the  
127 globe are indicators not of many individuals responding individually to fluctuating noise  
128 conditions, but rather the result of cultural or microevolutionary shifts at the population  
129 level.

130 We present here data from two behavioural domains: vocal learning and adult song  
131 plasticity. In particular, we present 1) the first experimental study of the effects of chronic  
132 exposure on song frequency in a songbird that is a frequent inhabitant of noise-polluted  
133 habitats and 2) tests of adult vocal plasticity in response to changing background noise  
134 conditions.

## 135 **Materials and methods**



136 ***Vocal ontogeny study***

137 *Birds and rearing conditions*

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

146 *Noise exposure and song tutoring*

147 The two noise treatments were city-like ("CITY") noise, consisting of filtered white noise 0 –  
148 3 kHz with a 500 Hz linear roll-off. The CITY noise was designed to simulate the average  
149 noise profile found in a busy urban area, while at the same time being more extreme both in  
150 spectral shape and temporal consistency (see Fig S1). Further details describing the degree  
151 of masking of the minimum frequency of each tutor song in each noise treatment is  
152 provided in the electronic supplement, Table S1. The control noise group was exposed to  
153 noise with the same bandwidth as the CITY group, but covering high, rather than low  
154 frequencies (3-6 kHz band-pass filtered white noise, 500 Hz linear fade-in, 100Hz linear roll-  
155 off). The CONTROL noise treatment was designed to control for non-auditory effects of  
156 noise exposure, but with noise in a different bandwidth than that of most traffic or urban  
157 noise sources. The sound pressure level (SPL) of the noise was 60-63 dB SPL (re. 20 $\mu$ Pa) at

158 the position of the middle perch in each cage. In addition to the noise playback, both groups  
159 of birds were tutored with 9 great tit songs (Fig S2) that were recorded the previous year  
160 from nine adult males in the quiet forested area where our nestlings were collected. Typical  
161 great tit song consists of several repetitions of identical song phrases (motifs). Phrases or  
162 motifs often contain 2 to 4 different notes or “syllables”, and the bird alternates between  
163 high and low pitched syllables to produce the characteristic great tit “teacher teacher” song  
164 (Fig. S2). Tutor songs were selected to include both the lowest and highest minimum  
165 frequency song types recorded in the population. As great tits probably learn songs from  
166 tutors during both their pre-dispersal time near their natal area and post-dispersal from  
167 neighbours during territory formation in the late winter/early spring the following year , we  
168 continued tutor playback until the birds were a full year old, when song is thought to  
169 crystallize and then remain constant throughout life [36]. Each tutor file was 30 seconds  
170 long and included 10-24 motifs in total. Playback consisted of 1 hour blocks of the 9 tutor  
171 files in randomized order. These 1 hour tutor playback blocks occurred 1-4 times per day  
172 during the first year post-hatch.

### 173 *Song recording*

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

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

### 183 *Song analyses*

184 From the 20 nestling birds, 9 CITY and 10 CONTROL birds survived to adulthood. Some birds  
185 did not sing enough for statistical analyses in the recording chambers, so were excluded  
186 from the analysis, leaving 7 birds in the CITY group and 6 birds in the CONTROL group.

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

191 Minimum frequency was measured in all three studies (this and the two below) at a set  
192 threshold below the peak frequency [38]. To this end, we used a script written in Igor Pro v.  
193 5 (Wavemetrics Inc.) that determined the peak frequency, and the frequency -22 dB below  
194 that peak in a power spectrum ( $f_s$  22 kHz, 1024 point FFT, Hamming window, 22 Hz  
195 frequency resolution) for each syllable. We used the -22 dB cut-off because this was the  
196 lowest threshold at which the minimum frequency could be measured given the signal-to-  
197 noise ratio of the quietest syllable type in noise for our set of recordings.

### 198 *Statistical analyses*

199 For each bird we took the mean minimum frequency of each song type in the bird's  
200 repertoire. The average number of syllables measured per song type was 41.7 (range 17-61)  
201 in the CITY noise condition, and 41.8 (range 14-60) in the CONTROL condition. For each bird,

202 an overall mean minimum frequency was calculated by taking the average of all these mean  
203 minimum frequencies for all the song types in their recorded repertoire. We then tested  
204 whether the mean of the minimum frequencies of all the songs in each bird's repertoire  
205 were different from those of tutor songs with a Kruskal-Wallis test.

## 206 ***Adult plasticity study 1***

### 207 *Syllable frequency plasticity – Birds and noise treatments, song analysis*

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

### 219 *Syllable frequency plasticity – Statistical analyses*

220 We compared the mean minimum frequency of each syllable type that each individual sang  
221 in more than one noise condition. We only included a syllable if the individual sang it at least  
222 10 times in each noise treatment. Syllables were given unique identifiers, so that even if  
223 more than one individual sang the same syllable type, these were only compared within a

224 bird, not between individuals. We compared differences in minimum frequency between  
225 noise treatments (CITY, CONTROL and NO-NOISE) using a linear mixed model with syllable  
226 nested inside bird ID as a random factor, the difference in min frequency as predicted value,  
227 and pairs of background noise treatments as a fixed factor (CITY vs. CONTROL, CITY vs. NO-  
228 NOISE, and CONTROL vs. NO-NOISE). The test was performed independent of noise  
229 presentation order. Because of low sample sizes, we have included effect sizes for each test  
230 in the three Adult plasticity studies (Fig S3 & S4). In significant models we calculated  
231 adjusted  $r^2$  for GLMs according to [39]. In non-significant models we plotted effect sizes  
232 with confidence intervals. We calculated significance using an Analysis of Deviance test with  
233 2 degrees of freedom. Statistical analyses were done with R 3.2.1 and the packages lme4  
234 and MuMIn.

235

## 236 ***Adult plasticity study 2***

### 237 *Syllable type usage – Birds, treatment, and song analysis*

238 To test whether birds might selectively use certain song types from within their repertoires  
239 to minimize overlap with current noise profiles, we recorded adult birds from our vocal  
240 ontogeny study (see above) in both their “home noise” and in the “opposite noise” (i.e. CITY  
241 noise for CONTROL birds, and CONTROL noise for CITY birds). We then compared the  
242 average minimum frequencies of all song types sung during 3 randomly selected 5-minute  
243 intervals in each background noise condition. If birds selectively sing song types with higher  
244 minimum frequencies in low frequency noise than they do in high noise or no noise, then

245 these differences should be detected in the mean minimum frequencies sung during these  
246 randomly chosen intervals in each noise condition.

### 247 *Syllable type usage – Statistical analyses*

248 We tested the influence of rearing conditions on the minimum frequency of songs during  
249 three 5-minute intervals, and whether the minimum song frequency changed when noise  
250 conditions changed, with linear mixed models (LMMs). Noise treatment during rearing was  
251 a fixed effect, and individual was a random effect. Songs were taken as repeated  
252 measurements of one individual without distinguishing different syllables. First we  
253 compared the minimum frequency of song of seven birds raised in the CITY-noise condition  
254 with four birds raised in CONTROL-noise (1553 songs total, 18 to 296 songs per bird). We  
255 tested with a Log-Likelihood test whether the model was better than a null-model without  
256 treatment.

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

### 266 ***Adult Plasticity Study 3***

267 *Lombard Effect - Animals, housing, noise playback and song recording*

268 Finally, we tested whether noise-induced increases in song amplitude correspond with  
269 increases in song frequency in adult birds. We caught 6 adult male great tits from quiet  
270 forest areas around Starnberg, Germany. Birds were housed singly in cages 125 x 44 x 40  
271 cm, which were placed on tables in a sound-shielded room and provided with food and  
272 water ad libitum. Birds were visually, but not acoustically isolated from each other, although  
273 the sounds of neighbouring birds were dampened by panels of sound-absorbing foam.

274 We exposed singing birds to filtered white noise (1-10 kHz band pass Butterworth filter) at  
275 levels between 65-70 dB(A) SPL, measured at the position of the perches. Noise was played  
276 from a computer with an external digital sound card (Edirol UA-25EX), fed through a Pioneer  
277 A-109 stereo amplifier to two JBL Control 1 Pro loudspeakers. During recording sessions, all  
278 but two perches were removed from the cages, with the 2 remaining perches placed at the  
279 same height and 15 cm apart. Sennheiser ME62 microphones were positioned 50 cm above  
280 the cage, equidistant between the two perches. We calibrated the recording microphones  
281 using a calibration microphone (Behringer ECM8000) and acoustic calibrator (Casella CEL-  
282 184). When birds were habituated to the room and singing regularly, we recorded their song  
283 in the quiet room (average ambient room noise 35 dB(A) SPL) and then again during noise  
284 playback. Recordings were made using Sound Analysis Pro version 2.063 at a sampling rate  
285 of 44.1 kHz and 16 bit resolution.

286 *Lombard effect - Song analysis*

287 Using calibrated song recordings in Avisoft SASLab Pro v. 5.2, we measured the root mean  
288 square (RMS) amplitude of each individual syllable in each motif in each song type that birds

289 sang in both no-noise and noise conditions. We then subtracted the noise using logarithmic  
290 computational rules [25] to determine the RMS values for the syllables themselves in both  
291 noise treatments.

292 For measuring the peak frequency of each syllable, we resampled the song recordings at  
293 22.05 kHz, and created power spectra for each syllable (1024 pt FFT, Hamming window, 22  
294 Hz frequency resolution). Because of the signal-to-noise ratios in these recordings and  
295 because of the wideband spectrum of the noise used to elicit the Lombard effect, minimum  
296 frequency would be difficult to measure reliably.

#### 297 *Lombard effect - Statistical analyses*

298 Amplitude and frequency of the same syllables between the noise-exposed and control  
299 treatments were analysed with LMMs, with noise or no-noise as fixed effects, and individual  
300 and syllable type nested in individuals as random factors. We tested for differences in the  
301 mean values between conditions of 55 syllables of 6 individuals (3 to 23 syllables each),  
302 which were sung at least 20 times in each treatment. The significance of the models was  
303 tested by comparing them to null models with a Log-Likelihood test.

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

310



311 **Results**

312 *Vocal Ontogeny Study*

313 Birds exposed to the CITY-noise did not sing with higher minimum frequencies than the  
314 tutors or than the CONTROL-noise birds, when singing in their “home” noise condition (Fig  
315 1) (Kruskal-Wallis chi-squared = 2.57, df = 2, p-value = 0.28).

316 We further examined the specific songs that birds in each group learned from tutors and  
317 found that city-noise birds did not preferentially learn or sing song types with the highest  
318 minimum frequencies. In fact, 3 of the 4 birds that copied tutor songs in the city-noise group  
319 learned and sang the tutor song with the lowest minimum frequency, even though this song  
320 type had the greatest degree of overlap with the background noise (Table S1). The control-  
321 noise birds also did not selectively copy tutor song types that would have minimized overlap  
322 between song and noise.

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

329

330 *Adult Plasticity Study 1- Syllable frequency plasticity*

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

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

#### 340 *Adult Plasticity Study 2 – Syllable type usage*

341 For each bird that sang in both noise conditions, we compared minimum frequency in three  
342 5-minute intervals during which the bird was actively singing..

343 We found no significant differences in the minimum frequency between birds raised in CITY  
344 noise and birds raised in CONTROL noise when they were singing in their "home" noise  
345 conditions (Fig 2) (Maximum Likelihood Test, model with treatment vs. model without,  
346  $\log\text{LikNull-Model} = -598.56$ ,  $Df = 1$ ,  $X^2 = 1.76$ ,  $P < 0.19$ ). In the model, 71 % of the variance  
347 can be attributed to the individuals (here we did not further distinguish between individual  
348 and syllable variation). When we tested whether average minimum frequency of songs  
349 used changed in a new noise condition , we again found no significant difference for either  
350 CITY or CONTROL birds singing in the opposite noise condition (Fig 2B)(Maximum Likelihood  
351 Test, model with treatment vs. model without,  $\log\text{LikNull-Model} = -275.98$ ,  $\log\text{Lik-Model} = -$

352 275.81, Df = 1,  $X^2 = 1.76$ ,  $P < 0.58$ )(Fig 2). Forty-two percent of the variation in the  
353 differences in minimum frequency could be attributed to individual.

#### 354 *Adult Plasticity Study 3 - Lombard effect*

355 All birds exhibited the Lombard effect when exposed to increased background noise levels  
356 (Fig 3A). Song amplitude was significantly higher in noise (Maximum Likelihood Test, model  
357 with treatment vs. model without,  $\log\text{Lik}_{\text{Null-Model}} = -381.03$ ,  $\log\text{Lik}_{\text{Model}} = -344.12$ , Df = 1,  $X^2 =$   
358  $73.82$ ,  $P < 0.0001$ ), rising, on average, by 10 dB (LMM, random factors 6 birds, 55 syllables;  
359 fixed effect, estimate for noise treatment is  $9.9 \pm 0.8$  dB, t-value = 12.20,  $r^2$  for fixed factors ,  
360  $r^2_{\text{GLIMM}} = 0.41$ ). In contrast, peak frequencies did not change significantly in noise (LMM, 6  
361 birds, 55 syllables; estimated noise effect:  $-43 \pm 27$  Hz, t-value = -1.575), and the model was  
362 not significantly different from the null hypothesis (Maximum Likelihood Test, model with  
363 treatment vs. model without,  $\log\text{Lik}_{\text{Null-Model}} = -837.65$ ,  $\log\text{Lik}_{\text{Model}} = -836.42$ , Df = 1,  $X^2 = 2.47$ ,  
364  $p = 0.12$ ).

#### 365 **Discussion**

366 We found that birds collected as nestlings from quiet forested areas, and reared in the  
367 laboratory with chronic exposure to noise did not sing songs that differed in frequency from  
368 those of the tutors from quiet forested areas. The minimum song frequencies were the  
369 same as those of the tutors both when we considered all the song types within their tutees'  
370 repertoires, and if we considered which song types they used in different noise conditions.  
371 While the commonest suggested reason for the higher frequency songs observed in urban  
372 birds has been that these pitch shifts are a response to low frequency noise, our  
373 experiments indicate that chronic exposure to city-like noise did not induce frequency shifts

374 within individual birds. Although there have only been a few studies that have examined the  
375 effects of noise exposure on individual song frequency shifts [27, 29, 30], our findings are  
376 contrary to those previous studies, but support the prediction of Slabbekoorn and den Boer-  
377 Visser that urban-forest population differences are likely to result from differences in song  
378 repertoire composition rather than individuals modifying the same song types [8].

379

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

388

389 A recent study found that young zebra finches that were exposed to low-frequency noise  
390 during early vocal ontogeny did not develop adult songs with higher minimum frequencies  
391 [42]. While this finding is important for a general understanding of the auditory feedback  
392 mechanisms during vocal production learning, it is not clear whether wild zebra finch  
393 populations differ at all in song pitch. Our data show that even in a species in which the  
394 urban upward shift of song frequencies is well documented, this pattern cannot be  
395 explained by developmental plasticity during vocal learning. Moreover, unlike zebra finches,  
396 great tits typically have a repertoire of several different song types and our results show

397 that males not only stayed on pitch when they copied songs from their tutors in noise, but  
398 also did not selectively acquire those song types into their repertoires that are particularly  
399 well-suited to the noise conditions under which they are memorized.

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

405

406 Previous studies reported individual frequency shifts related to noise exposure in several  
407 songbird species [e.g. 30-33]. Based on an experiment on free-ranging great tits, Halfwerk  
408 and Slabbekoorn suggested that males selected those song types from their repertoire that  
409 are particularly well suited for certain noise conditions [30]. However, the birds in our  
410 experiment did not show any tendency towards differential song type usage dependent on  
411 current noise conditions, as they neither sang higher frequency song types in low frequency  
412 noise nor lower frequency song types in high frequency noise. Can this conflicting evidence  
413 be explained by different methodology? Our noise treatment was different from that of  
414 Halfwerk & Slabbekoorn [30] and similar studies in other songbird species [27, 28], in that  
415 the period of noise exposure was of a much longer duration (2-3 weeks vs. several minutes).  
416 It may be that song type switching is a viable strategy only when dealing with transient  
417 increases in background noise level, but when faced with chronic noise, the need to display  
418 a large repertoire size, or to use song-type matching in territorial encounters with  
419 neighbours, overrides any potential benefits that might be gained by using only the higher

420 frequency subset of the song repertoire. However, if a switch in song types only occurs in  
421 response to short-term fluctuations in noise, this cannot account for the observed  
422 divergence in minimum frequency between birds in noisy and quiet habitats.

423

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

436 The change in song frequency observed in urban populations is often assumed to be an  
437 adaptive response to the low-frequency noise typical of these areas [but see 48]. However,  
438 environmental acoustics of cities differ from the original habitats of birds in more than just  
439 noise profile [49], and the combined effects of noise and altered habitat acoustics may  
440 impose a stronger selective force on vocal signalling than does traffic noise alone [50].

441 While several studies reported positive correlations between breeding bird species  
442 richness/abundance and proximity to noisy roads [reviewed in 1, 51], a recent study

443 attempted to disentangle the effects of traffic noise from the traffic itself and found that  
444 roads and vehicles on them explained the negative effects better than the noise per se [51].  
445 Male density may also contribute to changes in song [52] as has been found in urban  
446 Japanese great tits [6] and Northern cardinals (*Cardinalis cardinalis*) [53]. However, no  
447 correlation between male density and minimum frequency was found in cardinals [53] or  
448 Eurasian blackbirds [54]. Little is known about the impact of air or water pollution on the  
449 behaviour of wild animals, but as air pollutants such as ozone and nitrogen oxides  
450 irreversibly damage birds' lungs [55], and chronic exposure to hydrocarbons emitted by  
451 traffic is correlated with reduced growth rates [56] and body weight [57] in birds, it is not  
452 hard to imagine that air pollution could indirectly lead to changes in vocalizations.

453

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

466

467 **Ethics**

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

472 **Competing Interests**

473 We have no competing interests

474 **Author's contributions**

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

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483





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

490 **Figure 2.** Neither the birds reared in low frequency (CITY) noise (A), nor those reared in high  
491 frequency (CONTROL) noise (B) switched to song types that differed in minimum frequency  
492 when the background noise conditions changed. Mean minimum frequencies for three, 5-  
493 minute long randomly selected song bouts for each bird are shown. Means for each bird in  
494 each background noise condition are shown; error bars indicate 95% confidence intervals  
495 for the data. Colour denotes individuals.

496 **Figure 3.** Wild-caught adult male great tits sang with higher amplitudes when background  
497 noise levels increased (A), but did not increase song frequency as vocal amplitudes  
498 increased (B). Means, plus 95% confidence intervals for each individual are shown. Colour  
499 denotes individuals.

500

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