Extra-pair paternity variation in two passerine birds breeding in a gradient of urbanisation

Irene Di Lecce[1]*, Joanna Sudyka[2,3], Charles Perrier[4], Marta Szulkin[1]

[1]Institute of Evolutionary Biology, Biological and Chemical Research Centre, Faculty of Biology, University of Warsaw, Warsaw, Poland

[2]Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

[3]Groningen Institute for Evolutionary Life Sciences (GELIFES), Netherlands

[4]CBGP, INRAe, CIRAD, IRD, Montpellier SupAgro, University of Montpellier, France

*Correspondence: e-mail: irene.dilecce1@gmail.com

Abstract

Urbanisation has been increasing worldwide in recent decades, driving environmental change and exerting novel selective pressures on wildlife. Phenotypic differences between urban and rural individuals have been widely documented in several taxa. However, the extent to which urbanisation impacts mating strategies is less known. Here, we inferred extra-pair paternity variation in nestbox-breeding great tits (*Parus major)* and blue tits (*Cyanistes caeruleus)* in a gradient of urbanisation in Warsaw, Poland, over 3 breeding seasons. Urbanisation was quantified as the amount of impervious surface area (ISA), light pollution, noise pollution and tree cover within a 100 m radius around each nestbox. We successfully genotyped 1235 great tits and 1306 blue tits with a genotyping-by-sequencing method. Extra-pair paternity was inferred by computing a genomewide relatedness matrix on 9379 SNP markers in great tits and 12958 SNP markers in blue tits. We report higher extra-pair paternity in blue tits breeding in more urbanized areas (e.g. with higher ISA, light pollution and noise pollution, and lower tree cover). However, no such trend was found in great tits. Late-stage survival of individual nestlings in both species was not associated with paternity or urbanisation proxies, thus we were not able to detect fitness benefits or drawbacks of being within-pair or extra-pair offspring in relation to the inferred degree of urbanisation. Our results contribute to the growing body of knowledge reporting on the effects of urbanisation on avian ecology and behaviour and confirm species-specific and population-specific patterns of extra-pair paternity.

Keywords

Extra-pair paternity, urbanisation, nestbox, *Parus major, Cyanistes caeruleus*

Introduction

Urbanisation is responsible for creating ecologically novel habitats by fragmenting, isolating and ultimately replacing the natural environment (Marzluff & Ewing, 2001). In contrast to rural habitats, urban areas are characterized by specific environmental pressures, such as higher temperatures; noise, light, air and chemical pollution; higher population densities; lower availability of natural food sources and presence of anthropogenic food (Oke, 1973; Møller et al., 2012; Szulkin et al., 2020). Birds such as great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) are a valuable study system in urban evolutionary ecology, as they are present in both natural and man-made environments, and therefore allow inference into biological variation driven by urbanisation (Marzluff, 2017; Thompson 2022). There is now accumulating evidence that urbanisation may affect aspects of their phenotype, behaviour, life-history, demographics, and lead to altered fitness (Isaksson, 2018; Rodewald & Gehrt, 2014). For instance, studies report an earlier onset of reproduction, reduced brood sizes, higher rates of nest-failure and lower body mass of nestlings in urban environments compared to more natural ones (Peach et al., 2008; Hedblom & Soderstrom, 2012; Capilla-Lasheras et al., 2022; but see Chamberlain et al. 2009 for evidence of increased annual productivity in urban areas). In addition, birds from urban populations tend to be smaller and lighter, and tend to show more variable body sizes compared to conspecifics from rural areas (Corsini et al., 2020; Thompson et al., 2022).

While the impact of urbanisation on phenotypes is increasingly well understood, knowledge on the extent to which cities impact sexual traits and sexual selection is more limited (Sepp et al., 2020). Divergent sexual selection pressures driven by urbanisation could lead to differences in behavioural, physiological, morphological or life-history traits in signal emitters and/or signal receivers between urban and non-urban populations, ultimately driving adaptation and speciation (Cronin et al., 2022; Thompson et al., 2018). For instance, urban blackbirds (*Turdus merula*) advance their dawn song and become active earlier in the morning due to noise and light pollution (Nordt & Klenke, 2013), similarly to European robins (*Erithacus rubecula*) breeding in noisy areas during the day (Fuller et al., 2007) and to great tits (*Parus major*) in artificially lighted areas (Da Silva et al., 2014). It has also been shown that urban great tits and urban blackbirds sing songs with higher frequency (i.e. pitch) compared to forest birds (Slabbekoorn & Peet, 2003; Nemeth & Brumm, 2009). There is also evidence regarding urbanisation and plumage coloration: polymorphic species living in cities show darker plumages than those in forests (Jacquin et al., 2013). Furthermore, birds from urban environments have duller carotenoid-based plumage (Jones et al., 2010; Salmón et al., 2023) and less conspicuous sexual plumage traits (Chatelain et al., 2017; Senar et al., 2014). These alterations in phenotype, singing behaviour, communication efficiency, mate preference, breeding phenology, activity patterns and densities may thus affect the outcome of female-male interactions in a sexual selection context, and consequently influence extra-pair paternity in the urban environment.

Mating outside the social bond has been widely observed in socially monogamous species with biparental care (Brouwer & Griffith 2019). Extra-pair paternity is a long studied mating behaviour, where offspring are sired by males other than the female's social partner and for which great variation is observed among individuals, populations and species (Griffith, 2002). Many theories have been suggested to explain this aspect of avian mating systems (reviewed in Birkhead & Møller, 1992, 1996; Gowaty 2006). Males can maximize their reproductive success by copulating with as many females as possible, with few risks and costs, because their parental investment is lower than that of females. On the other hand, females may incur much higher costs by copulating with males outside their social bond, and yet they have been observed seeking extra-pair copulations, for instance as a bet-hedging strategy or to maximise genetic diversity of their offspring (Westneat & Stewart, 2003).

By inducing physiological and/or behavioral responses, the urban environment might act as an ecological driver for altered patterns of interaction and communication between females and males and consequently of extra-pair paternity. For instance, Kempenaers et al., 2010 showed that male blue tits occupying edge territories with street lights advanced their dawn song and obtained more extra-pair partners than males in non-edge territories or in edge territories without street lights. In another study, it was reported that under high environmental noise the preference of females for their pair-bonded males decreased in zebra finches (*Taeniopygia guttata*), suggesting that the noisy urban environment might lead to higher rates of extra-pair paternity (Swaddle & Page, 2007). In great tits, Halfwerk & Slabbekoorn (2009) showed short-term flexibility in singing response to noisy conditions, which impair male-female and male-male communications. For instance, in the presence of low-frequency "city-life" conditions emulating traffic noise, birds advantageously shifted to high-frequency songs, with consequences for female fidelity and reproductive success in the urban environment, as females showed a preference for low-frequency songs during their peak of fertility (Halfwerk et al., 2011). Pipoly et al., 2019 further showed higher frequency of extra-pair offspring in great tit broods in urban habitats relative to forest habitats. Additionally, it has been suggested that habitat structure (in particular vegetation density) may affect opportunities for extra-pair copulations, by influencing mate-guarding efficiency by males or extra-territorial forays of both males and females to gain extra-pair partners (Sherman & Morton 1988; Westneat & Stewart 2003). For instance, Mays & Ritchison (2004) found that high vegetation density negatively affected male proximity to their mate in yellow-breasted chats (*Icteria virens*), although it was not clear whether this affected extra-pair paternity. The urban environment is characterized by lower structural complexity of vegetation and lower tree cover (Gil & Brumm, 2013; Szulkin et al., 2020), which might remove visual obstacles and might therefore facilitate males in guarding their mates and/or in keeping intruders off their territories, leading to lowered extra-pair paternity rates. Conversely, the presence of buildings and concrete walls may represent a larger obstacle than dense vegetation, obstructing the visual component of mate-guarding behavior and favouring concealment to engage in extra-pair copulations. In addition, because of the patchy distribution of vegetation cover and higher instability of food sources in urban habitats (lower availability of high-quality food such as caterpillars for tit species and presence of low-quality anthropogenic food) (Mackenzie, et al., 2014), birds might have to travel much further than their territories to forage, allowing females to escape guarding behavior by their social mate and allowing neighbouring males to gain access to females. Alternatively, the lower food availability of the urban environment might lead females to engage more often in extra-pair copulations in order to gain more resources (Gray, 1997). Indeed, higher rates of extra-pair paternity have been observed in lower quality territories (in terms of vegetation cover and food availability; Charmantier & Blondel, 2003; Rubenstein, 2007).

Here, we tested whether environmental features of the urban space affect rates of extra-pair paternity. During three consecutive seasons, we recorded breeding events of wild great tits and blue tits taking place in nestboxes set in a gradient of urbanisation in eight study sites within and outside the city of Warsaw, Poland. We predicted higher rates of extra-pair paternity (specifically presence of extra-pair offspring in the brood and proportion of extra-pair offspring per brood) in nests surrounded by higher Impervious Surface Area (ISA), higher light pollution, higher noise pollution and lower tree cover (Figure 1). We hypothesized that the structural features of the urban environment (higher ISA) might hinder mate-guarding behaviour, leading to higher rates of extra-pair paternity. We expected higher light pollution to correlate with higher rates of extra-pair paternity, based on previous evidence indicating that it might facilitate seeking and obtaining extra-pair fertilizations. We also expected higher noise pollution to correlate with higher extra-pair paternity based on previous evidence showing disruptions of male-female and male-male communication patterns in urban environments. In addition, we predicted that the lower environmental quality of the urban habitat, exemplified by lower tree cover, would induce females to engage more often in extra-pair paternity to increase survival of their offspring and/or allow both females and males greater opportunity to seek extra-pair matings.

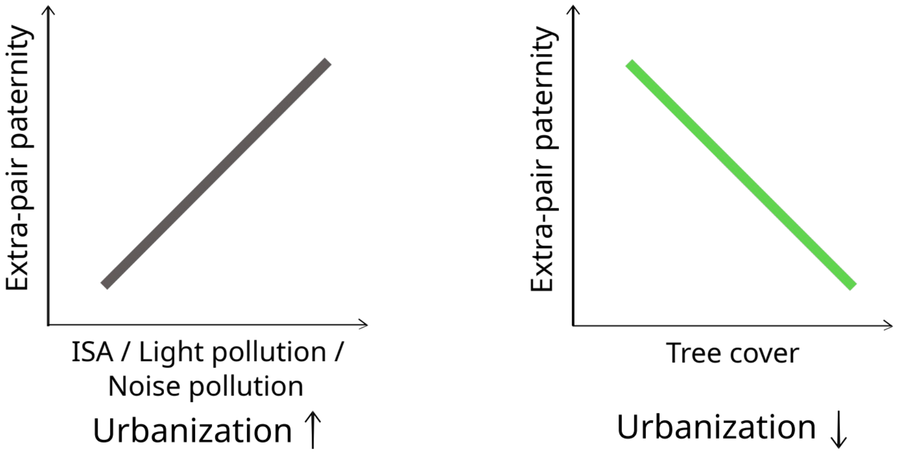

Figure 1. Schematic representation of the predictions of the study.

Materials and Methods

Study sites and field methods

The study was carried out on wild great tits and blue tits breeding in 500 nestboxes set up in eight study sites within and outside the city limits of Warsaw, Poland. A characteristic of all sites, ordered in decreasing distance to the city centre, is presented in Table 1 and a map of the study sites is presented in Figure 2.

Table 1. A description of the eight study sites.

	Site name	Coordinates	Nr nestbox	Description
A	**Palmiry**	20°46'48.9748" E - 52°22'11.3382" N	47	suburban village
B	**Kampinoski Park Narodowy**	20°47'14.3867" E - 52°21'22.5409" N	110	mixed-coniferous forest outside the city limits
C	**Osiedle Olszyna**	20°57'39.37097" E - 52°16'23.71883" N	52	residential area
D	**Las Olszyna**	20°57'33.93652" E - 52°16'10.55093" N	21	deciduous, wet alder forest
E	**Cmentarz Żydowski**	20°58'23.44285" E - 52°14'52.45584" N	91	urban woodland area
F	**Muranów**	20°59'5.74332" E - 52°14'52.17925" N	46	residential area
G	**Kampus Ochota Uniwersytetu Warszawskiego**	20°59'8.85224" E - 52°12'43.77676" N	28	Warsaw University Science campus
H	**Pole Mokotowskie**	21°0'6.98321" E - 52°12'46.66874" N	105	urban park

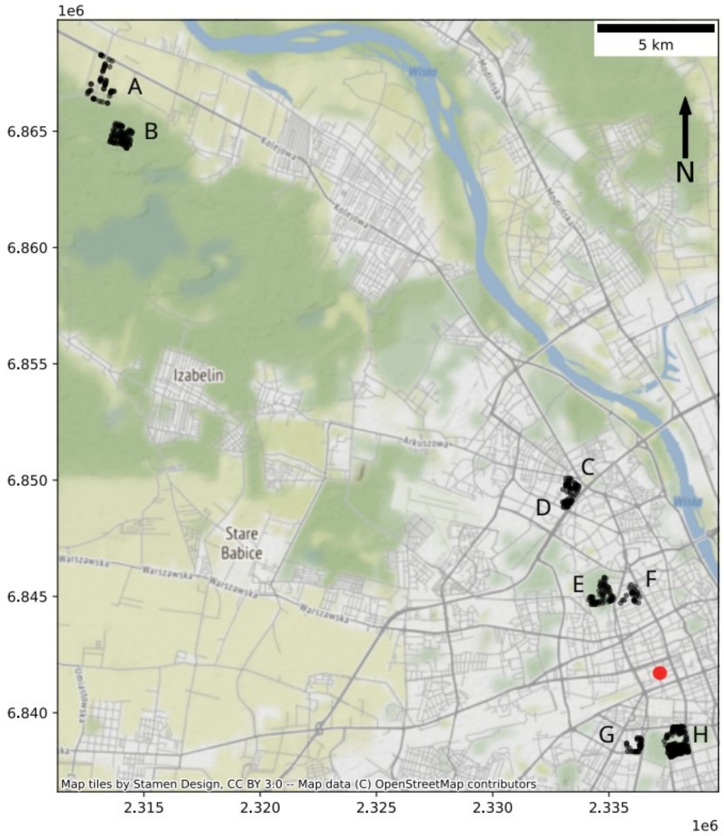

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Figure 2. Study sites (A-H) representing a mosaic of heterogeneous habitats within and outside of Warsaw, Poland. Black dots represent individual nestboxes in each site and the red dot is the Palace of Culture and Science (city centre).

Data was collected over three consecutive breeding seasons, from 2017 to 2019. In each study site, woodcrete Schwegler 1b nestboxes, with a 32 mm diameter entrance, were hanged 50 meters apart at a height between 2.5 and 3 m with a random orientation. Protective devices against predation were used in Palmiry and Kampinos National Park, due to high predation rate (personal observations). Nestboxes were cleaned at the end of each breeding season and in February/early March to remove nesting material from winter roosts. From the beginning of April, nestboxes were inspected on a weekly basis to record all breeding events. From the start of incubation, each nest was monitored individually. Egg laying date, clutch size, number of hatchlings and number of fledged birds were recorded for each nest. Blood samples were collected from adults at catching when nestlings were at least 12 days old (hatching day = day 1) and from nestlings at 15 days of life (based on the oldest nestling in the brood in case of asynchrony of hatching), by puncturing the brachial vein with a sterile needle into heparin-free capillary tubes. A tail feather was collected in case of unsuccessful blood sampling and tissue samples were collected from nestlings that died between hatching and day 15. Samples were preserved in 99% ethanol at +4 °C until DNA isolation. Only first clutches were included in the analyses, as opportunities and constraints for extra-pair copulations may differ between first and second clutches (Weatherhead & McRae, 1990; Dietrich et al., 2004). For instance, males providing post-fledging care to first brood offspring might be less able to guard their mates that are laying a new clutch, compared to males during a first nesting attempt. Additionally, females initiating a second nesting attempt might have easier access to males whose mates are not initiating a second brood. Alternatively, males from pairs not initiating a second brood might pursue extra-pair copulations more intensely, as they are free from duties related to nesting with their mate. Thus, a threshold was calculated by adding 30 days to the earliest laying date within each study site and year, after which a clutch was considered a second breeding attempt and removed from analyses (this way we excluded only actual second clutches and not repeated clutches after failure of first breeding attempts; Van Balen, 1973). To remain within the 30 days timeline, we discarded 104/335 (31%) great tit nests and 34/260 (13%) blue tit nests for which we had information on egg laying date.

Genetic analysis, parentage and sex assignment

Genomic DNA was extracted from 2606 blood samples using the Blood Mini kit and from 193 feather and tissue samples using the Genomic Mini kit (A&A Biotechnology, Gdynia, Poland), collected from 1272 great tits and 1334 blue tits. We modified the manufacturer’s protocol by incubating blood and tissue samples overnight at 37 °C. DNA concentration and purity was assessed with a DeNovix DS-11 spectrophotometer. Genotyping by sequencing was performed at 9379 SNP markers in great tits and 12958 SNP markers in blue tits by Diversity Arrays Technology Pty, Ltd (Canberra, AU) using the DarTseqLD method, which employs genomic complexity reduction using restriction enzyme pairs (Kilian et al. 2012). Detailed information on the method can be found in Supplementary Material and Di Lecce et al., 2023. All subsequent analyses were performed in R (version 4.1.2) (R Core Team, 2021) separately for each species. After filtering SNP data for individuals and loci with call rate lower than 70% using dartR (version 1.9.9.1; Gruber et al., 2018), 7344 SNP markers and 1235 individuals were kept in great tits and 9366 SNP markers and 1306 individuals in blue tits. A genomewide relatedness matrix (GRM) was computed among individuals from pairs of neighbouring sites using the function snpgdsGRM with the method GCTA (Yang et al., 2011) implemented in *SNPRelate* (version 1.26.0) (Zheng et al., 2012). Extra-pair offspring, extra-pair fathers and broods containing half-siblings, where the social father was not sampled, were identified based on discrepancies between the GRM and a social pedigree of all individuals ringed in the field created using *ggroups* (version 2.1.0) (Nilforooshan et al., 2020). Figure 3 shows the distribution of GRM values zoomed in on related individuals from all study sites and Figure 4 shows the comparison between GRM and social relatedness values.

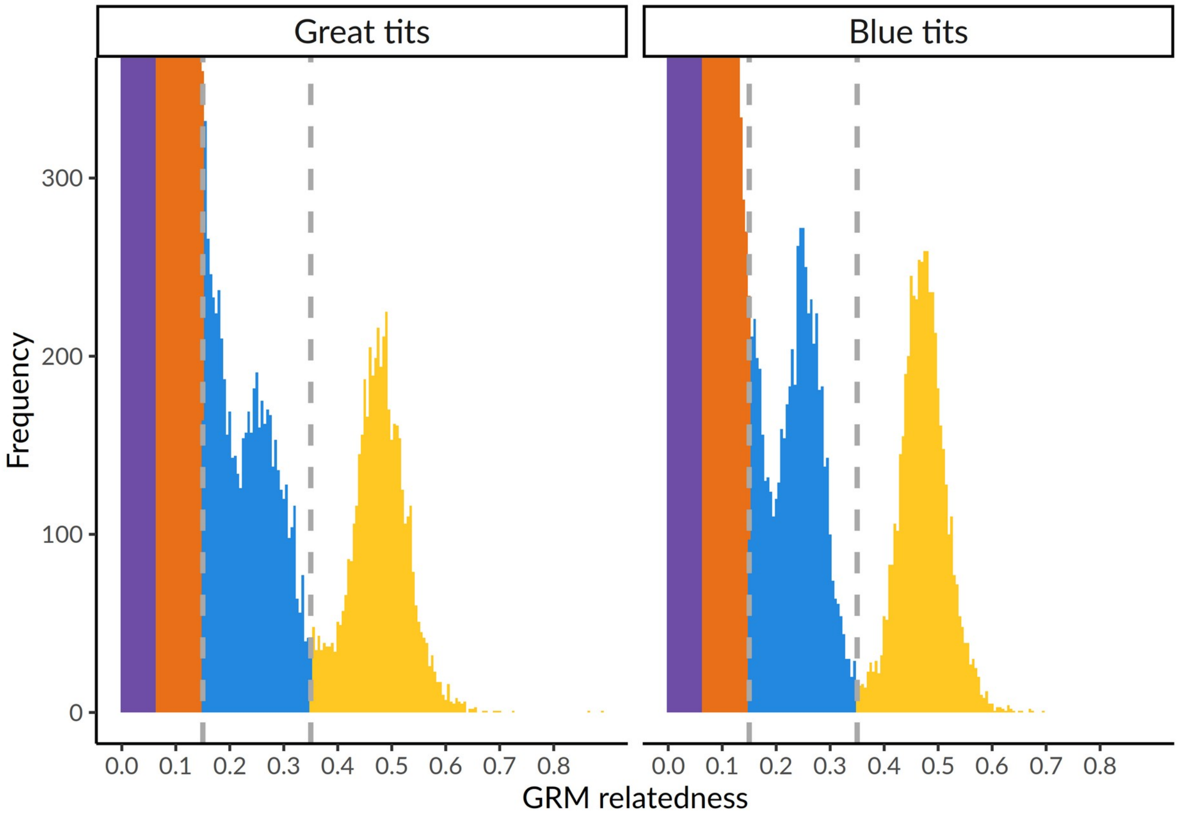

Figure 3. Distribution of GRM values zoomed in on related individuals. Color intervals are indicative of different coefficients of relatedness: purple indicates 1/16; orange 1/8; blue 1/4 (reflecting half-sib relatedness) and yellow 1/2 (full sibling and parent-offspring relatedness). Vertical dotted lines indicate cut-off values used to identify half-siblings, full-siblings and extra-pair paternities.

Following Perrier et al. (2018), father – offspring pairs (social relatedness = 0.5) with GRM relatedness estimates below 0.15 were classified as instances of extra-pair paternity (falling within the orange/purple distribution in Figure 4). Adult males with GRM relatedness estimates above 0.35 with offspring from other nests (social relatedness = 0) were identified as extra-pair fathers (falling within the yellow distribution in Figure 4). In nests where the social father was not sampled (e.g. when catching attempts failed), pairs of siblings within a given nest (social relatedness = 0.5) with GRM estimates between 0.15 and 0.35 were classified as half-siblings (falling within the blue distribution in Figure 4) and above 0.35 as full siblings (falling within the yellow distribution in Figure 4). Nestlings with GRM relatedness estimates below 0.1 to both social parents and social siblings (social relatedness = 0.5) were classified as instances of brood parasitism (falling within the orange/purple distribution in Figure 4). Sex was assigned to 1966 nestlings with *assignPOP* (version 1.2.2) (Chen et al., 2018) using markers that diverge between females and males (Brelsford et al., 2017; Trenkel et al., 2020). We identified 11 markers in blue tits and 7 in great tits which showed sex differences in heterozygosity, high FST and by running BayeScan 2.1 with default parameter options (outlier SNPs were identified with a q-value below 0.05; Foll & Gaggiotti 2008).

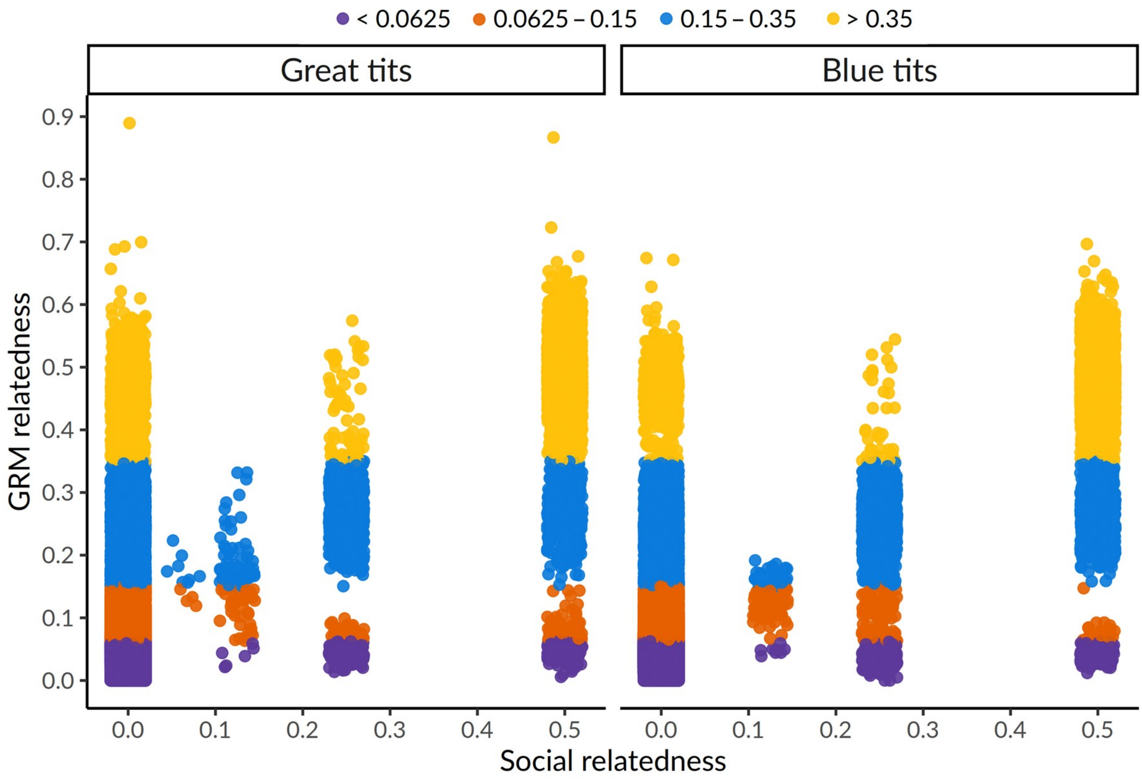

Figure 4. Biplot of GRM and social relatedness values among all individuals illustrating the concordances and discrepancies between the two. GRM and social information are not always concordant and GRM gives in-depth information on relatedness that is not captured by social pedigrees. Color intervals are indicative of different coefficients of relatedness: purple indicates 1/16; orange 1/8; blue 1/4 (reflecting half-sibling relationships) and yellow 1/2 (reflecting full sibling and parent-offspring relationships).

Environmental variables connected to urbanisation

Various environmental variables in this urban setting have been readily demonstrated to alter avian physiology, nestling development and reproductive outputs (Corsini et al., 2020; Chatelain et al., 2021; Maraci et al., 2022). Urbanisation is also likely to change interaction patterns among breeding birds – in terms of foraging, diurnal activity or communication patterns – possibly driving changes in extra-pair paternity rates in the population. Consequently, the following environmental variables describing the habitat within a 100 m radius surrounding each nestbox were estimated: (a) Impervious Surface Area (ISA), that is the percentage of soil area covered by impervious materials, such as buildings, roads and other infrastructure (Yuan & Bauer 2007); (b) light pollution, that is artificial night lighting, calculated as average pixel brightness (uncelebrated digital number) from a night-time digital photograph of Warsaw taken by the International Space Station with a resolution of 10 m per pixel (Kyba et al., 2015); (c) noise pollution, recorded over four days throughout the field season, three times per day, on DbC scale using hand-held sound level meters equipped with a microphone, and (d) tree cover, that is the percentage of tree cover density of the surface. Previous work in this study site demonstrated a positive relationship of ISA with light pollution and noise pollution, and a negative relationship with tree cover (Szulkin et al., 2020). A radius of 100 m around each nestbox corresponds to a literature-based conservative estimate of the range of parental foraging distance during nestling feeding, assessed in blue tits to be on average 53.2 m (±22.9 SD) in natural, food poor, environments (Tremblay et al., 2004). More details on how these variables were quantified can be found in Szulkin et al. (2020).

Statistical analysis

We used generalized linear mixed models to test whether extra-pair paternity covaried with urbanisation, whilst including breeding pair identity as random effect. As response variables we tested (i) the occurrence of extra-pair paternity in the brood (presence/absence of extra-pair offspring in the brood) in a model with binomial error structure and logit link function within the R package *lme4* (Bates et al., 2015) and (ii) the proportion of extra-pair offspring per brood (calculated as number of extra-pair offspring/total number of offspring in the brood) in a model with betabinomial error structure and logit link function within *glmmTMB* (Brooks et al., 2017). As a proxy for urbanisation we used ISA, light pollution, noise pollution and tree cover. Because these variables were highly correlated ($R > |0.5|$), they were separately included in the models. To assess the relative importance of these distinct environmental variables on variation in extra-pair paternity rates, we reported the percentage of variance explained by each of them, calculated by subtracting the marginal r-squared value of the model not including the urbanisation variable from r-squared values of full models. Due to mortality recorded between hatching and time of sampling, we included all broods sampled for at least 50% of the original number of hatched nestlings in both models, in order to gain a representative sample size of the original brood (22 nests in blue tits and 18 in great tits were removed). We included year as categorical variable ($n = 3$ years), and laying date and clutch size as fixed effects in all models. Squared laying date was initially included in the models (as food availability is distributed non-linearly across a breeding season; Wesołowski and Rowiński 2014) and later removed as the variable was always non-significant (all $P > 0.1$). We introduced site identity as random effect but encountered convergence issues in all models and therefore retained only breeding pair identity in the final models. In the model testing the occurrence of extra-pair paternity in the brood we first included an interaction between the urbanisation variable and year and later removed it from final models as always non-significant (all P > 0.1). Finally, we investigated the effect of each urbanisation variable at the individual level, with a generalized linear mixed model with binomial error distribution and logit link function. Offspring status as within-pair or extra-pair (0/1) was the response variable and nest identity was introduced as random effect. We also tested for the effect of being extra-pair on a proxy of fitness (i.e. successful fledging of offspring ringed and blood sampled 15 days after hatching, hereafter late-stage survival) in a similar model. Nestlings that died before day 15 (101 great tits and 79 blue tits) were therefore excluded. The response variable was offspring status as fledged (1) or not fledged (0) and nest identity was fitted as random effect. In both these models, year (n = 2 years) and sex were included as fixed factors, and body condition index, laying date and clutch size were fitted as covariates. Body condition index was calculated as ‘scaled mass index’ following Peig & Green (2009) for 2018 and 2019, because we did not measure tarsus in 2017. We repeated the analyses with body mass instead of body condition index to include all 3 sampled years (Table S2; Table S3). In the model for late-stage survival, offspring status as within-pair or extra-pair was also included as fixed factor and a focal two-way interaction between the urban variable and offspring status was tested. All models were checked for dispersion, zero inflation and multicollinearity (VIF scores in each model never exceeded 2) (Hartig, 2022). All statistical analyses were performed in R 4.1.3 (R Core Team, 2021), separately for each species. R-squared was calculated with the function r.squaredGLMM in *MuMIn* (Barton, 2009) and r2_nakagawa in *performance* (Lüdecke et al., 2021).

Results

Genetic paternity and extra-pair paternity

In great tits, 944 nestlings from 161 broods and 309 adults were successfully sequenced, together with 1022 blue tit nestlings from 157 broods and 295 blue tit adults. In each species, the distribution of GRM values (Figure 3) highlights the presence of parent-offspring links (yellow), full sibling (yellow) and half sibling relationships (blue). The comparison of relatedness values between the GRM and the social pedigree revealed some discrepancies, indicating extra-pair paternity and brood parasitism. Males with social relatedness of 0.5 and GRM relatedness < 0.1 with their social offspring represented cuckolded fathers [n = 42 out of 125 in great tits (34%) and n = 66 out of 137 in blue tits (48%); Figure 4]. Males with social relatedness 0 and GRM relatedness > 0.35 with offspring from other nests were identified as extra-pair fathers (n = 17 out of 216 great tits and n = 24 out of

189 blue tits; Figure 4). Extra-pair offspring were identified by having social relatedness 0.5 and GRM relatedness < 0.1 with their social father and social relatedness 0 and GRM relatedness > 0.35 with fathers from other nests (n = 79 out of 753 in great tits and n = 133 out of 903 in blue tits; Figure 4). Thus, 11% of great tit offspring, and 15% of blue tit offspring were classified as extra-pair offspring. In nests with unknown social father identity, nestlings with 0.5 social relatedness and GRM relatedness between 0.15 and 0.35 with each other were identified as half siblings (n = 11 out of 36 nests and n = 69 out of 191 nestlings in great tits; n = 7 out of 20 nests and n = 49 out of 120 nestlings in blue tits; Figure 4). Nestlings with GRM relatedness estimates < 0.1 to the other nestlings in the nest and to both social parents (0.5 social relatedness) were classified as instance of brood parasitism (n = 2 blue tit nestlings; Figure 4). In great tits, 32% (52/161) of broods were of mixed-paternity, and in blue tits 47% (73/157). Figure S1 reports the number of broods by number of extra-pair offspring per species.

Extra-pair paternity and urbanisation at the nest level

In blue tits, the occurrence of extra-pair paternity in the brood, modeled as absence (0) or presence (1) of extra-pair offspring, was positively associated with ISA around the nest (Table 2; Figure 5). Among the studied variables, ISA explained the greatest amount of variance in the presence or absence of extra-pair offspring in the brood (Table 3). In great tits, our models showed no association between urbanisation and presence of extra-pair offspring in the brood (Table 2; Figure 5). The interaction between each urban variable and year was never significant and removed from final models (in great tits: ISA x year: $\chi^2 = 0.248$, $P = 0.884$; light pollution x year: $\chi^2 = 1.198$, $P = 0.549$; noise pollution x year: $\chi^2 = 0.538$, $P = 0.764$; tree cover x year: $\chi^2 = 0.466$, $P = 0.792$, and blue tits: ISA x year: $\chi^2 = 0.348$, $P = 0.840$; light pollution x year: $\chi^2 = 0.115$, $P = 0.944$; noise pollution x year: $\chi^2 = 0.665$, $P = 0.717$; tree cover x year: $\chi^2 = 1.016$, $P = 0.602$).

Table 2. Generalized linear mixed models with presence of extra-pair offspring in the brood (0/1) as the dependent variable. Year, laying date, clutch size, ISA (model a), light pollution (model b), noise pollution (model c) and tree cover (model d) were included as predictors. Social pair identity was introduced as random effect. Reference level for year was 2017. Significant differences ($P < 0.05$) are in bold, trends ($P < 0.2$) in italics. Marginal (R^2m) and conditional (R^2c) R-squared are shown.

Presence of extra-pair offspring in the brood

	 **(n = 120)**			 **(n = 120)**		
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
a	**ISA**	0.042 (0.218)	0.846	**ISA**	0.456 (0.229)	**0.047**
	Year		0.280	**Year**		*0.062*
	Year 2018	0.339 (0.654)		**Year 2018**	-0.949 (0.549)	
	Year 2019	0.894 (0.632)		**Year 2019**	0.271 (0.513)	
	Laying date	-0.412 (0.320)	*0.198*	**Laying date**	-0.002 (0.219)	0.994
	Clutch size	0.099 (0.222)	0.655	**Clutch size**	0.101 (0.207)	0.625
	R^{2}m	0.053		**R^{2}m**	0.099	
	R^{2}c	0.086		**R^{2}c**	0.133	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
b	**Light pollution**	-0.003 (0.217)	0.991	**Light pollution**	0.330 (0.221)	*0.136*
	Year		0.272	**Year**		*0.086*
	Year 2018	0.366 (0.651)		**Year 2018**	-0.860 (0.540)	
	Year 2019	0.914 (0.630)		**Year 2019**	0.264 (0.511)	
	Laying date	-0.418 (0.320)	*0.192*	**Laying date**	-0.012 (0.217)	0.956
	Clutch size	0.085 (0.222)	0.703	**Clutch size**	0.070 (0.205)	0.731
	R^{2}m	0.052		**R^{2}m**	0.079	
	R^{2}c	0.084		**R^{2}c**	0.118	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
c	**Noise Pollution**	-0.205 (0.234)	0.382	**Noise Pollution**	-0.003 (0.216)	0.989
	Year		0.246	**Year**		*0.096*
	Year 2018	0.550 (0.682)		**Year 2018**	-0.863 (0.531)	
	Year 2019	1.031 (0.650)		**Year 2019**	0.189 (0.499)	
	Laying date	-0.481 (0.333)	*0.149*	**Laying date**	-0.083 (0.225)	0.712
	Clutch size	-0.002 (0.232)	0.992	**Clutch size**	0.004 (0.204)	0.986
	R^{2}m	0.060		**R^{2}m**	0.056	
	R^{2}c	0.096		**R^{2}c**	0.090	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
d	**Tree cover**	-0.005 (0.217)	0.983	**Tree cover**	-0.162 (0.220)	0.460
	Year		0.272	**Year**		*0.082*
	Year 2018	0.361 (0.657)		**Year 2018**	-0.901 (0.539)	

Year 2019	0.912 (0.628)		**Year 2019**	0.236 (0.506)	
Laying date	-0.417 (0.322)	*0.196*	**Laying date**	-0.022 (0.225)	0.923
Clutch size	0.087 (0.222)	0.695	**Clutch size**	0.023 (0.200)	0.909
R^2m	0.052		**R^2m**	0.061	
R^2c	0.085		**R^2c**	0.100	

Table 3. Amount of variance in extra-pair paternity, recorded as (a) presence or absence of extra-pair offspring in the brood and (b) proportion of extra-pair offspring per brood, explained by distinct environmental variables in blue tits. The contribution of each urbanisation variable was obtained by subtracting marginal r-squared values of the model not including the urbanisation variables from those of models in Table 2 and Table 4.

	(a) Presence of extra-pair offspring in the brood	**(b) Proportion of extra-pair offspring per brood**
	Marginal r-squared (%)	Marginal r-squared (%)
ISA	4.3	7.9
Light Pollution	2.3	5.2
Noise Pollution	0	3.4
Tree Cover	0.6	5.4

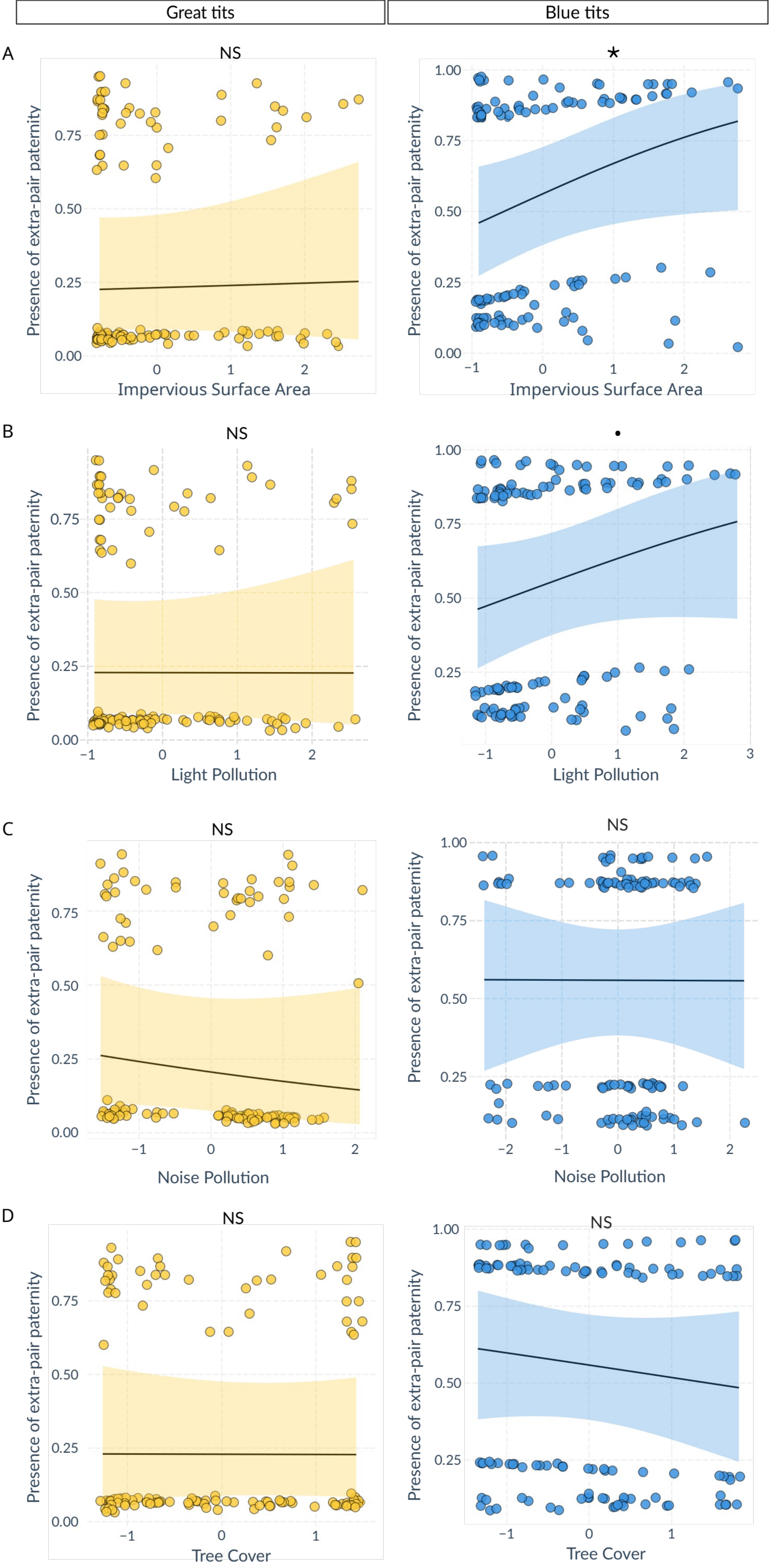
Great tits
Blue tits
A
NS
*
Presence of extra-pair paternity
0.00
0.25
0.50
0.75
1.00
−1
0
1
2
Impervious Surface Area
B
NS
•
0.00
0.25
0.50
0.75
1.00
−1
0
1
2
3
Light Pollution
C
NS
NS
0.00
0.25
0.50
0.75
1.00
−2
−1
0
1
2
Noise Pollution
D
NS
NS
0.00
0.25
0.50
0.75
1.00
−1
0
1
Tree Cover

Figure 5. Presence of extra-pair offspring in the brood in relation to urbanisation: a) ISA; b) light pollution; c) noise pollution and d) tree cover within 100 m around the nest in great tits and blue tits (based on values reported in Table 2). Predicted values with 95% confidence interval are shown. NS = non-significant relationship; * denotes a significant relationship with 0.01≤p≤0.05 and • a trend (p < 0.2).

Models in blue tits provided statistical support for an effect of urbanisation on the proportion of extra-pair offspring per brood for all the urban variables tested: the proportion of blue tit extra-pair offspring increased with increasing ISA, light pollution and noise pollution, and decreased with increasing tree cover around the nest (Table 4). ISA explained the greatest amount of variance in the proportion of extra-pair offspring per brood (Table 3). In great tits, there was no association between the proportion of extra-pair offspring per brood and the urban-related variables (Table 3). Models testing the probability of being extra-pair at the individual level in relation to the urbanisation variables are presented in Table S1 and Table S2.

Table 4. Generalized linear mixed models with proportion of extra-pair offspring per brood as the dependent variable. Year, laying date, clutch size, ISA (model a), light pollution (model b), noise pollution (model c) and tree cover (model d) were included as predictors. Social pair identity was introduced as random effect. Reference level for year was 2017. Significant differences (P < 0.05) are in bold, trends (P < 0.2) in italics.

Proportion of extra-pair offspring per brood

	 **(n=101)**			 **(n = 110)**		
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
a	**ISA**	0.025 (0.221)	0.909	**ISA**	0.632 (0.185)	**0.001**
	Year		0.220	**Year**		**<0.001**
	Year 2018	0.789 (0.675)		**Year 2018**	-1.371 (0.470)	
	Year 2019	1.069 (0.619)		**Year 2019**	0.340 (0.424)	
	Laying date	-0.552 (0.335)	0.099	**Laying date**	0.093 (0.184)	0.612
	Clutch size	0.058 (0.233)	0.803	**Clutch size**	-0.178 (0.184)	0.334
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
b	**Light pollution**	0.014 (0.228)	0.951	**Light pollution**	0.502 (0.188)	**0.008**
	Year		0.216	**Year**		**0.001**

	Year 2018	0.797 (0.677)		**Year 2018**	-1.273 (0.479)	
	Year 2019	1.073 (0.618)		**Year 2019**	0.346 (0.436)	
	Laying date	-0.554 (0.3350)	0.098	**Laying date**	0.097 (0.191)	0.611
	Clutch size	0.053 (0.236)	0.822	**Clutch size**	-0.204 (0.188)	0.279
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
c	**Noise Pollution**	0.018 (0.249)	0.944	**Noise Pollution**	0.436 (0.219)	**0.047**
	Year		0.229	**Year**		**0.001**
	Year 2018	0.791 (0.692)		**Year 2018**	-1.369 (0.487)	
	Year 2019	1.068 (0.629)		**Year 2019**	0.204 (0.438)	
	Laying date	-0.550 (0.345)	0.111	**Laying date**	0.125 (0.202)	0.537
	Clutch size	0.058 (0.262)	0.825	**Clutch size**	-0.171 (0.197)	0.387
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
d	**Tree cover**	-0.104 (0.211)	0.622	**Tree cover**	-0.566 (0.207)	**0.006**
	Year		0.218	**Year**		**<0.001**
	Year 2018	0.734 (0.674)		**Year 2018**	-1.358 (0.468)	
	Year 2019	1.060 (0.617)		**Year 2019**	0.405 (0.432)	
	Laying date	-0.522 (0.338)	0.122	**Laying date**	0.179 (0.196)	0.360
	Clutch size	0.091 (0.229)	0.691	**Clutch size**	-0.211 (0.185)	0.253

Fitness of extra-pair offspring in a gradient of urbanisation

In both species, late-stage survival was systematically associated with individual body condition, but not with any of the urbanisation proxies nor with offspring status as extra-pair or within-pair (Table 5). The interaction between each urban variable and offspring status was always non-significant and removed from final models (in great tits: ISA x epo: χ^2 = 0.007, P = 0.797; light pollution x epo: χ^2 = 0.089, P = 0.765; noise pollution x epo: χ^2 = 0.022, P = 0.883; tree cover x epo: χ^2 = 0.025, P = 0.875, and blue tits: ISA x epo: χ^2 = 0.936, P = 0.333; light pollution x epo: χ^2 = 0.815, P = 0.367; noise pollution x epo: χ^2 = 0.112, P = 0.738; tree cover x epo: χ^2 = 0.498, P = 0.480). This analysis was repeated by including body mass instead of body condition index to test all 3 years of the dataset, yielding equivalent results (Table S3).

Table 5. Generalized linear mixed models testing late-stage survival (0/1; dependent variable) of nestlings. Variables included as predictors were: offspring status as within-pair or extra-pair (0/1), year, sex, clutch size, body condition index, ISA (model a), light pollution (model b), noise pollution (model c) and tree cover (model

d). Reference levels were: 2018 (year), female (sex) and within-pair (epo). Significant differences ($P < 0.05$) are in bold, trends ($P < 0.2$) in italics. Marginal (R^2m) and conditional (R^2c) R-squared are shown.

Late-stage survival of nestlings

	 **(n = 526)**			 **(n = 567)**		
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
a	**ISA**	-0.577 (1.116)	0.605	**ISA**	0.033 (0.304)	0.913
	Year	-0.264 (3.219)	0.935	**Year**	0.809 (0.669)	0.226
	Laying date	1.856 (2.491)	0.456	**Laying date**	0.965 (0.581)	*0.096*
	Clutch size	-0.272 (1.423)	0.849	**Clutch size**	0.913 (0.526)	*0.083*
	Sex	0.283 (0.847)	0.738	**Sex**	-0.336 (0.357)	0.347
	Epo	0.473 (3.604)	0.896	**Epo**	0.187 (0.595)	0.753
	Body condition index	0.619 (0.478)	0.196	**Body condition index**	0.477 (0.219)	**0.029**
	R^2m	0		**R^2m**	0.092	
	R^2c	0		**R^2c**	0.358	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
b	**Light pollution**	-0.395 (1.180)	0.738	**Light pollution**	-0.015 (0.320)	0.963
	Year	-0.221 (3.284)	0.946	**Year**	0.798 (0.664)	0.229
	Laying date	1.823 (2.502)	0.466	**Laying date**	0.948 (0.579)	*0.101*
	Clutch size	-0.183 (1.374)	0.894	**Clutch size**	0.891 (0.511)	*0.081*
	Sex	0.282 (0.847)	0.739	**Sex**	-0.335 (0.357)	0.349
	Epo	0.397 (3.672)	0.914	**Epo**	0.193 (0.594)	0.745
	Body condition index	0.612 (0.478)	0.201	**Body condition index**	0.476 (0.219)	**0.029**
	R^2m	0		**R^2m**	0.092	
	R^2c	0		**R^2c**	0.358	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
c	**Noise Pollution**	0.436 (1.530)	0.776	**Noise Pollution**	-0.472 (0.382)	0.216
	Year	0.574 (3.717)	0.877	**Year**	0.773 (0.659)	0.241
	Laying date	2.492 (3.219)	0.439	**Laying date**	0.750 (0.579)	0.195
	Clutch size	0.105 (1.486)	0.944	**Clutch size**	0.682 (0.514)	0.185

	Sex	0.273 (0.845)	0.747	**Sex**	-0.319 (0.358)	0.374
	Epo	0.568 (3.587)	0.874	**Epo**	0.245 (0.592)	0.679
	Body condition index	0.604 (0.475)	0.204	**Body condition index**	0.447 (0.220)	**0.042**
	R^2m	0		**R^2m**	0.116	
	R^2c	0		**R^2c**	0.366	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
d	**Tree cover**	0.263 (1.576)	0.867	**Tree cover**	0.042 (0.344)	0.904
	Year	-0.191 (3.540)	0.957	**Year**	0.786 (0.673)	0.243
	Laying date	1.863 (2.634)	0.479	**Laying date**	0.932 (0.594)	*0.117*
	Clutch size	-0.171 (1.406)	0.903	**Clutch size**	0.880 (0.515)	*0.088*
	Sex	0.280 (0.846)	0.741	**Sex**	-0.335 (0.357)	0.349
	Epo	0.549 (3.610)	0.879	**Epo**	0.196 (0.594)	0.742
	Body condition index	0.596 (0.476)	0.210	**Body condition index**	0.476 (0.218)	**0.029**
	R^2m	0		**R^2m**	0.092	
	R^2c	0		**R^2c**	0.358	

Discussion

This study reports on a consistent trend for extra-pair paternity to positively covary with urbanisation in blue tits, but not in great tits. Rates of extra-pair paternity in the studied population were within the ranges that have been previously documented (Brouwer & Griffith, 2019). In great tits, 32% of broods and 11% of offspring were sired by extra-pair males. These values were even higher in blue tits, with 47% of broods and 15% of nestlings were sired by extra-pair males, which is consistent with previous reports (Brouwer & Griffith, 2019). In accordance with our expectations, we observed higher extra-pair paternity when nests were surrounded by higher urbanisation, although only in one of the two studied species. In blue tits, extra-pair paternity occurred more often in nests with higher amount of impervious surfaces (ISA) and the proportion of extra-pair offspring per brood was higher in nests with higher ISA, light pollution and noise pollution, and lower tree cover. No association between urbanisation and extra-pair paternity was detected in great tits. In both species, extra-pair and within-pair offspring did not differ in their late-stage survival, irrespective of how urbanized the nest surroundings were.

Several mechanisms might explain a positive relationship between extra-pair paternity rates and urbanisation. Previous evidence has shown that, compared to within-pair matings, extra-pair copulations occur in more secluded places, to ensure their success while avoiding the high costs of repercussions from the social male, i.e. punishment or reduction/loss of parental care (Tryjanowski et al., 2007). The structural features of the urban environment (higher ISA coupled with lower tree cover) might hinder mate-guarding behaviour and result into higher rates of extra-pair paternity. Visual occlusion provided by impervious surface areas might prevent males from guarding their mates as efficiently as in more open habitats or even in forest habitats (Sherman & Morton, 1988). Thus, females in the urban environment might be more able to foray on their own within and outside of their territories and be approached by extra-pair males and/or solicit extra-pair copulations (Ramos et al., 2014; Tryjanowski et al., 2007). As predicted by the habitat structure hypothesis by Sherman and Morton's (1988), Mays & Ritchison (2004) found that structural habitat characteristics can predict the probability of extra-pair paternity (but see contrasting results by Biagolini-Jr et al., 2017). Furthermore, increasing vertical habitat structure (causing visual occlusion) led to higher extra-pair paternity in the scissor-tailed flycatcher, *Tyrannus forficatus* (Roeder et al., 2022). Indeed ISA explained the highest amount of variance in models testing both the presence of extra-pair offspring in the brood and the proportion of extra-pair offspring per brood.

Another potential mechanism leading to higher extra-pair paternity in the urban environment is related to the presence of artificial night at light. The earlier start of daily activity induced by light pollution might facilitate seeking and obtaining extra-pair copulations, because of the link between dawn song and individual quality. In addition, the noisy conditions characterizing the urban environment may disrupt communication among males and the sexes, with possible consequences for the reproductive outcome of female-male interactions (Swaddle & Page, 2007; Halfwerk & Slabbekoorn, 2009; Halfwerk et al., 2011). Furthermore, higher extra-pair paternity rates in urban populations might be related to higher breeding densities often observed in urban habitats compared to rural habitats (Møller et al., 2012). Urban birds might therefore have more spatio-temporal opportunities for extra-pair copulations than their conspecifics breeding in forest habitats. Several studies have shown that an increased proximity among breeding individuals can favour encounter rates and reduce searching costs for an extra-pair partner (Mayer & Pasinelli, 2013; Stewart et al., 2010). On the other hand, several other studies have failed to find any support for the density hypothesis (Chuang et al., 1999; Tarof et al., 1998). Among the identified extra-pair fathers (17 in great tits and 24 in blue tits), we had geographical coordinates of the nest for 9 in great tits and for 19 in blue tits. 5 extra-pair fathers (56%) in great tits and 8 in blue tits (42%) were closest neighbours of the nest where they sired extra-pair offspring, while the remaining ones bred within one or two territories from where they sired extra-pair offspring. Given the limited number of extra-pair fathers, it is difficult to shed further light on the relationship between extra-pair paternity and density in our population.

The observed higher extra-pair paternity rates in the urban space might also be driven by lower food availability characterizing urban habitats. Previous work has shown how the patchy structure, altered species composition and lower vegetation cover of urban areas, coupled with high levels of pollution, negatively affected the abundance, richness and size of arthropods available to birds (Mackenzie et al., 2014; Shochat et al., 2004; Zvereva et al., 2010). A lower abundance of caterpillars, the main food source for nestlings, was found in urban parks compared to a nearby forest (Marciniak et al., 2007). Another study showed that urban caterpillars were more abundant and heavier than rural ones, however they had lower carotenoid concentration, suggesting that urbanisation exerts an influence on both food quantity and quality (Isaksson & Andersson, 2007; Isaksson, 2009). Indeed, it has been shown that lower environmental quality, i.e. consistent low food availability and/or high parasite infestation rates, correlates with higher rates of extra-pair paternity in natural habitats (Charmantier & Blondel, 2003; Rubenstein, 2007). It is therefore possible that urban birds might be more likely to engage in extra-pair matings while foraging further away from their nests, allowing females to escape guarding by their social mate and encounter males from neighbouring territories. Females breeding in low quality environments could also gain access to additional food resources and/or parental care by engaging in extra-pair copulations (Gray, 1997). For instance, female red-winged blackbirds (*Agelaius phoeniceus*) that copulated with extra-pair males were allowed to feed on their territories (Gray, 1997) and male black-capped chickadees (*Parus atricapillus*) that lost their social mate during breeding fed the extra-pair offspring that they likely sired (Otter et al. 1994). There is also evidence that female pied flycatchers (*Ficedula hypoleuca*), house wrens (*Troglodytes aedon*) and tree swallows (*Tachycineta bicolor*) solicited copulations from neighbouring males, once their social mates were experimentally removed after egg laying, and thus obtained assistance in brood rearing (Gjershaug et al., 1989; Freed, 1987; Robertson, 1990).

Similarly to what was observed at the nest level (Table 2), individual probability of being an extra-pair offspring was positively associated with ISA, light pollution and noise pollution, and negatively with tree cover (with these correlations being marginally significant for light pollution, noise pollution and tree cover in Table S1; Table S2). Interestingly, late-stage survival of offspring (from 15 days after hatching until successful fledging) was not associated with being extra-pair or within-pair offspring. This contrasts with previous studies on blue tits reporting, for instance, that extra-pair offspring were more likely to fledge than their within-pair half siblings

(Kempenaers et al., 1997; Charmantier et al., 2004). Our results suggest that there are no detectable benefits or drawbacks of being within-pair or extra-pair offspring in the studied urbanized environment. This might however stem from the fact that we investigated mortality at a later stage, that is from d15 to fledging, whereas most of mortality might occur earlier during development (Corsini et al., 2020). Unfortunately, we could assess offspring status as within-pair or extra-pair only for a subset of offspring that died before day 15 (31/101 in great tits and 38/79 in blue tits; i.e. only in those nests where it was possible to sample the social father). Additionally, as we did not collect blood samples right after hatching, our data on offspring status and survival until day 15 for dead nestlings only includes those offspring that died and were not removed from the nest by parents. Nevertheless, we found no apparent difference in the proportion of extra-pair vs within-pair offspring between offspring that died before day 15 and those that survived until day 15 (in great tits: X-squared = 3.765, df = 1, p-value = 0.052; in blue tits: X-squared = 0.148, df = 1, p-value = 0.701).

Conclusions

Extra-pair paternity in blue tits consistently covaried with urbanisation variables quantified within a 100 m radius around the nestbox. Higher ISA, light pollution and noise pollution, together with lower tree cover, were associated with higher rates of extra-pair paternity. Despite the urbanisation variables being highly intercorrelated (Szulkin et al. 2020), each of them is likely to be involved in different biological and ecological pathways. While promiscuity was not assessed *per se*, extra-pair paternity is generically used as a proxy variable for promiscuity; we therefore argue that promiscuity in blue tits is likely to increase with urbanisation – similarly to what was found earlier in other species (great tits: Pipoly et al., 2019; spotted towhees *Pipilo maculatus:* Smith et al., 2016; humans: Larmuseau et al., 2019). Furthermore, we found no association between offspring late-stage survival (from day 15 to fledging) and urbanisation variables in either species, irrespective of offspring status as within-pair or extra-pair. Our results complement previous evidence of alterations in phenotype, breeding phenology, density, physiology, nestling development and reproductive outputs observed in the urban environment. The fact that we did not detect higher extra-pair paternity in great tits suggests that different species and populations might react differently to different urban environments and/or that different urban environments might exert different selective pressures on wildlife. Further work is needed to shed light on the mechanisms leading to increased rates of extra-pair paternity in cities across avian species.

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Data Accessibility Statement

The data that support the findings of this study will be available in FigShare at 10.6084/m9.figshare.24512656 upon publication.

Author Contributions

I.D.L., J.S. and M.S. conceived the study and collected data in the field. I.D.L. performed lab work and analyzed the data, with contributions from all authors. I.D.L. wrote the first draft and all authors revised successive versions and approved the final manuscript. M.S. funded the project.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Supplementary Material

Genotyping

DNA sequencing was outsourced to Diversity Arrays Technology Pty, Ltd and performed using DArTseqLD, a high-throughput genotyping by sequencing method that employs genomic complexity reduction using restriction enzyme pairs (Kilian et al., 2012). DArTseq™ represents a combination of DArT complexity reduction methods and next generation sequencing platforms (Sansaloni et al., 2011; Kilian et al., 2012; Courtois et al., 2013; Raman et al., 2014; Cruz et al., 2013). Therefore, DArTseq™ represents a new implementation of sequencing of complexity reduced representations (Altshuler et al., 2000) and more recent applications of this concept on next generation sequencing platforms (Baird et al., 2008; Elshire et al., 2011). Similarly to DArT methods based on array hybridization, the technology is optimized for each organism and application by selecting the most appropriate complexity reduction method (both the size of the representation and the fraction of a genome selected for assays). Based on testing several enzyme combinations for complexity reduction, Diversity Arrays Technology Pty Ltd selected the SbfI-HpaII method for the two species of interest – the great tit Parus major and the blue tit Cyanistes caeruleus. DNA samples were processed in digestion/ligation reactions principally following Kilian et al. (2012) but replacing a single SbfI-compatible adaptor with two different adaptors corresponding to two different Restriction Enzyme (RE) overhangs. The SbfI-compatible adapter was designed to include Illumina flowcell attachment sequence, sequencing primer sequence and “staggered”, varying length barcode region, similar to the sequence reported by Elshire et al., 2011). Reverse adapter contained flowcell attachment region and HpaII-compatible overhang sequence. Only “mixed fragments” (SbfI-HpaII) were effectively amplified in 30 rounds of PCR using the following reaction conditions:

1. 94°C for 1 min
2. 30 cycles of: 94°C for 20 sec
   58°C for 30 sec
   72°C for 45 sec
3. 72°C for 7 min

After PCR equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina) bridge PCR followed by sequencing on Illumina Hiseq2500. The sequencing (single read) was run for 77 cycles. Sequences generated from each lane were processed using proprietary DArT analytical pipelines. In the primary pipeline, the fastq files were first processed to filter away poor quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence. In that way, the assignments of the sequences to specific samples carried in the “barcode split” step were highly reliable (Kilian et al., 2012).

Filtering was performed on the raw sequences using the following parameters:

Filter	Filter Parameters
Barcode region	Min Phred pass score 30, Min pass percentage 75
Whole read	Min Phred pass score 10, Min pass percentage 50

Approximately 1,410,000 sequences per sample were identified and used in marker calling. Finally, identical sequences were collapsed into “fastqcoll files”. The fastqcoll files were “groomed” using DArT PL’s proprietary algorithm which corrects low quality base from singleton tag into a correct base using collapsed tags with multiple members as a template. The “groomed” fastqcoll files were used in the secondary pipeline for DArT PL’s proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14). For SNP calling, all tags from all libraries included in the DArTsoft14 analysis were clustered using DArT PL’s C++ algorithm at the threshold distance of 3, followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on the analysis of approximately 1,000 controlled cross populations. Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating true allelic variants from paralogous sequences. In addition, multiple samples were processed from DNA to allelic calls as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers. Calling quality was assured by high average read depth per locus (Average across all markers was over 20 reads/locus).

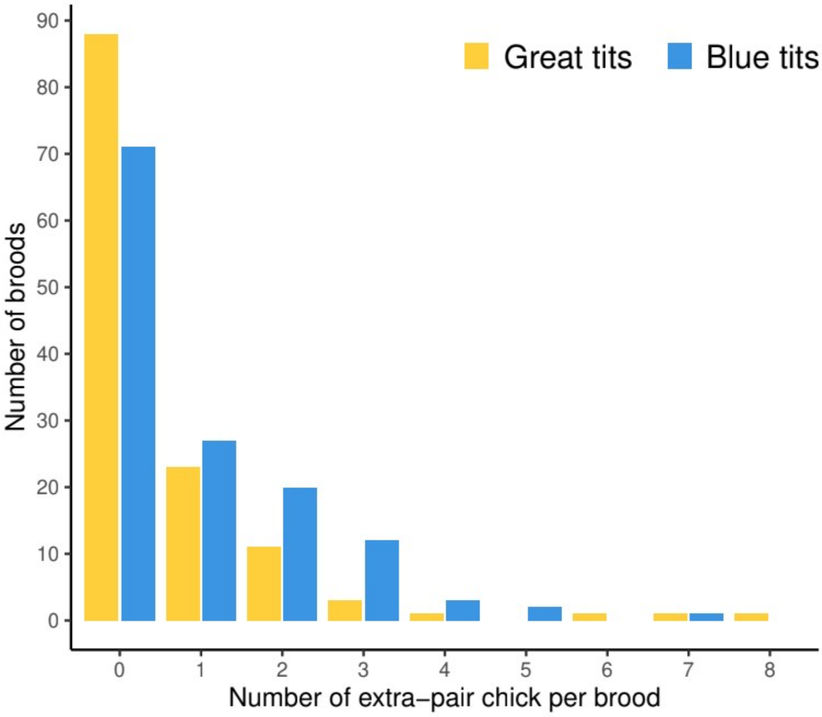

Figure S1 – Frequency of broods with number of extra-pair offspring per species. Only broods whose parents were caught were included, as the number of extra-pair offspring can be identified comparing relatedness of each nestling to the social father (31 nests were excluded in great tits and 16 in blue tits).

Table S1 - Generalized linear mixed models testing individual probability of being within-pair or extra-pair offspring (0/1; dependent variable), with year, sex, clutch size, body index, ISA (model a), light pollution (model b), noise pollution (model c) and tree cover (model d) as predictors. Reference levels were: 2018 (year) and female (sex). Significant differences ($P < 0.05$) are in bold, trends ($P < 0.2$) in italics. Marginal (R^2m) and conditional (R^2c) R-squared are shown.

Probability of being extra-pair offspring

	 **(n = 526)**			 **(n = 579)**		
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
a	**ISA**	0.011 (0.245)	0.965	**ISA**	0.609 (0.225)	**0.007**
	Year	0.125 (0.564)	0.825	**Year**	1.488 (0.495)	**0.003**
	Laying date	-0.659 (0.414)	*0.111*	**Laying date**	0.171 (0.350)	0.624
	Clutch size	0.077 (0.260)	0.768	**Clutch size**	-0.232 (0.301)	0.440
	Sex	0.325 (0.341)	0.339	**Sex**	-0.866 (0.310)	**0.005**
	Body condition index	0.086 (0.221)	0.698	**Body condition index**	0.053 (0.108)	0.622
	R^2m	0.017		**R^2m**	0.091	
	R^2c	0.188		**R^2c**	0.285	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
b	**Light pollution**	-0.037 (0.259)	0.886	**Light pollution**	0.432 (0.239)	*0.070*
	Year	0.111 (0.565)	0.844	**Year**	1.411 (0.509)	**0.006**
	Laying date	-0.668 (0.412)	*0.105*	**Laying date**	0.119 (0.362)	0.742
	Clutch size	0.059 (0.263)	0.822	**Clutch size**	-0.330 (0.308)	0.284
	Sex	0.325 (0.340)	0.340	**Sex**	-0.857 (0.311)	**0.006**
	Body condition index	0.090 (0.221)	0.684	**Body condition index**	0.056 (0.109)	0.604
	R^2m	0.017		**R^2m**	0.075	
	R^2c	0.188		**R^2c**	0.291	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
c	**Noise pollution**	0.158 (0.265)	0.550	**Noise pollution**	0.471 (0.274)	*0.086*
	Year	0.188 (0.565)	0.7391	**Year**	1.445 (0.506)	**0.004**
	Laying date	-0.609 (0.412)	*0.139*	**Laying date**	0.206 (0.372)	0.580
	Clutch size	0.149 (0.269)	0.598	**Clutch size**	-0.238 (0.321)	0.458
	Sex	0.327 (0.341)	0.337	**Sex**	-0.853 (0.311)	**0.006**
	Body condition index	0.089 (0.219)	0.685	**Body condition index**	0.067 (0.108)	0.536
	R^2m	0.018		**R^2m**	0.078	
	R^2c	0.184		**R^2c**	0.289	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
d	**Tree cover**	-0.317 (0.263)	0.229	**Tree cover**	-0.414 (0.256)	*0.106*
	Year	0.291 (0.566)	0.607	**Year**	1.523 (0.507)	**0.003**
	Laying date	-0.565 (0.403)	0.162	**Laying date**	0.185 (0.371)	0.618
	Clutch size	0.165 (0.252)	0.512	**Clutch size**	-0.314 (0.310)	0.310

	Sex	0.333 (0.340)	0.328	**Sex**	-0.851 (0.311)	**0.006**
	Body condition index	0.103 (0.218)	0.636	**Body condition index**	0.049 (0.110)	0.655
	R^2m	0.023		**R^2m**	0.074	
	R^2c	0.182		**R^2c**	0.284	

Table S2 - Generalized linear mixed models testing individual probability of being within-pair or extra-pair offspring (0/1; dependent variable), with year, sex, clutch size, body mass, ISA (model a), light pollution (model b),noise pollution (model c) and tree cover (model d) as predictors. Reference levels were: 2018 (year) and female (sex). Significant differences ($P < 0.05$) are in bold, trends ($P < 0.2$) in italics.

Probability of being extra-pair offspring

	 **(n = 684)**			 **(n = 853)**		
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
a	**ISA**	0.060 (0.223)	0.788	**ISA**	0.589 (0.165)	**< 0.001**
	Year		0.564	**Year**		**0.007**
	Year 2018	0.390 (0.648)		**Year 2018**	-0.838 (0.477)	
	Year 2019	0.695 (0.652)		**Year 2019**	0.620 (0.462)	
	Laying date	-0.036 (0.251)	0.885	**Laying date**	-0.092 (0.230)	0.691
	Clutch size	0.126 (0.249)	0.614	**Clutch size**	-0.161 (0.202)	0.425
	Sex	0.273 (0.310)	0.379	**Sex**	-0.672 (0.253)	**0.008**
	Body mass	0.010 (0.180)	0.957	**Body mass**	0.367 (0.151)	**0.015**
	R^{2}m	0.007		**R^{2}m**	0.080	
	R^{2}c	0.188		**R^{2}c**	0.266	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
b	**Light pollution**	0.054 (0.239)	0.821	**Light pollution**	0.533 (0.170)	**0.002**
	Year		0.558	**Year**		**0.017**
	Year 2018	0.397 (0.647)		**Year 2018**	-0.718 (0.485)	
	Year 2019	0.702 (0.652)		**Year 2019**	0.617 (0.472)	
	Laying date	-0.040 (0.252)	0.875	**Laying date**	-0.051 (0.237)	0.831
	Clutch size	0.123 (0.250)	0.622	**Clutch size**	-0.175 (0.206)	0.395
	Sex	0.272 (0.310)	0.380	**Sex**	-0.660 (0.253)	**0.009**
	Body mass	0.012 (0.184)	0.948	**Body mass**	0.362 (0.153)	**0.018**
	R^{2}m	0.007		**R^{2}m**	0.072	
	R^{2}c	0.188		**R^{2}c**	0.268	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
c	**Noise pollution**	0.200 (0.233)	0.392	**Noise pollution**	0.585 (0.199)	**0.003**
	Year		0.562	**Year**		**0.011**
	Year 2018	0.311 (0.641)		**Year 2018**	-0.869 (0.488)	
	Year 2019	0.672 (0.642)		**Year 2019**	0.543 (0.474)	
	Laying date	-0.033 (0.245)	0.893	**Laying date**	0.060 (0.246)	0.807
	Clutch size	0.188 (0.253)	0.458	**Clutch size**	-0.101 (0.214)	0.637
	Sex	0.267 (0.309)	0.388	**Sex**	-0.668 (0.254)	**0.009**
	Body mass	0.030 (0.178)	0.865	**Body mass**	0.371 (0.154)	**0.016**
	R^{2}m	0.009		**R^{2}m**	0.075	
	R^{2}c	0.183		**R^{2}c**	0.274	

		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
d	**Tree cover**	-0.250 (0.230)	0.278	**Tree cover**	-0.572 (0.192)	**0.003**
	Year		0.502	**Year**		**0.005**
	Year 2018	0.299 (0.635)		**Year 2018**	-0.861 (0.478)	
	Year 2019	0.721 (0.637)		**Year 2019**	0.657 (0.467)	
	Laying date	-0.021 (0.243)	0.931	**Laying date**	0.023 (0.238)	0.922
	Clutch size	0.182 (0.243)	0.454	**Clutch size**	-0.211 (0.202)	0.297
	Sex	0.269 (0.309)	0.385	**Sex**	-0.639 (0.252)	**0.011**
	Body mass	0.033 (0.178)	0.852	**Body mass**	0.325 (0.150)	**0.030**
	R^2m	0.010		**R^2m**	0.074	
	R^2c	0.182		**R^2c**	0.264	

Table S3 - Generalized linear mixed models testing late-stage survival of nestlings (0/1; dependent variable). Variables included as predictors were: offspring status as within-pair or extra-pair (0/1), year, sex, clutch size, body mass, ISA (model a), light pollution (model b),noise pollution (model c) and tree cover (model d). Reference levels were: 2018 (year), female (sex) and within-pair (epo). Significant differences ($P < 0.05$) are in bold, trends ($P < 0.2$) in italics.

Late-stage survival of nestlings

	 **(n = 679)**			 **(n = 847)**		
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
a	**ISA**	-0.195 (0.918)	0.832	**ISA**	0.162 (0.280)	0.564
	Year		0.816	**Year**		**0.009**
	Year 2018	-2.045 (3.511)		**Year 2018**	-2.667 (0.973)	
	Year 2019	-1.530 (2.746)		**Year 2019**	-0.898 (0.981)	
	Laying date	2.376 (2.198)	0.280	**Laying date**	-0.338 (0.469)	0.471
	Clutch size	-0.035 (1.021)	0.973	**Clutch size**	0.498 (0.466)	0.285
	Sex	-0.637 (0.766)	0.405	**Sex**	-1.03 (0.417)	**0.013**
	Epo	4.142 (3.977)	0.298	**Epo**	-0.615 (0.635)	0.333
	Body mass	0.634 (0.394)	*0.107*	**Body mass**	2.077 (0.306)	**< 0.001**
	R^{2}m	0		**R^{2}m**	0.036	
	R^{2}c	0		**R^{2}c**	0.058	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
b	**Light pollution**	0.024 (0.950)	0.980	**Light pollution**	0.209 (0.309)	0.498
	Year		0.806	**Year**		**0.009**
	Year 2018	-2.150 (3.519)		**Year 2018**	-2.632 (0.974)	
	Year 2019	-1.501 (2.714)		**Year 2019**	-0.866 (0.983)	
	Laying date	2.468 (2.234)	0.269	**Laying date**	-0.310 (0.470)	0.510
	Clutch size	0.038 (0.989)	0.969	**Clutch size**	0.490 (0.464)	0.292
	Sex	-0.641 (0.765)	0.403	**Sex**	-1.029 (0.416)	**0.013**
	Epo	4.105 (3.968)	0.301	**Epo**	-0.614 (0.634)	0.333
	Body mass	0.641 (0.397)	*0.107*	**Body mass**	2.080 (0.305)	**< 0.001**
	R^{2}m	0		**R^{2}m**	0.036	
	R^{2}c	0		**R^{2}c**	0.058	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
c	**Noise pollution**	0.122 (1.176)	0.918	**Noise pollution**	0.215 (0.370)	0.562
	Year		0.811	**Year**		**0.008**
	Year 2018	-2.303 (3.845)		**Year 2018**	-2.756 (0.977)	
	Year 2019	-1.562 (2.765)		**Year 2019**	-0.994 (0.967)	
	Laying date	2.570 (2.401)	0.285	**Laying date**	-0.296 (0.472)	0.530
	Clutch size	0.093 (1.138)	0.935	**Clutch size**	0.509 (0.472)	0.281
	Sex	-0.642 (0.765)	0.402	**Sex**	-1.029 (0.416)	**0.013**

	Epo	4.100 (3.965)	0.301	**Epo**	-0.609 (0.634)	0.337
	Body mass	0.643 (0.396)	0.105	**Body mass**	2.0819 (0.307)	< **0.001**
	R^{2}m	0		**R^{2}m**	0.036	
	R^{2}c	0		**R^{2}c**	0.058	
		Estimate (SE)	**Pr(>χ^2)**		**Estimate (SE)**	**Pr(>χ^2)**
d	**Tree cover**	0.292 (1.219)	0.811	**Tree cover**	-0.195 (0.334)	0.560
	Year		0.828	**Year**		**0.008**
	Year 2018	-1.923 (3.559)		**Year 2018**	-2.705 (0.975)	
	Year 2019	-1.521 (2.767)		**Year 2019**	-0.939 (0.973)	
	Laying date	2.333 (2.218)	0.293	**Laying date**	-0.307 (0.473)	0.516
	Clutch size	-0.073 (1.057)	0.945	**Clutch size**	0.499 (0.466)	0.284
	Sex	-0.636 (0.765)	0.406	**Sex**	-1.030 (0.416)	**0.013**
	Epo	4.111 (3.956)	0.299	**Epo**	-0.604 (0.632)	0.339
	Body mass	0.634 (0.394)	*0.108*	**Body mass**	2.072 (0.304)	< **0.001**
	R^{2}m	0		**R^{2}m**	0.036	
	R^{2}c	0		**R^{2}c**	0.058	