Consistency and plasticity of risk-taking behaviour towards humans at the nest in urban and forest great tits, *Parus major*

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Urban animals often show bolder behaviour towards humans than their nonurban conspecifics. However, it is unclear to what extent this difference is due to consistent individual characteristics or to plasticity such as habituation. To address this question, we investigated parental risk-taking behaviour in 371 female great tits in urban and forest populations by checking their nest repeatedly (several times per week, for up to nine breeding episodes) and recording their behavioural responses to this recurring disturbance during incubation. We found that urban females were bolder, as they stayed on the nest more often than females in forests. Furthermore, great tits produced alarm calls around the nests more frequently in urban than in forest habitats. There was no habitat difference in the frequency of an antipredator behaviour, the hissing threat display on the nest, although this was rare in both habitats. We also tested the consistency and plasticity of risk-taking behaviour on three different temporal scales (within breeding attempts, between broods within a year and across years). Staying on the nest was highly repeatable within females, whereas alarm calls had low repeatability within pairs at all three temporal scales. The probability of staying on the nest increased within breeding attempts, whereas the probability of alarm calls increased across years. Neither consistency nor plasticity in these components of risk taking differed between urban and forest habitats. We conclude that urban birds are bolder in multiple behavioural measures and, overall, both stable individual differences and behavioural plasticity may have contributed to the higher risk taking we often see in urban populations. Furthermore, staying on the nest appears to be an individually consistent trait in female great tits regardless of habitat urbanization, providing a low-impact measurement of risk taking, which may potentially facilitate field studies related to individual differences in behaviour.

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Urbanization is one of the most important environmental changes of our time, generating opportunities for frequent interactions between humans and populations of wild animals (Chamberlain et al., 2009; Seress & Liker, 2015). Several species thrive in urban areas, and their behaviour often differs greatly between urban and nonurban populations (Ditchkoff, Saalfeld, & Gibson, 2006; Griffin, Netto, & Peneaux, 2017; Ritzel & Gallo, 2020; Sol, Lapiedra, & González-Lagos, 2013). Humans, despite their ubiquity in cities, pose relatively little risk to urban animals, because they commonly show a neutral or positive attitude towards most wildlife (Clucas & Marzluff, 2012). Therefore, tolerating human presence and activity can be adaptive for urban animals by saving time from fleeing and hiding that can be allocated to other activities such as foraging and parental care. Additionally, the presence of humans can deter other types of predators and thus reduce predation risk, and this 'human shield effect' can also favour generally bolder phenotypes (Geffroy et al., 2020). According to these ideas, many studies have found that urban animals show bolder behaviour (i.e. higher risk taking) towards humans; for example, they flee at shorter distances (Atwell et al., 2012; Samia

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et al., 2017; Sol et al., 2018), or return to their predisturbance activities faster following human disturbance (Vincze et al., 2019).

There is increasing interest in the extent to which the differences between urban and nonurban animals' risk-taking behaviour are due to consistent variation among individuals or behavioural plasticity (Miranda, 2017). One mechanism through which urban populations can become bolder on average than nonurban populations is differential colonization (Møller, 2010; also referred to as 'personality-dependent habitat selection' by; Carrete & Tella, 2011). This hypothesis suggests that individuals with consistently higher risk-taking behavioural phenotypes are those that settle in the novel, urban habitats. Another mechanism that can lead to behavioural differences between populations is local adaptation (Atwell et al., 2012; Møller, 2008; Partecke, Schwabl, & Gwinner, 2006), i.e. individuals with consistently higher risk-taking behaviour realize higher fitness in urban habitats compared to lower risk-taking individuals. Both of the above mechanisms assume stable behavioural differences between individuals. Alternatively, the difference between urban and forest populations can be due to behavioural plasticity. For example, urban animals may habituate to anthropogenic disturbance, i.e. learn that humans, which they initially perceive as threatening, are actually not dangerous, and therefore reduce their avoidance responses towards humans, leading to more risk-taking behaviour compared to their nonurban conspecifics (Cavalli, Baladrón, Isacch, Biondi, & Bó, 2018; Vincze et al., 2016).

Consistency and plasticity are not mutually exclusive, because a behaviour with large among-individual variation can also show high within-individual plasticity, and vice versa (Dingemanse, Kazem, Réale, & Wright, 2010). Therefore, stable behavioural differences and plasticity may both contribute to the increased risk taking of urban animals, but the relative importance of these two processes may differ between species or contexts. Some studies suggest faster habituation to humans in urban than in rural animals, indicating that plasticity can be an important factor behind the differences in boldness between habitats (Cavalli et al., 2018; Vincze et al., 2016). By contrast, other studies have found individual consistency in risk-taking behaviour towards humans and no habituation over repeated observations, suggesting that stable among-individual variation is relatively more important for these behavioural differences between habitats (Carrete & Tella, 2013; Holtmann, Santos, Lara, & Nakagawa, 2017; Sprau & Dingemanse, 2017). There is also evidence for genetic differences which may underlie stable behavioural differences between urban and nonurban individuals: a handful of studies have found differences between urban and nonurban populations in candidate genes related to boldness and risk-taking behaviour (Mueller, Partecke, Hatchwell, Gaston, & Evans, 2013; Riyahi, Björklund, Mateos-Gonzalez, & Senar, 2017; van Dongen, Robinson, Weston, Mulder, & Guay, 2015). Studying habituation and consistent differences between individuals simultaneously may aid our understanding of the mechanisms of urban adaptation, yet no study to our knowledge has compared both individual consistency and plasticity in risk-taking behaviour towards humans between urban and nonurban conspecifics.

In this study, we monitored the parental risk-taking behaviour of great tits during the incubation period in two urban and two forest populations, over 6 years, to test its differences between habitats and its individual consistency and plasticity in each habitat. Great tits breed successfully in both urban habitats and forests, and different populations along the urbanization gradient show significant differences in behavioural traits such as territorial aggression (Hardman & Dalesman, 2018), problem-solving performance (Preiszner et al., 2017), exploration (Charmantier, Demeyrier, Lambrechts, Perret, & Grégoire, 2017; but see ;

Grunst, Grunst, Pinxten, & Eens, 2019), neophobia (Grunst et al., 2019) and boldness towards humans (Vincze et al., 2019). Here we studied three different proxies of risk-taking behaviour parents can exhibit in response to an approaching human: alarm calling outside the nest, flight from the nest and threat display on the nest. We predicted that urban birds would take more risks than their forest conspecifics in each of these behavioural contexts, because the former may have adapted and/or adjusted their behaviour to the low cost of risk taking associated with the low level of threat in cities posed by people and/or by predators (Geffroy et al., 2020).

We also tested the individual consistency and plasticity of risk taking and compared them between urban and nonurban populations. To do this, we estimated the repeatability of these behaviours while also estimating their change over time (Nakagawa & Schielzeth, 2010). We predicted that risk-taking behaviours will be highly repeatable if consistent behavioural variation among individuals was an important driver of the differences between urban and nonurban populations through differential colonization, local adaptation or developmental plasticity (i.e. individual plasticity in early life that leads to long-term, often irreversible changes in phenotype; Miranda, 2017). By comparing repeatability between urban and nonurban populations, we may gain further insight into the underlying processes of behavioural adaptations to urbanization, because colonization of urban habitats may select for a subset of individuals with low interindividual variation and high risk taking (Carrete & Tella, 2011), whereas risk-taking behaviour may become heterogeneous among individuals again when a population has been urbanized for a longer period (Møller, 2010). Regarding plasticity, we predicted that if habituation to humans plays a major role in the urban−forest differences in risk-taking behaviour, then the birds will systematically reduce their response to frequent human disturbances, i.e. nest checks, over repeated encounters. Also, we predicted that in this case behavioural change would be faster in urban birds because they have more opportunities to habituate to humans (i.e. more frequent encounters with nonthreatening humans that may speed up the habituation process). Furthermore, urban animals may have a higher capacity to habituate even with the same frequency of encounters compared to nonurban conspecifics, as suggested by earlier evidence on both birds (Vincze et al., 2016) and reptiles (Pellitteri-Rosa et al., 2017).

METHODS

Data Collection

We monitored great tit populations breeding in nestboxes at four different study sites in Hungary. The two urban sites, consisting of green areas such as public parks, graveyards and university campuses, were in the cities of Veszprém (47°05′17″N, 17°54′29″E) and Balatonfüred (46°57′30″N, 17°53′34″E). The other two sites were deciduous forests at Vilma-puszta (47°05′06″N, 17°51′51″E) and near Szentgál (47°06′39″N, 17°41′17″E). Further details of the study sites, including the degree of urbanization of the city sites, are provided in Seress et al. (2018).

We checked the active nest boxes regularly (at least twice a week) from early March to mid-July from 2013 to 2018. The boxes were hanging on trees, 1.5−4.7 m (mean ± SD = 3.61 ± 0.50) and 2.2−4.8 m (mean ± SD = 3.16 ± 0.50) from the ground in urban and forest sites, respectively. To check them, we took them off the tree using a 3 m pole with a hook at the end, which we also used for returning the nestbox to its original location.

During each check, we recorded the number of eggs and/or nestlings along with the behaviour of parents inside and outside the nest (see below). We determined the first day of the incubation

period for each clutch as the day of laying the last egg (which is a conservative approach, as great tits often start incubating a few days before the last egg), and the last day of the incubation period as the day before the hatching of the first nestling. The observed parental behaviours (see below) showed considerable variation among individuals and among observations during the incubation period, but not in the preceding egg-laying and the succeeding chick-rearing periods when parents were almost never on the nest. Therefore, we analysed data only from the incubation period. As male great tits do not incubate their eggs (Kluijver, 1950), we always assumed that the bird we found on the nest was the female. This was also supported by our field observations as we only observed females (identified either by plumage characteristics or unique colour ring combinations, see below) staying in the nestboxes during these nest checks.

We recorded female behaviour at each nest check as one of the following categories (Fig. 1): (1) 'Off-nest': the female was neither found on the nest nor seen flying off the nest. (2) 'Flying off': the female was seen flying off the nest. Most of the time this happened while we were taking the nest off the tree (83.1%); occasionally it happened when we approached the nestbox within 15 m but before we took it off the tree (2.2%) or after we opened the nestbox lid (14.7%). (3) 'Staying': the female stayed on the nest after we opened the nestbox lid. If the female stayed, we also recorded whether or not she showed threat display by fanning her tail feathers, snapping her wings and making a hissing sound, a behaviour observed in incubating females of several Paridae species (Grunst et al., 2019; Koosa & Tilgar, 2016; Krams et al., 2014; Sibley, 1955; Thys, Lambreghts, Pinxten, & Eens, 2019; Thys, Pinxten, & Eens, 2021; Zhang et al., 2020).

Regardless of female location, we also recorded whether or not we heard great tit alarm calls around the nestbox during each nest check. While in many cases we were able to determine the sex of the birds making the alarm calls (either by their unique colour ring combinations, see below, or by plumage patterns), this was not always possible because of poor visibility due to dense foliage and/

or because of multiple individuals making the alarm calls. Therefore, we treated all great tit alarm calls the same in our analysis, regardless of whether they came from the mother, the father or, potentially, other great tits, which can happen if birds from neighbouring territories join a 'mobbing chorus' (Regelmann & Curio, 1983). For this reason, alarm calling in our study does not represent individual characteristics of the female, but rather characteristics (i.e. risk-taking level) of the pair. Nevertheless, our observations during the nest checks when we could identify the alarm-calling bird suggest that when the mother stays on the nest, most alarm calls are made by her mate, whereas when she flies off the nest, most alarm calls are made by her. If the female is not present on the nest, both parents may make alarm calls, although the literature suggests male great tits defend their nest more vigorously than females do (Regelmann & Curio, 1986).

Following their first incubation period, we attempted to capture all ringless females with a nestbox trap (Seress et al., 2017) during the chick-rearing period, and individually ringed them with a unique combination of one metal ring and three plastic colour rings. In subsequent breeding attempts, both within the same year and in subsequent years, we identified birds by these colour ring combinations. We also increased the proportion of colour-ringed individuals in our populations by capturing birds at bird feeders and night roosts during the winter (16.2% of colour-ringed females, 60 individuals, were captured this way). Before 2014, we also put colour rings on nestlings, some of which were resighted as breeding adults (8.1% of colour-ringed females, 30 individuals). The proportion of females caught with each method was similar at all four sites (percentage of birds colour-ringed during their first brood, before their first brood as adults and as nestlings, respectively: Veszprém city: 76.8%, 17.2% and 6.0%; Balatonfüred city: 78.0%, 14.2% and 7.8%; Szentgál forest: 76.6%, 14.8% and 8.5%; Vilmapuszta forest: 74.6%, 14.3% and 11.1%). Over the 6 years, we recorded the behaviour of females during 2957 nest checks from 954 breeding attempts. However, breeding attempts where the female was neither captured nor identified by colour rings (229 breeding
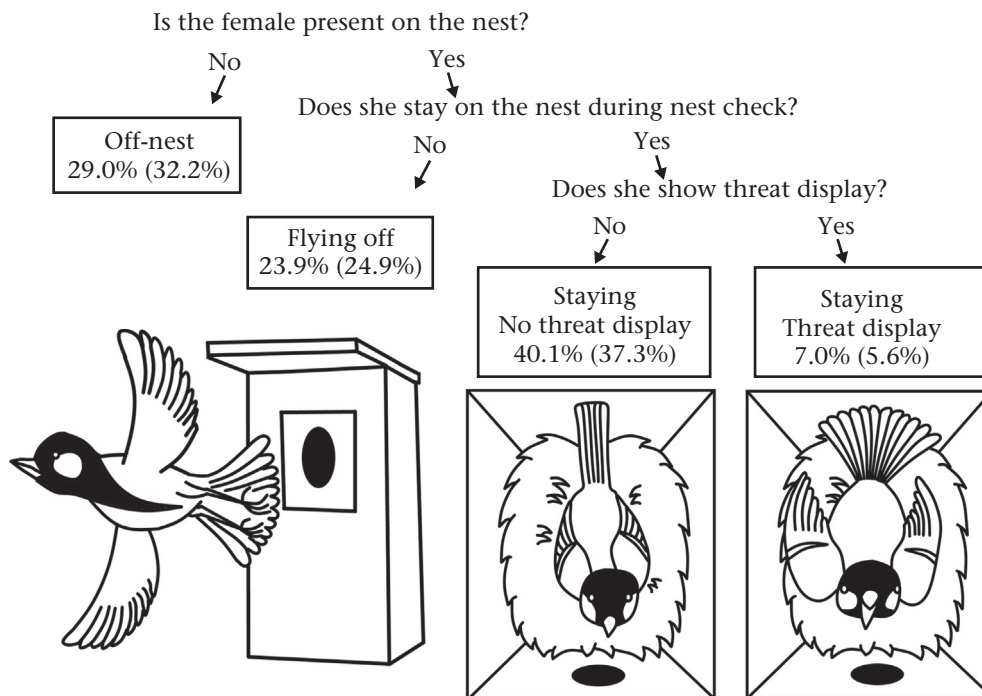

Figure 1. Female behavioural categories (female location) and their relative frequency as the percentage of all nest checks. For each behaviour, the first percentage only includes observations where the female's identity is known; the second percentage (in parentheses) includes observations both with identified and with unidentified females.

attempts, 24.0%) were excluded from our analyses, because in these cases we were not certain about the female's identity, which could potentially bias estimates of between-brood consistency and plasticity. To estimate the influence of unidentified birds on the overall results, we compared the frequency of each of the recorded behaviours between sites and between the two habitat types with chi-square tests, both including and excluding the nests of unidentified females, which did not qualitatively change any of the results (Table A1). The final sample size for the study was 725 breeding attempts (out of which 180 were second broods within a year) by 371 females, with behaviour recorded during a total of 2165 nest checks. More than 90% of the females were checked more than once during incubation (Table A2). We included birds with single observations in our sample because this provides a more accurate (unbiased) estimate for among-individual variation, thereby for repeatability and urban−forest contrasts. However, we repeated the analyses excluding females with single observations, which did not change our results qualitatively (Table A3).

Statistical Analyses

All analyses were run in the R computing environment (Version 3.6.1., R Core Team, 2019). To quantify parental risk taking, we defined three behavioural variables: (1) 'alarm calling': whether we recorded alarm calls in the proximity of the nest (1) or not (0); (2) 'staying': whether a female stayed on the nest (1) or flew off (0) during a nest check, i.e. from the start of the observer's approach until the nestbox was put back on the tree; (3) 'threat display': whether a female showed threat display (1) or not (0) on the nest. From the 'staying' variable, we excluded observations where the female was off-nest at the beginning of the nest check, yielding a lower sample size (1537 nest checks from 687 nesting attempts of 354 females). The female was present on the nest at a similar frequency (72.2−72.6%) in three of the four habitats, but less frequently in Vilma-puszta forest (62.9%; Table A1). For the 'threat display' variable, we used only those nest checks where the female stayed on the nest, resulting in a sample size of 1019 nest checks from 479 nesting attempts of 245 females.

To test whether the frequency of threat displays differed between habitats, we used nonparametric tests. First, we compared the frequency of observations with threat display between the four sites and between the two habitat types with chi-square tests (Table A1). Second, we also compared the number of urban and forest females that showed threat display at least once versus those that never did with a chi-square test. Because threat displays on the nest were rare (Fig. 1), we could not run complex models to test their repeatability or change over time.

For 'staying' and 'alarm calling', we built generalized linear mixed-effects models with binomial error distribution and logit-link function. Each model included the following predictors: study site (as a four-level factor), clutch size, 'within-brood experience' (i.e. how many times we checked that specific nest in the current incubation period before that particular observation), 'within-year experience' (i.e. whether it was the first or second nesting attempt of that female in that given year that we subjected to a nest check, treated as a two-level factor) and 'across-years experience' (i.e. for how many years we monitored the bird's nesting attempts until the current brood since 2013). The latter three variables were included to test short-, medium- and long-term temporal change in the behaviour, respectively. All continuous variables, specifically clutch size, within-brood experience and across-years experience were centred around their respective means (of the entire data set). Additionally, the model with alarm calling as response variable included the fixed effect of a variable called 'female location' (a three-level factor coding female

behaviour as 'off-nest', 'flying off' or 'staying'; Fig. 1.) and a female location*study site interaction, because the frequency of alarm calls and the identity of the individual making them can depend on how the female behaves (as explained above). To test whether short-, medium- and long term change in risk-taking behaviours differed between urban and nonurban habitats, we initially also included interactions between site and the three temporal fixed effects (i.e. plasticity due to short-, medium- and long-term changes in experience), as well as the interaction between site and clutch size (i.e. plasticity due to variation in clutch size). We had relatively few data points per breeding event (most frequently three observations per breeding event; Table A2), and there was no variation in our binary data within some females; this latter phenomenon (called separation in logistic models) did not allow us to reliably fit individual random slopes in our binomial models, so we could not estimate plasticity on the individual level (Dingemanse et al., 2010). Owing to this constraint we estimated one slope per site in our full models, which assumes that the within-population average of all individual slopes is adequately approximated by fitting a single slope to each population (DeWitt & Scheiner, 2004; Pigliucci, 2001). Since none of the interactions was statistically significant and none improved the model fit significantly (see Results and Table A4), we calculated the effect of urbanization and repeatability (as described below) from the simplified final models without these interactions (assuming a common slope across all populations for each plasticity gradient, i.e. each aspect of time, and clutch size). We had data from every year from 2013 to 2018 from all four study sites; the effect of 'year' as a six-level fixed factor was nonsignificant in all models ($P > 0.05$ in Type II analysis-of-deviance tables) and did not improve the model fit, as indicated by the increased Akaike information criterion (AIC) of the models including year (alarm calling, full model: $P_{year} = 0.262$, $\Delta AIC = 27.1$; alarm calling, final model: $P_{year} = 0.230$, $\Delta AIC = 3.1$; staying on the nest, full model $P_{year} = 0.438$, $\Delta AIC = 4.8$; staying on the nest, final model: $P_{year} = 0.746$, $\Delta AIC = 7.2$); therefore, we present all models without including 'year'. All models included the following random factors, nested within each other: brood ID (grouping observations of the same nest, for within-brood repeatability), mother-year ID (grouping observations of the same female from the same year, for within-year repeatability) and mother ID (grouping all observations of the same female, for across-years repeatability). The models were built using the function 'glmer' in the R package lme4 (Bates, 2007), with the BOBYQA optimizer (Powell, 2009) with 30 000 as the maximum number of function evaluations. Both binomial models showed underdispersion (dispersion parameter for staying: 0.232; for alarm calls: 0.672), which indicates that our results are conservative (i.e. false positives are unlikely, with some chance of false negatives).

To test the effect of urbanization on alarm calling and staying, we used preplanned comparisons (Ruxton & Beauchamp, 2008) by calculating urban−forest linear contrasts from each model's estimated marginal means, using the emmeans package (Lenth, Singmann, Love, Buerkner, & Herve, 2019), similarly to other studies we conducted on the same populations (Pipoly et al., 2019; Vincze et al., 2019). With this method, we estimated urban−forest differences while controlling for the potential confounding effect of site identity without running into the statistical problems of having too few random factor levels (Bolker et al., 2009; Piepho, Büchse, & Emrich, 2003). For staying, we also calculated contrasts for each pair of sites (Table A5). For alarm calling, we calculated urban−forest linear contrasts separately for each level of the female location variable (i.e. off-nest, flying off and staying). For repeated comparisons from the same model, we adjusted P values with the false discovery rate (FDR) method (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001).

To calculate repeatability (*R*) of staying and alarm calling, we first extracted the components of variance (s^2) for each random factor from the above final models. Then we followed the formula described by Nakagawa and Schielzeth (2010) to calculate adjusted repeatability (i.e. repeatability corrected for variance from fixed effects) on the original scale from models implementing additive overdispersion. We adjusted this formula to calculate within-brood, within-year and across-years repeatability estimates following the method suggested by Araya-Ajoy, Mathot, and Dingemanse (2015). According to this method, long-term repeatability is calculated by dividing the long-term component of variance by the total variance, whereas short-term repeatability is calculated by dividing the sum of short- and long-term components of variance by the total variance (therefore short-term repeatability is inherently higher than long-term repeatability). Therefore, on our three temporal scales, across-years repeatability included only the across-years component of variance, within-year repeatability included the across-years and within-year components of variance, and within-brood repeatability included the across-years, within-year and within-brood components of variance. Then, to estimate 95% confidence intervals of repeatability estimates, we ran 1000 parametric bootstraps for each model (confidence intervals at the three temporal scales were calculated from the same boot-strapping procedure, and thus are not independent from each other). To test whether repeatability differed between urban and forest habitats, we built separate models for the subset of urban birds and the subset of forest birds, and ran repeatability analyses separately; then we compared the 84% confidence intervals of the repeatability estimates between the two habitat types. We used 84% because the lack of overlap between two 84% confidence intervals indicates that the 95% confidence interval of the difference does not include zero (Payton, Greenstone, & Schenker, 2003). See the Supplementary Material for our full custom script.

Ethical Note

All procedures were in accordance with the ASAB/ABS Guidelines for the Use of Animals in Research and with Hungarian laws, licensed by the Middle Transdanubian Inspectorate for Environmental Protection, Natural Protection and Water Management (permit numbers: 31559/2011, 24861/2014 and VE-09Z/03454-8/2018). The human disturbance to which the birds were exposed was not higher than what is typical for a regular nest-monitoring regime. We never removed incubating females from the nest.

RESULTS

Habitat Differences in Risk Taking

Alarm calls were observed most frequently when the female flew off the nest, intermediately when she was not present, and least frequently when she stayed on the nest (Fig. 2). Regardless of female location, alarm calls were more frequent in urban than in forest habitats (34.3% versus 28.3% of observations; Table 1, Table A1, Fig. 2).

Forest birds flew off the nest significantly more often than urban birds (Table 1), but there were considerable differences between sites within a habitat type, especially in forest (Fig. 3). The significant habitat difference was primarily driven by the difference between Veszprém and Szentgál (the two sites with the largest sample sizes, with 643 and 467 of 1537 observations, respectively; the female stayed on the nest in 79.3% versus 49.3% of observations in Veszprém and Szentgál, respectively; Tables A1 and A5, Fig. 3).

We observed threat display at least once in 22% of the identified females (24.4% and 23.7% in the cities of Balatonfüred and Veszprém, 20% and 19.7% in the forests of Vilma-puszta and Szentgál). The number of females showing threat display at least once did not differ significantly between urban and forest habitats
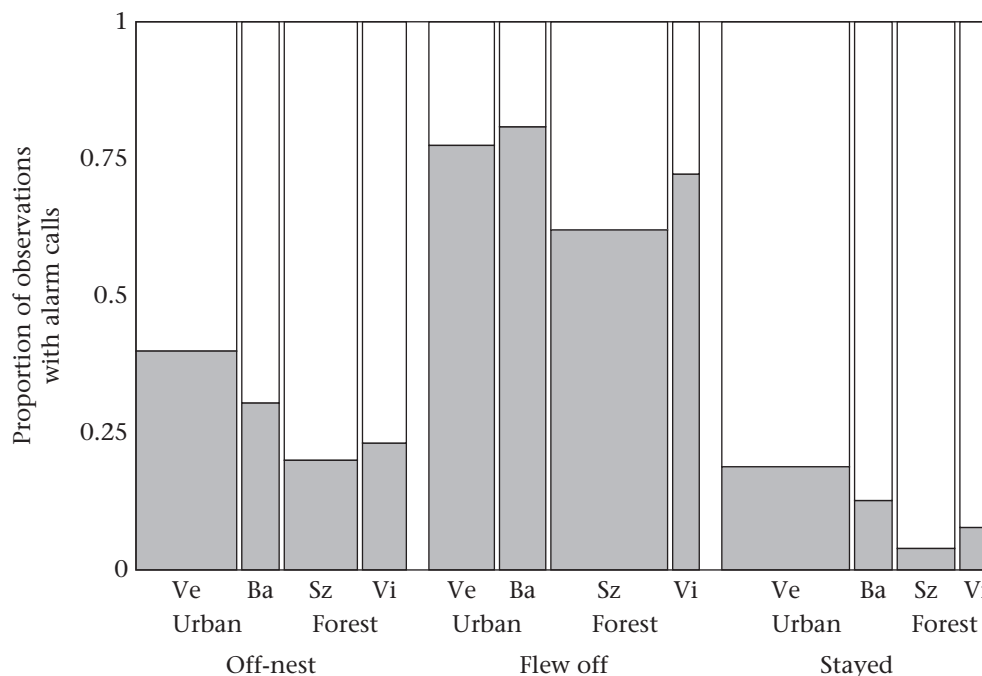

Figure 2. Proportions of observations with alarm calls outside the nest (a) when the female was off-nest, (b) when the female flew off during the nest check and (c) when the female stayed on the nest, at the two urban and two forest sites (Ve: Veszprém, Ba: Balatonfüred, Sz: Szentgál, Vi: Vilma-puszta). Proportions with alarm calls are indicated by the grey portion of the columns, and column widths are proportional to sample sizes (Veszprém: 891 observations, 116 females; Balatonfüred: 336 observations, 64 females; Szentgál: 647 observations, 128 females; Vilma-puszta: 291 observations, 63 females).

Table 1
Differences (linear contrasts) in the occurrence of risk-taking behaviours between urban and forest sites

Behavioural variable	Estimate	SE	z	P
Staying	1.720	0.692	2.480	0.013
Alarm calling, female off nest	0.745	0.273	2.728	0.010
Alarm calling, female flies off	0.651	0.331	1.969	0.049
Alarm calling, female stays	1.015	0.337	3.010	0.008

For alarm calling, separate contrasts were calculated for each female location (the focal female was off, flew off and stayed on the nest), and we adjusted the P values for three comparisons using the FDR method. Contrasts were calculated from mixed-effects models with binomial error (Table A6), estimates are on the logit-link scale with positive values indicating greater probability of staying and alarm calling in urban than in forest habitats.

(chi-square test comparing four sites: $\chi^2_3 = 0.877$, $P = 0.831$; chi-square test comparing two habitat types: $\chi^2_1 = 0.593$, $P = 0.898$). We obtained qualitatively the same results when we compared the frequency of observations (rather than females) with threat displays between sites or habitats (see Table A1).

Temporal Plasticity of Risk Taking

The frequency of alarm calls increased across years (estimate ± SE = 0.265 ± 0.104, $z = 2.557$, $P = 0.011$; Table A6), but did not change within or between breeding attempts within years (Table 2, Table A6). Experiencing repeated disturbance was associated with increased probability of staying on the nest within a breeding attempt, i.e. females were more likely to stay on the nest at later nest checks over the incubation period (estimate ± SE = 0.254 ± 0.106, $z = 2.395$, $P = 0.017$; Table A6). There was no significant change in staying on the nest across nesting events between broods within a year or across years (Table 2, Table A6). Behavioural change over time did not differ significantly between urban and forest habitats for either alarm calling or staying on any temporal scale, as indicated by the nonsignificant site*experience interactions (Table 2, Table A4). Furthermore, the probabilities of staying on the nest and making alarm calls were unrelated to clutch size (Table 2, Table A6), and the

interaction between cutch size and habitat type was also nonsignificant (Table 2, Table A6).

Repeatability of Risk Taking

Probability of alarm calling had low repeatability ($R < 0.2$) at all three temporal scales in both habitats. The 95% confidence intervals included zero for across-years repeatability when we tested all birds pooled and urban and forest birds separately; for within-year repeatability, the 95% confidence intervals included zero for forest birds, but not for urban birds or all birds pooled. For within-brood repeatability, confidence intervals did not include zero in any of the three analyses (Table 3).

Probability of staying on the nest was repeatable ($R = 0.5-0.7$) at all three temporal scales in both habitats, with the 95% confidence intervals never including zero (Table 3). None of the repeatability estimates, for alarm calling or for staying on the nest, differed significantly between urban and forest habitats as indicated by the overlapping 84% confidence intervals (Table 3).

DISCUSSION

By monitoring great tits during incubation in two urban and two forest populations, we tested for between-habitat differences in their risk-taking behaviour as well as in these behaviours' consistency and plasticity. First, we found that in urban habitats, birds made more alarm calls than in forest habitats, and females stayed on their nests more often upon human disturbance, but we found no such urban−forest difference in the females' threat display behaviour when disturbed on the nest. Second, within breeding attempts, the probability of the female staying on her nest during nest checks increased over time but the probability of the parents making alarm calls did not, whereas across years, the probability of alarm calling increased, but the probability of staying on the nest did not change. None of the risk-taking variables showed significant change from first to second broods within a year. Third, staying on the nest was highly repeatable within individual females, whereas alarm calls had low repeatability, at all three temporal scales.
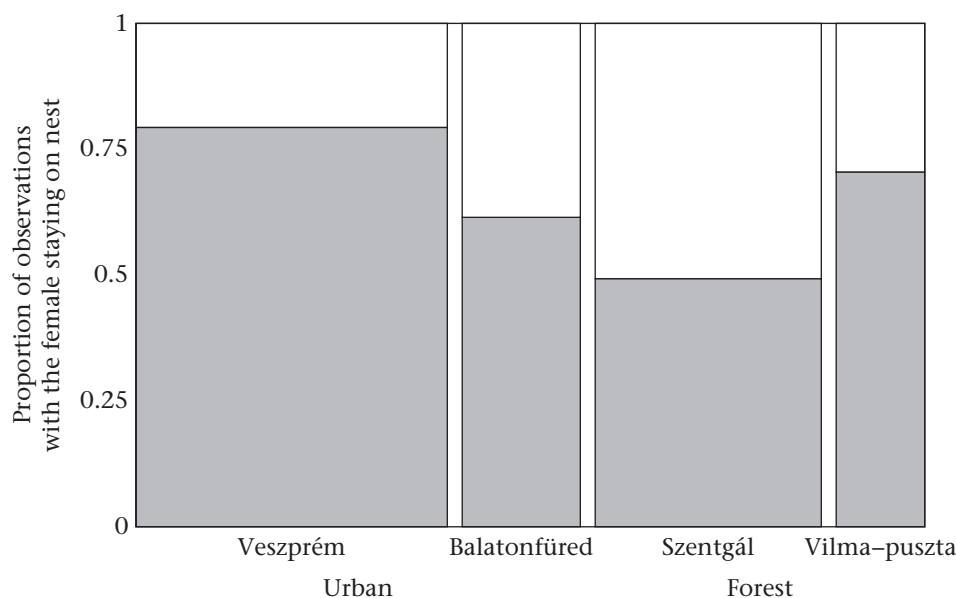
Figure 3. Proportion of observations with females staying on the nest (grey areas of the bars) at the two urban and two forest sites. Only observations where the female was present at the beginning of the nest check are included. Column widths are proportional to the number of observations (Veszprém: 643 observations, 113 females; Balatonfüred: 244 observations, 62 females; Szentgál: 467 observations, 119 females; Vilma-puszta: 183 observations, 60 females).

Table 2
Type 2 ANOVA results of the full models (with interactions) and the final models (without interactions) for 'alarm calling' and 'staying'

Behaviour	Predictor	Full models		Final models	
		χ^2	P	χ^2	P
Alarm calling	Site	23.592	<0.001*	24.395	<0.001*
	Female location	279.534	<0.001*	280.570	<0.001*
	Within-brood experience	0.028	0.866	0.054	0.815
	Within-year experience	0.016	0.898	0.009	0.924
	Across-years experience	6.673	0.010	6.537	0.011*
	Clutch size	1.417	0.234	1.709	0.191
	Site*Female location	5.173	0.522	4.841	0.564
	Site*Within-brood experience	3.044	0.384	–	–
	Site*Within-year experience	1.257	0.739	–	–
	Site*Across-years experience	0.914	0.822	–	–
	Site*Clutch size	2.751	0.432	–	–
Staying	Site	17.125	<0.001*	23.073	<0.001*
	Within-brood experience	5.871	0.015*	5.737	0.016*
	Within-year experience	2.262	0.133	2.199	0.139
	Across-years experience	0.819	0.366	0.955	0.328
	Clutch size	0.443	0.506	0.226	0.635
	Site*Within-brood experience	3.391	0.335	–	–
	Site*Within-year experience	2.561	0.464	–	–
	Site*Across-years experience	1.308	0.727	–	–
	Site*Clutch size	1.075	0.783	–	–

P values below 0.05, indicating statistically significant effects, are marked with asterisks. Within-brood, within-year and across-years experience indicate plasticity on these timescales; the interaction terms indicate whether plasticity differed between sites. 'Site' refers to the four study sites; 'female location' refers to whether the female was away from the nest, was on the nest but flew off or stayed on the nest. Parameter estimates for the same models are presented in Table A6.

Finally, neither plasticity nor repeatability of risk-taking behaviours differed significantly between urban and forest habitats.

Habitat Differences in Risk Taking

We found that urban birds showed bolder behaviour than forest birds when their nest was disturbed by a human. Similar studies of breeding birds by Cavalli, Baladrón, Isacch, Biondi, and Bó (2016) and Kunca and Yosef (2016) also found that urban birds are more likely to stay on their nest when approached by a human and more likely to show aggressive, mobbing behaviour towards the person checking their nest compared to rural birds. These findings are in line with a large number of studies, conducted in various other contexts (e.g. foraging or chick rearing), finding bolder behaviour in urban than in nonurban animals (Abolins-Abols, Hope, & Ketterson, 2016; Atwell et al., 2012; Ritzel & Gallo, 2020; Samia, Nakagawa, Nomura, Rangel, & Blumstein, 2015; Vincze et al., 2016, 2019).

Despite the significant differences between urban and nonurban habitats in staying on the nest, we also found significant contrasts between sites within habitat type (Table A5). Specifically, the significant difference between habitat types was mostly due to the large difference between the Veszprém and Szentgál sites, whereas females at the other two sites showed intermediate risk taking. This heterogeneity between sites within habitat types might be due to the smaller sample sizes at Balatonfüred and Vilma-puszta, which may have resulted in less reliable estimates than for the other sites. Furthermore, there is evidence that variation in human disturbance within a habitat type (such as distance from roads or human activity) can lead to variation in risk-taking phenotypes (Carrete & Tella, 2010; Shannon, Cordes, Hardy, Angeloni, & Crooks, 2014; Sprau & Dingemanse, 2017). Balatonfüred is a smaller city with a lower human population than Veszprém, with most human activity occurring in the summer when the birds' breeding season has already ended; thus, the birds in Balatonfüred may have experienced less human disturbance than in Veszprém. Similarly, although human disturbance is very low in both of our forest sites, Vilma-puszta is more frequently visited by recreational trekkers than Szentgál, with some tourist paths directly crossing the nestbox sites; therefore, birds may be exposed to more frequent human

activity. Alternatively, or additionally, differences in blue tit population density between sites might contribute to differences in risk-taking behaviour, especially alarm calling. For example, in a more densely populated area, more birds from neighbouring territories can join the 'mobbing chorus', although in our experience this is rare.

Unlike staying and alarm calling, the frequency of on-nest threat display (i.e. 'hissing') behaviour did not differ significantly between habitat types. This result is somewhat surprising, because threat display is an effective strategy against nest predators, increasing the survival of both the females and their offspring (Krams et al., 2014), and there is evidence that nest predation is less common in cities than in natural habitats (Eötvös, Magura, & Lövei, 2018; Vincze et al., 2017), including our study population (percentage of nests destroyed by predators: Balatonfüred: 2.7%; Veszprém: 1.7%; Szentgál: 9.6%; Vilma-puszta: 3.4%; E. Vincze, personal observation). In our study, however, threat display was relatively uncommon (7% of observations), compared to studies that tested this behaviour experimentally (to woodpecker dummies; see Krams et al., 2014; Koosa & Tilgar, 2016; Grunst et al., 2019; Thys et al., 2019) and found a hissing response to be common (61–78%). There is evidence that tit species give differential responses to different types of threats (Curio, Klump, & Regelmann, 1983; Suzuki, 2011; Vincze et al., 2019), which may explain why humans in our study did not elicit a hissing response as often as the woodpecker dummy did in other studies (i.e. the majority of birds may not perceive humans as potential nest predators in either habitat type). This threat specificity may also explain the lack of urban–forest difference, as the frequency of the 'threat' of humans checking nests did not differ between habitats.

Consistency and Plasticity of Risk Taking

We tested consistency and plasticity of great tits' parental risk-taking behaviour on three temporal scales: across years, within years between first and second broods and within breeding attempts. Out of the two traits we tested for consistency, staying on the nest, showed high repeatability values on all three temporal scales, even across years, indicating that this is a stable within-

Table 3
Repeatability estimates (R) for alarm calling and staying in the short, medium and long term

Behaviour	Temporal scale	Habitat	R	CI (95%)	CI (84%)
Alarm calling	Within-brood	All birds	0.122	0.069—0.147	—
		Urban	0.160	0.075—0.201	0.092—0.178
		Forest	0.123	0.054—0.164	0.068—0.147
	Within-year	All birds	0.078	0.024—0.126	—
		Urban	0.103	0.032—0.171	0.048—0.149
		Forest	0.082	0.000—0.147	0.017—0.128
	Across-years	All birds	0.038	0.000—0.073	—
		Urban	0.068	0.003—0.126	0.019—0.105
		Forest	0.007	0.000—0.075	0.000—0.050
Staying	Within-brood	All birds	0.681	0.356—0.709	—
		Urban	0.769	0.038—0.802	0.058—0.775
		Forest	0.708	0.472—0.734	0.517—0.706
	Within-year	All birds	0.636	0.332—0.686	—
		Urban	0.697	0.112—0.760	0.040—0.725
		Forest	0.704	0.462—0.733	0.513—0.700
	Across-years	All birds	0.556	0.273—0.615	—
		Urban	0.606	0.003—0.686	0.007—0.628
		Forest	0.613	0.372—0.686	0.436—0.655

We present both 95% and (for habitat comparison) 84% confidence intervals (CI). The repeatability values are on original scales (Nakagawa & Schielzeth, 2010). For components of variance from which these values were calculated see Table A7.

individual trait of female great tits. This suggests that, despite being a simple binary measurement, staying on the nest might provide a biologically meaningful proxy for the risk-taking axis of animal personality. The advantages of this proxy are that it is fast, cheap and easy to categorize even by inexperienced observers. Most importantly, it is a low-impact measurement, as it requires no extra disturbance for the birds besides regular nest checks that are routinely conducted in many study systems for collecting other data such as clutch size and hatching date. Although such low-impact measurements cannot replace finer-scale behavioural assays overall, they may represent a useful alternative in situations when the latter approach cannot be applied effectively. To assess the utility of this approach, future studies need to carefully explore the relationships between the propensity to stay on the nest upon human disturbance and other, finer-scale measurements of risk taking with already established ecological relevance in great tits and possibly also other species.

The probability of alarm calling showed no significant repeatability across years and low repeatability within years and within breeding attempts. This low repeatability may reflect low consistency in alarm calling. Alternatively, this may be the result of the limitation that we could not always identify the alarm-calling individuals, and thus we estimated the consistency of the pair, which is dependent on the behaviour of both the female and the male, as well as the chance of observing them at the nest. Higher repeatability at the level of pairs would have indicated either assortative mate choice, i.e. that birds pair with partners with similar risk-taking behaviour as their own (Both, Dingemanse, Drent, & Tinbergen, 2005; Pogány et al., 2018), or behavioural synchronization, i.e. the male and the female adjust their behaviour to one another or to the same brood quality (Dostálková & Špinka, 2007); the low repeatabilities we found support neither of these mechanisms.

We also tested population level plasticity on three temporal scales. The probability of the females staying on the nest increased significantly over time within broods, but we did not find systematic change in this behaviour on longer timescales. Within-brood plasticity can be influenced by at least two factors: the age of eggs and the parents' experience with nest checks. The former explanation, often referred to as the 'brood value hypothesis',

suggests that eggs closer to hatching are more valuable for the parents (Montgomerie & Weatherhead, 1988), which can explain why females were less likely to fly off the nest during later nest checks. However, the variable we tested (number of nest checks, rather than egg age) is more directly related to the birds' experience with the humans checking the nests. Thus, habituation to these nest checks is also a likely explanation: the females became more and more likely to stay on the nest because they learned to tolerate the brief disturbances associated with regular nest checks. A third alternative explanation may be that the apparent change in time is due to sampling bias, for example because females that consistently stayed on the nest were checked more times to determine clutch size than females that did not stay. To test this latter option, we looked at all pairs of consecutive observations of the same individual, and found that of 244 observations where the female flew off, 54 were followed by an observation where she stayed on the nest (22.1%), whereas of 511 observations where the female stayed on the nest, only 33 were followed by the female flying off (6.5%). This difference was statistically significant ($\chi^2_1 = 38.27$, $P < 0.001$), supporting the notion that the within-brood plasticity of staying on the nest that we found at the population level is likely to be due to a within-individual increase in the probability of staying.

The lack of a similar across-years habituation in staying on the nest might have resulted from the time passing between broods (typically 1 year, occasionally several years) being long enough for the birds to recover from habituation to nest checks (Rankin et al., 2009). Alternatively, it may be explained by 'dishabituation', which can be caused by the trapping of the parents which may provide a strong negative experience cancelling out the results of earlier habituation (Rankin et al., 2009). As 73.4% of the observations in our study came from the first two broods of each female (Table A2), between which they were trapped for ringing, dishabituation from the first to the second brood might be strong enough to mask across-brood habituation for later broods. In addition, our earlier study showed that female great tits are also sensitive to the trapping of their mates (Seress et al., 2017), which may have resulted in further dishabituation.

The probability of alarm calling increased across years, but showed no short-term plasticity. This across-years plasticity, again, may be explained by at least two factors: the parents' age and/or their experience with disturbance from their previous broods. One prediction of the reproductive value hypothesis is that the older the birds, the lower the value of their future reproduction, thus the more valuable the current brood (Williams, 1966), which may explain why older females and their mates defend their nests by making alarm calls more often. The increase in alarm calls may also be explained by a long-term habituation: over years, birds got more used to the disturbances around the nests and thus stayed nearby, making alarm calls, rather than flying away. Conversely, it may also be explained by sensitization: owing to negative experiences, such as getting captured and ringed in the chick-rearing period of their first brood, birds may recognize people as a threat and therefore make more alarm calls during later breeding episodes. Lasting recognition and more intensive alarm calling towards individuals who captured birds (i.e. individual researchers) is also present in some species (Levey et al., 2009; Marzluff, Walls, Cornell, Withey, & Craig, 2010), although in our earlier study we did not find evidence for recognition of individual humans in a different situation in great tits (Vincze et al., 2019). Finally, it is also possible that this across-years change is due to changes in the populations' composition: individuals that made alarm calls less often may have been less likely to breed in our nestboxes in subsequent years, resulting in a greater proportion of alarm-calling individuals from the second year onward compared to the first year.

Owing to the low frequency of threat displays, we were not able to test either its consistency or its plasticity. However, other studies that investigated this behaviour experimentally found that it was repeatable within a nesting attempt (Grunst et al., 2019; Koosa & Tilgar, 2016; Krams et al., 2014; Thys et al., 2019) and across years (Thys et al., 2021), and also found within-brood plasticity (Thys et al., 2019), which is a pattern similar to what we found for staying on the nest.

Differences Between Habitats

To our knowledge, our study is the first to test the differences between urban and forest populations in both consistency and plasticity of behaviour towards humans. Interestingly, we did not find any significant urban–forest difference in the consistency of risk-taking behaviour. It has been proposed that lower among-individual variation in urban than nonurban populations may indicate differential colonization or microevolution (Carrete & Tella, 2011), although this prediction may apply only for populations that have recently adapted to urban habitats (Møller, 2010). As great tits have been present in urban habitats in Hungary at least since the 1920s (Møller et al., 2012), it is possible that they have been urbanized long enough for their risk-taking behaviour to become heterogeneous again. Therefore, our finding of similar repeatability of risk taking in urban and forest populations neither supports nor refutes the possibility that urban great tit populations are bolder due to colonization by bold individuals and/or local selection for boldness. Nevertheless, the high individual consistency of staying on the nest suggests that these processes (which rely on stable individual differences) were possible, although we cannot exclude that developmental plasticity also played a role in this high individual consistency.

On the other hand, if risk-taking behaviour is primarily shaped by habituation, we predicted higher plasticity in urban than in forest habitats. This prediction was not supported by our results, as change over time in staying on the nest and alarm calling did not differ between habitat types. There is evidence of faster habituation rates in urban than in nonurban animals (Pellitteri-Rosa et al., 2017; Vincze et al., 2016), but one study found the opposite pattern, with rural animals showing faster and greater habituation (Cavalli et al., 2018). Notably, the first two studies were both performed in captivity and the animals habituated to a novel disturbance. By contrast, the latter was performed in the animals' natural habitat and to a stimulus (approaching human) that they frequently encounter even outside the experimental situation. Therefore, in this latter study the urban animals may have already had a high baseline risk-taking behaviour when the experiment started, owing to earlier habituation. Our observations were also performed in a natural habitat, but at least in the first brood, the nest check was a novel disturbance stimulus. Thus, it is possible that we found no urban–forest difference in plasticity because the two aforementioned effects cancelled each other out: although urban birds are more capable of fast habituation rates than forest birds, they also had bold baseline behaviour (i.e. stayed on the nest and made alarm calls relatively more often even during the first nest check), leaving little room for habituation. Note, however, that our statistical models had low dispersion parameters, and underdispersed models tend to be more conservative; thus, we cannot exclude the possibility that the interactions were all nonsignificant because of limited power. Nevertheless, our data did not support the prediction that either plasticity or consistency of risk taking would be generally more salient in urban habitats than in forests, which suggests that both habituation and stable between-individual differences (due to personality-dependent habitat choice and/or local evolution of boldness) might play similarly important roles in adaptations to human disturbances in the cities.

Supplementary Material

Supplementary material associated with this article can be found online at https://doi.org/10.1016/j.anbehav.2021.06.032.

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Appendix

Table A1
Proportion of observations with birds showing a given behaviour at each site and in each habitat type, and results of chi-square tests

Behaviour	Unidentified females	Urban sites		Forest sites		χ^2	P
		Veszprém	Balatonfüred	Szentgál	Vilma-puszta		
Alarm calling	Included	0.307	0.361	0.278	0.258	13.682	0.003
			0.322		0.272	8.581	0.003
	Excluded	0.334	0.366	0.296	0.254	11.493	0.009
			0.343		0.283	8.424	0.004
Female present on nest	Included	0.679	0.706	0.682	0.639	4.570	0.206
			0.686		0.669	0.938	0.333
	Excluded	0.722	0.726	0.722	0.629	10.755	0.013
			0.723		0.693	2.171	0.141
Staying	Included	0.779	0.613	0.459	0.659	157.84	<0.001
			0.732		0.463	98.359	<0.001
	Excluded	0.793	0.615	0.493	0.705	113.49	<0.001
			0.744		0.552	60.887	<0.001
Threat display	Included	0.136	0.144	0.112	0.131	1.378	0.711
			0.138		0.119	0.800	0.371
	Excluded	0.143	0.153	0.143	0.171	0.686	0.877
			0.145		0.153	0.057	0.810

Degrees of freedom = 3 for site comparisons and 2 for habitat type comparisons.

Table A2
Frequency of the numbers of repeats within the total sample

Repeats	Obs/female	Obs/year	Obs/brood	Brood/female	Year/female	Brood/year
1	27	38	58	200	255	367
2	44	77	163	83	68	180
3	80	154	297	38	36	
4	52	104	165	24	9	
5	34	59	32	13	3	
6	19	43	5	7		
7	26	46	2	3		
8	20	18	2	1		
9	8	4	0	2		
10	11	4	1			
11	7					
12	9					
13	5					
14	2					
15	5					
16	4					
18	6					
19	3					
20	2					
23	3					
25	2					
26	1					
27	1					

Obs/female: number of females with 1, 2, etc. observations; obs/year: number of female-year combinations with 1, 2, etc. observations; obs/brood: number of nesting attempts with 1, 2, etc. observations; brood/female: number of females with 1, 2 etc. broods included; year/female: number of females with broods from 1, 2, etc. years included; brood/year: number of female-year combinations with 1 or 2 broods included.

Table A3
Type 2 ANOVA results of the full models (with interactions) and the final models (without interactions) for 'alarm calling' 'staying', with single-observation individuals excluded

Behaviour	Predictor	Full models		Final models	
		χ^2	P	χ^2	P
Alarm calling	Site	22.417	<0.001*	23.177	<0.001*
	Female location	274.812	<0.001*	275.738	<0.001*
	Within-brood experience	0.051	0.822	0.095	0.758
	Within-year experience	0.015	0.902	0.006	0.939
	Across-years experience	6.571	0.010*	6.346	0.012*
	Clutch size	1.380	0.240	1.661	0.197
	Site*Female location	5.893	0.435	5.443	0.488
	Site*Within-brood experience	3.738	0.291	–	–
	Site*Within-year experience	1.209	0.751	–	–
	Site*Across-years experience	0.925	0.820	–	–
	Site*Clutch size	2.096	0.553	–	–
Staying	Site	17.459	0.001	20.556	<0.001*
	Within-brood experience	0.064	0.801	4.090	0.043
	Within-year experience	4.242	0.039	1.010	0.315
	Across-years experience	0.941	0.332	0.378	0.539
	Clutch size	0.375	0.541	0.001	0.978
	Site*Within-brood experience	0.077	0.994	–	–
	Site*Within-year experience	3.234	0.357	–	–
	Site*Across-years experience	2.767	0.429	–	–
	Site*Clutch size	0.902	0.825	–	–

P values below 0.05, indicating statistically significant effects, are marked with asterisks. For models with single-individual observations included, see Table 2.

Table A4
Likelihood ratio tests of model fit of alternative models with 'alarm calling' and 'staying' as dependent variable

Model	df	AIC	BIC	logLik	Deviance	χ^2	df	P
Alarm calling								
Without interactions	19	2057.9	2165.8	-1010.0	2019.9			
With interactions	31	2074.1	2250.2	-1006.1	2019.1	7.762	12	0.803
Staying								
Without interactions	11	1318.7	1377.4	-648.35	1296.7			
With interactions	23	1330.0	1452.7	-641.98	1284.0	12.744	12	0.388

The model 'with interactions' includes the within-brood experience*study site, within-year experience*study site, across-year experience*study site and clutch size*study site interactions.

Table A5
Pairwise comparisons (linear contrasts) between sites for the frequency of the female staying on the nest, calculated from our models

Comparison	Estimate	SE	z	P
Szentgál–Balatonfüred	−1.930	0.967	−1.997	0.069
Szentgál – Veszprém	−4.740	0.999	−4.746	<0.001
Szentgál – Vilma-puszta	−3.240	1.053	−3.077	0.006
Balatonfüred – Veszprém	−2.810	0.950	−2.960	0.006
Balatonfüred – Vilma-puszta	−1.310	1.075	−1.218	0.223
Veszprém – Vilma-puszta	1.500	0.915	1.640	0.121

P values are corrected using the FDR method.

Table A6
Parameter estimates (on logit scale) of the linear mixed-effects models for the probability of alarm calling outside the nest and the female staying on the nest

Behaviour	Predictor	Estimate	SE	z	P
Alarm calling	Intercept (Szentgál, off nest)	−1.665	0.256	−6.499	<0.001
	Site: Balatonfured (urban)	0.564	0.391	1.442	0.149
	Site: Veszprem (urban)	1.065	0.314	3.396	0.001*
	Site: Vilma-puszta (forest)	0.140	0.391	0.357	0.721
	Female location: Fly off	2.300	0.295	7.798	<0.001*
	Female location: Stay	−1.964	0.436	−4.507	<0.001*
	Within-brood experience	−0.013	0.056	−0.233	0.815
	Within-year experience	0.019	0.195	0.096	0.924
	Across-years experience	0.265	0.104	2.557	0.011*
	Clutch size	−0.056	0.043	−1.307	0.191
	Balatonfüred − Fly off	0.527	0.524	1.007	0.314
	Veszprém − Fly off	−0.296	0.424	−0.698	0.485
	Vilma-puszta − Fly off	0.419	0.553	0.758	0.449
	Balatonfüred − Stay	0.740	0.595	1.242	0.214
	Veszprém − Stay	0.547	0.484	1.130	0.259
	Vilma-puszta−Stay	0.746	0.629	1.186	0.236
Staying	Intercept (Szentgál forest)	−0.921	0.586	−1.572	0.116
	Site: Balatonfüred (urban)	1.930	0.967	1.997	0.046*
	Site: Veszprém (urban)	4.741	0.999	4.746	<0.001*
	Site: Vilma-puszta (forest)	3.240	1.053	3.077	0.002*
	Within-brood experience	0.254	0.106	2.395	0.017*
	Within-year experience	0.673	0.455	1.479	0.139
	Across-years experience	0.238	0.243	0.977	0.328
	Clutch size	0.054	0.115	0.475	0.635

P values below 0.05, indicating statistically significant effects, are marked with asterisks.

Table A7
Components of variance (s^2) extracted from linear mixed-effects models with alarm calling and staying as response variable

Component of variance	Alarm calling			Staying		
	All birds	Urban	Forest	All birds	Urban	Forest
Within-brood	0.493	0.487	0.493	1.322	2.705	0.078
Within-year	0.448	0.305	0.906	2.329	3.222	1.909
Between-year	0.434	0.585	0.081	16.228	22.639	12.819
Residual	2.451	1.940	3.010	4.405	2.644	1.534