

## RESEARCH ARTICLE

# Risk-taking coping style correlates with *SERT* SNP290 polymorphisms in free-living great tits

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## ABSTRACT

The coping style of an individual in relation to potentially dangerous situations has been suggested to be inherited in a polygenic fashion, *SERT* being one of the candidate genes. In this study, we assessed in free-living great tits (*Parus major*) the association between SNP290 in the *SERT* promoter and three standard fear-related behaviors: the response of the birds to a black-and-white flag fixed to the top of the nest box, distress calling rate of the birds in the hand once captured and the hissing call of incubating females when approached by a predator. We found a strong association between SNP290 polymorphism and the three risk-taking behaviors, with birds with genotype CT entering the nest box with the flag faster and displaying more distress calls and fewer hissing calls. CT birds could therefore be described as more proactive than CC individuals. These results also suggest that hissing behavior should be regarded as a fear-induced shy behavior, and confirm that *SERT* has an important function in relation to risk aversion behaviors and coping style.

**KEY WORDS:** Personality, Risk aversion, Serotonin transporter, Distress calling, Hissing calling, *Parus major*

## INTRODUCTION

In recent years, it has been shown that individuals facing environmental challenges and stressful situations display differences in behavior that are stable over time and across contexts (Carere et al., 2010; MacKay and Haskell, 2015; Sih et al., 2004). Such individual differences in coping with stress have been categorized into different coping styles, varying along a continuum from proactive to reactive copers. Proactive individuals are more aggressive, explorative, neophilic and risk-prone than reactive individuals (Carere et al., 2010).

Coping styles can be heritable (Dochtermann et al., 2015; van Oers and Sinn, 2013). However, in spite of the large heritable component of coping styles and their important ecological and evolutionary implications (Carere and Maestriperi, 2013), the genetic loci underlying coping styles are relatively unknown. One of the most promising candidate genes for coping style traits is the serotonin transporter (*SERT*), which controls the uptake of the serotonin neurotransmitter in the neuronal synaptic clefts. In humans and laboratory animals, *SERT* is known to be involved in harm avoidance (Ebstein, 2006), anxiety behavior (Canli and Lesch,

2007; Savitz and Ramesar, 2004; Serretti et al., 2006), novelty seeking (Holmes et al., 2003; Murphy et al., 2008) and decision-making behavior (van den Bos et al., 2013). Modified methylation pattern or several genetic polymorphisms in the *SERT* gene can also lead to cognitive and psychiatric disorders in humans (Homberg and Lesch, 2011; Ouellet-Morin et al., 2013; van IJzendoorn et al., 2010).

Although there is quite ample knowledge on the function of *SERT* in humans and laboratory animals, few studies have investigated the role of *SERT* polymorphisms in the inter-individual variation of coping style traits in wild animals. Mueller et al. (2013) and Riyahi et al. (2015) found significant variation in the allelic frequencies in this gene between different populations linked to differences in coping style between urban and rural blackbirds (*Turdus merula*) and great tits (*Parus major*). In dunnocks (*Prunella modularis*), Holtmann et al. (2016) found a significant association between *SERT* polymorphisms and flight initiation distance, which is related to proactivity (Carrete and Tella, 2017; delBarco-Trillo, 2018). Bubac et al. (2021) also found one locus within *SERT* in grey seals (*Halichoerus grypus*) to be related to boldness. In addition, Timm et al. (2018) found a significant association between a risk-taking behavior (novel object test) of great tits and *SERT* single nucleotide polymorphisms (SNPs), and later they related *SERT* polymorphism to hissing behavior and anti-predator behavior of incubating females (Timm et al., 2019). However, Edwards et al. (2015) did not find any correlation between *SERT* polymorphisms and boldness or exploratory behaviors in the Seychelles warbler (*Acrocephalus sechellensis*), and no association was found with aggressiveness and dominance in macaques (*Macaca fascicularis*) (Miller-Butterworth et al., 2007). Similarly, Thys et al. (2021) found no association between *SERT* polymorphism and female–female aggression, and the association with hissing rate was marginally non-significant. More data are therefore needed to establish a generality for the role of *SERT* in natural populations, which helps to discern real associations from all the individual variation found (Savitz and Ramesar, 2004).

In this study, we preferred to focus on SNPs that *a priori* could be related to coping style phenotypes, rather than going on a fishing expedition by genotyping many SNPs and trying to determine a correlation of any of them with proactivity. In our former study on the *SERT* gene of *P. major*, we identified 10 SNPs in the promoter. We discovered that the promoter of this gene does not contain any CpG islands and that there are only two CpG dinucleotides in this area (Riyahi et al., 2015). This is important because methylation, and hence epigenetic effects, a key factor modulating the expression of *SERT* (van IJzendoorn et al., 2010), need the presence of these dinucleotides. The second CpG site, located 288 bp from the transcriptional start site (TSS), is abolished by a genomic variant (SNP290), behaving as an allele-specific methylation (ASM) site. Therefore, the methylation level at the second CpG site clearly stratified into three groups dictated by SNP

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290 genotype: homozygous A birds were ~8.1% (s.e.=0.55%) methylated, GA heterozygous individuals were ~31.3% methylated (s.e.=1.03%) and homozygous G birds were ~49.0% methylated (s.e.=0.85%) (Riyahi et al., 2015). In addition, we found that methylation level at this site was related to exploration score in urban great tits (Riyahi et al., 2015). Therefore, *SERT* SNP290 could be an ideal candidate SNP to relate to coping style.

As a measure of proactivity in our great tits, we decided to use three standard behavioral tests associated with consistent risk-taking or fear-related behavior. The first experiment was risk-taking behavior in reaction to a flag that was attached to the top of the nest box at the time of feeding of the nestlings. The method was previously used and tested by Cole and Quinn (2014), and reflects the proactivity of the birds in front of a new object. Time to enter into the flagged nest box correlated with additional assays with the same birds in captivity, in which exploration rate in a novel environment was measured. This novel environment assay additionally stresses the trade-off between risk-taking and chick provisioning.

The second test focused on distress calling rate during handling, which has also been previously recognized as a rate of proactivity (boldness): in black-capped chickadees (*Poecile atricapillus*), distress calling rate was positively related to exploration rate, which is one of the main characteristics of the proactive coping style (Guillette and Sturdy, 2011). In siskins (*Carduelis spinus*), individuals uttering more often distress calls also displayed bolder behaviors in front of a novel object (Mateos-González and Senar, 2012; Pascual and Senar, 2014). Distress calling has therefore been used as a proxy of proactivity in several studies (Andersen, 2012; Pascual and Senar, 2014; Richardson et al., 2016; Senar et al., 2017; Thorsteinsen, 2015).

The third test focused on the propensity of incubating females to utter hissing calls towards a nest predator. This behavior has been used recently in two studies to investigate the link between the *SERT* gene and coping style (Thys et al., 2021; Timm et al., 2019). This behavior was considered originally as an anti-predatory nest defense behavior related to proactivity (Krams et al., 2014), but Kalb et al. (2019) have recently stressed that more data are necessary to ascertain whether females uttering hissing calls are really proactive or reactive. Our study can therefore provide some additional information on the topic.

Here, we tested the prediction that between-individual variation in proactivity, as measured in the flag, distress calling and female hissing tests, is associated with genotypes in *SERT* SNP290. This study will help us to understand how an SNP can shape the behaviour of a species, and will confirm the role of *SERT* in coping style variation.

## MATERIALS AND METHODS

### General field methods

The study was carried out during the breeding season of 2015 (April–June) in the Can Catà Field Station, located in Collserola National Park, 3 km from Barcelona city. This area consists of mixed forest, dominated by pure oak (*Quercus ilex* and *Quercus cerroides*) stands at the bottom of the valleys and Aleppo pine (*Pinus halepensis*) forests in the hills. There are approximately 200 nest boxes in this area. The nest boxes were checked two to three times a week during the breeding period to measure breeding parameters. Breeding pairs were captured using nest-box traps during days 12–15 post-hatch. The great tits were ringed with numbered aluminium and PVC plastic rings. In addition, the birds were sexed and aged based on Svensson (1992) and Jenni and

Winkler (1994). We distinguish between birds in their first calendar year (yearlings) and adult birds (birds after a complete molt). Finally, blood samples were collected from a brachial vein from all of the birds and stored in pure ethanol (at 4°C) until DNA extraction. Birds were sampled, handled and ringed with special authorization (001501-0402.2009), following Catalan regional ethical guidelines for the handling of animals in research, from Servei de Protecció de la Fauna, Flora i Animal de Companyia, according to Decree 214/1997/30.07, Generalitat de Catalunya.

### Behavioral experiments

Behavioral experiments were carried out on 62 breeding pairs of great tits (62 males+62 females). We intended to conduct three behavioral experiments in our breeding pairs in all of the nest boxes. However, the number of individuals involved in each test varied because we were unable to perform some tests on some nests because the chicks died before conducting the experiment or because the parents abandoned the nests or were not captured. This is why sample sizes for the different experiments differ.

The first test was risk-taking behavior in response to a black-and-white flag (148×105 mm) that was fixed to the nest box, similarly to the experiment by Cole and Quinn (2014). This experiment is also very similar to that used by Timm et al. (2018), where instead of a flag, they used an Eppendorf box placed on the rooftop of the nest box. This experiment was conducted when the chicks had a median age of 9 days (range 9–12 days) by video recording at the distance of 7–36 m from the nests (median=16 m), depending on the presence of vegetation around each nest. Distance from the observer to the nest was set as a covariate in the analysis. To ascertain the effect of the flag (novel object) in our experiment, we first recorded the normal latency of the birds to enter the nest box without any flag. Then, we recorded the behavior of the birds with the flag attached at the top of the nest box. We recorded the behavior of the birds with a video camera, and corrected for the distance of the camera from the nest, as this could affect to the time of appearance of the bird in the video. The flag was removed after 40 min. This behavior was tested in 34 individuals, using the birds that entered the nest box during the control experiment (with no flag). However, when considering only the birds that entered the nest box during the flag trial, sample size was reduced to 24 birds. The trait has been shown previously to be repeatable (Cole and Quinn, 2014).

The second test included measuring the distress calling rate of the birds during handling (Senar et al., 2017). This was tested during the capture of the parents, normally at age 15 days of the chicks. Captured animals were kept in a ringing bag for a period of approximately 5 min to calm down. Just after extracting the bird from the ringing bag, distress calling rate was quantified by counting the number vocalized distress calls that were emitted by the birds during the next 15 s of handling while holding their legs and moving a straight finger positioned 1–2 cm from the beak of the focal bird (Markó et al., 2013; Senar et al., 2017). We recorded this behavior in a total of 64 birds. The trait has been shown previously to be repeatable (Senar et al., 2017).

The third test recorded the hissing response of incubating females to a mouse model while we inserted the model head into the entrance of the nest box. The head of the mouse was kept in this position for 15 s. We counted the number of calls of incubating females in that period. As soon as 15 s was over, the mouse was removed and the observer silently moved away. The method has been used in several other papers, although using a woodpecker as a nest predator (Krams et al., 2014; Thys et al., 2021; Timm et al., 2019). We recorded this behavior in a total of 34 females. The trait

has been found previously to be highly repeatable (Koosa and Tilgar, 2016; Krams et al., 2014).

### Genotyping

DNA was extracted from blood samples using an Ecogen MasterPure DNA purification kit (MCD85201). Primers were designed using the great tit genome (assembly 1.3, accession number: SRS1185780; Laine et al., 2016) blasting with the reverse complement of the *SERT* promoter interval of the great tit (GenBank: accession number KP869099) (Riyahi et al., 2015). The primer sequences were as follows: forward primer 5'-TTCAGCAATGCACAAAGTCCAG-3', reverse primer 5'-ACTCCAGGTCCTCCCTGTCCTC-3'. Note that for our previous paper (Riyahi et al., 2015), we sequenced the sense DNA strand, but here we used another primer and sequenced the antisense strand of DNA (the other strand). Because they are complementary sequences, the AA is equivalent to TT, GA to CT and GG to CC.

Genetic polymorphisms within the *SERT* loci were genotyped by standard PCR amplification and direct sequencing of the resulting amplicon. A total of 50 ng genomic DNA was amplified in 25  $\mu$ l reactions containing 10 $\times$  reaction buffer. All samples were sequenced at the Genomics Core Facility of the University of Pompeu Fabra (Barcelona, Spain) using an Applied Biosystems sequencing platform. All of the sequences were aligned using Sequencher v4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and visually checked for polymorphisms (SNPs). The names of the SNPs were given by the position of each SNP in the resulting amplicon.

Additionally to *SERT* SNP290, *SERT* SNP234 could also be of interest, because in our previous work we found it to be related to neophilia and hence proactivity (Riyahi et al., 2015). However, this time we were unable to detect the *SERT* SNP234. To be sure about this SNP, we genotyped again, using our new primers (for the antisense strand) our previous samples from Riyahi et al. (2015), which presumably contained the *SERT* SNP234 variant. However, although all SNPs described in our previous paper were detected, SNP234 was not detected, thus it was probably an artefact peak from the previously used sequencing machine. As a consequence, we decided to drop this SNP and focus only on *SERT* SNP290.

### Statistical analysis

The frequency distributions of the three behavioral measurements of proactivity used in this study were highly skewed to the right (skewness response to the flag=2.63 $\pm$ 0.47, distress calling rate=1.39 $\pm$ 0.30, hissing calling rate=1.61 $\pm$ 0.40) and did not follow a normal distribution (Shapiro–Wilk test: response to the flag,  $W=0.58$ ,  $P<0.001$ ; distress calling rate,  $W=0.73$ ,  $P<0.001$ ; hissing calling rate,  $W=0.67$ ,  $P<0.001$ ). Therefore, and because we were dealing with counts, the standard approach to test for the relationship between *SERT* SNP290 SNP and these three behaviors was a generalized linear model analysis (GLZ) with a Poisson distribution and log-link function (McCullagh and Nelder, 1998).

When analyzing risk-taking behavior in reaction to the flag, we used the latency to enter the nest box after the bird appeared in the video as a dependent variable and we included the distance of the camera from the nest as a covariate, as this could affect to the time of appearance of the bird in the video. We included sex, age of the birds and *SERT* SNP290 alleles as categorical factors. We only considered CC ( $N=19$ ) and CT (15) birds, as the TT genotype had a very low frequency in our population ( $N=4$ ). In this analysis, we only considered the birds that entered the nest box during our experiment without the flag, because if birds are not detected,

this could be because they simply have a low nest-box attendance rate.

When analysing distress calling rate during handling, we considered distress calling rate as the dependent variable and sex, age and *SERT* SNP290 as categorical factors. Again, we only considered CC ( $N=37$ ) and CT ( $N=27$ ) birds. Hissing rate was also analyzed using a GLZ analysis, considering calling rate as the dependent variable and age and *SERT* SNP290 as categorical factors. Because only females incubate, we only included females in analyses. In order to control for collateral effects, we also introduced variables number of eggs and laying date (number of days from 1 April) as continuous covariates. Again, we only considered CC ( $N=23$ ) and CT ( $N=11$ ) birds.

A chi-square test was performed to determine whether genotype frequencies of the *SERT* SNP290 in our population followed the Hardy–Weinberg equilibrium. We also used a chi-square test to evaluate the association between the decision to enter the nest box during the flag treatment in relation to SNP290 genotypes (CC and CT).

We used the program STATISTICA v. 12 for all statistical analyses.

### RESULTS

The resulting amplicon contained seven SNP variants: four SNPs located in the promoter and three SNPs located in exon 1. Our SNP of interest (*SERT* SNP290) was in Hardy–Weinberg equilibrium ( $\chi^2=0.52$ ,  $P=0.76$ ).

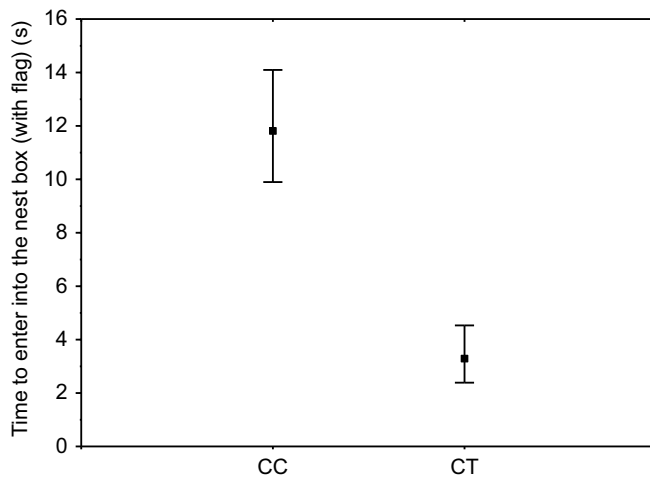
Great tits took significantly longer to return to the nest during the flag experiment than the control group (Wilcoxon matched pairs test:  $Z=3.34$ ,  $P<0.001$ ,  $N=36$ ). This confirmed that they were reacting specifically to the novel object. Considering the birds that entered the nest box during the control experiment (with no flag), we found that there was no association between the decision to enter the nest box during the flag treatment in relation to SNP290 genotypes (CC and CT) ( $\chi^2_1=0.20$ ,  $P=0.66$ ,  $N=34$ ). From the 10 birds not entering the nest box, five had the CC genotype and five the CT. However, considering the birds that entered the nest box during the flag trial, we found that CT birds were faster to enter the box than CC birds (Table 1, Fig. 1). No difference appeared between genotypes during the control trial (with no flag) (Wald statistic=1.38,  $P=0.24$ ). We also found during the control trial that females entered the nest box faster than males (latency males: 4.8 $\pm$ 0.15 s,  $N=17$ ; females: 0.7 $\pm$ 0.28 s,  $N=17$ ; Wald statistic=28.05,  $P<0.001$ ), and adults faster than young birds (latency yearlings: 3.5 $\pm$ 0.19 s,  $N=11$ ; adults: 1.0 $\pm$ 0.23 s,  $N=23$ ; Wald statistic=17.91,  $P<0.001$ ). During the flag trial, we also found that females entered the nest box faster than males (latency males: 8.5 $\pm$ 0.13 s,  $N=11$ ; females: 4.6 $\pm$ 0.13 s,  $N=13$ ; Table 1), but young birds entered the nest box faster than adults (latency yearlings: 4.8 $\pm$ 0.10 s,  $N=8$ ; adults: 8.1 $\pm$ 0.10 s,  $N=16$ ; Table 1).

**Table 1. Generalized linear model analysis (GLZ) (Poisson distribution) on latency to enter the nest box after the bird appeared in the video in relation to sex and *SERT* SNP290 alleles (CC and CT) of the birds**

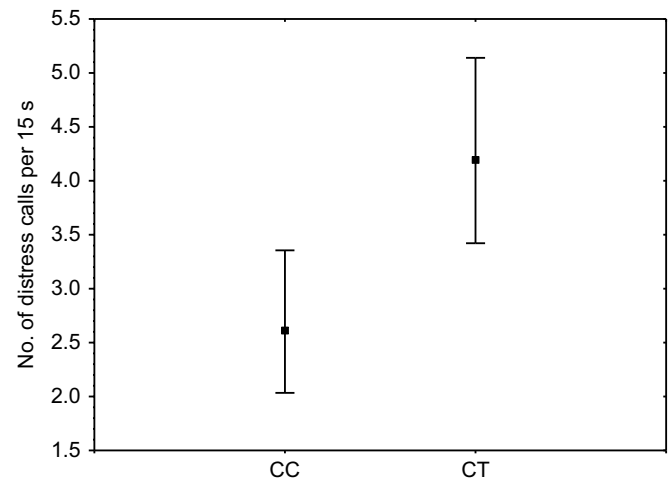
	Wald statistic	d.f.	<i>P</i>
Sex	14.49	1	<0.001
Age	7.06	1	<0.01
SNP290	56.41	1	<0.001
Distance to nest box	5.27	1	<0.05

We included as a covariate the distance of the video camera to the nest box. No interactions were significant and hence were not included in the table.  $N=24$  individuals.





**Fig. 1. Great tit latency to enter into the nest box (covariate-adjusted means) after noticing the flag on the nest box in relation to the *SERT* SNP290 genotypes (CC versus CT).** Sample size was 14 individuals for CC birds and 10 for CT birds.



**Fig. 2. Distress calling rate (adjusted means) of great tits during handling in relation to the *SERT* SNP290 genotypes (CC versus CT).** Sample size was 37 individuals for CC birds and 27 for CT birds.

We found an association between distress calling rate and SNP290 genotypes for the general genetic model, with CT birds uttering more distress calls than CC birds (Table 2, Fig. 2). Sexes did not differ in the number of distress calls uttered, but adults uttered more distress calls than young birds (yearlings:  $2.8 \pm 0.15$  calls,  $N=17$ ; adults:  $3.9 \pm 0.08$  calls,  $N=47$ ; Table 2).

We found that 44% ( $N=34$ ) of females uttered hissing calls. We also found an association between hissing call rate and SNP290 genotypes for the general genetic model, with CT birds uttering fewer distress calls than CC birds (Table 3, Fig. 3). Yearling females displayed more hissing calls than adult females (yearlings:  $6.4 \pm 0.12$  calls,  $N=15$ ; adults:  $2.4 \pm 0.15$  calls,  $N=19$ ; Table 2).

Latency to enter the nest box in response to a black-and-white flag was not correlated with distress calling rate ( $r=-0.09$ ,  $P=0.61$ ,  $N=31$ ) nor with hissing rate ( $r=0.11$ ,  $P=0.69$ ,  $N=17$ ). Distress calling rate was also not correlated with hissing rate ( $r=0.06$ ,  $P=0.73$ ,  $N=38$ ).

## DISCUSSION

We have shown that *SERT* SNP290 genotypes relate to coping style in three independent experiments. Great tits with the CT genotype entered the nest box faster than CC birds when the flag was displayed on the nest box, which indicates, according to the rationale by Cole and Quinn (2014), that CT birds were bolder than CC birds. Great tits with the CT genotype also displayed higher distress calling rate than CC birds, which again indicates that CT birds were bolder than CC birds (Senar et al., 2017). Results concerning the hissing call rate are at first sight unexpected because we found that CT birds uttered fewer hissing calls than CC birds.

**Table 2. GLZ (Poisson distribution) on the number of distress calls uttered in the 30 s period during handling in relation to sex, age and *SERT* SNP290 alleles (CC and CT) of the birds**

	Wald statistic	d.f.	<i>P</i>
Sex	3.25	1	0.07
Age	3.95	1	<0.05
SNP290	10.36	1	<0.001
Sex×SNP290	4.12	1	<0.05

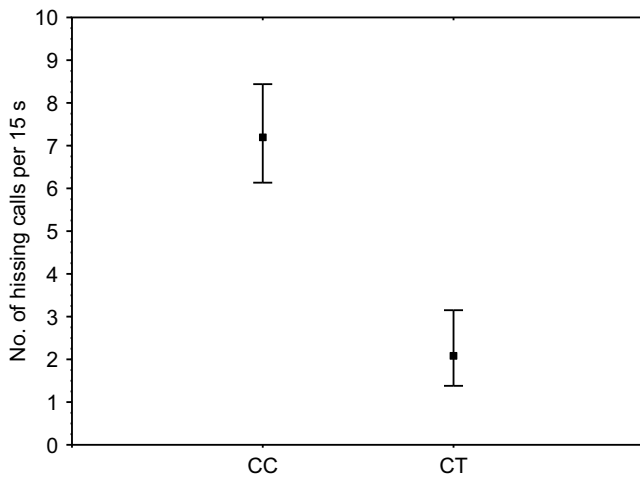
Only significant interactions are included in the table.  $N=64$  individuals.

However, it is not certainly clear whether this call is uttered by bold or shy individuals. Originally, Krams et al. (2014) assumed that this display should be considered as a bold type of antipredator response because it reduced the mortality risk of incubating females, but they also recognized that this link to boldness should be tested. This need to assess what hissing calls reflect was also expressed by Kalb et al. (2019). The link between hissing calls and personality was tested by van den Brink et al. (2012), who found that individuals uttering more hissing calls were also more docile, stayed calm when handled and feigned death, concluding that hissing calls mirrored a shy behavior. In line with this, we also found that less experienced yearling females hissed more often than adult ones, and Tilgar and Koosa (2019) found that hissing females had lower breeding success. Hence, if we define hissing behavior as a fear-induced shy behavior, CT females uttering fewer hissing calls is consistent with results from distress calling and latency to return to the nest box with a flag on top, which indicate that CT birds are bolder than CC birds. Here, we have to stress that yearling birds were not fully consistent. Yearling birds uttered fewer distress calls than adults and had longer latency times to enter the nest box during the control trial (with no flag), which is consistent with a shy behavior (Cole and Quinn, 2014; Senar et al., 2017). However, in the experimental trial with the flag, they had a shorter latency to return to the nest when compared with adults, which implies that they were bolder than adults (Cole and Quinn, 2014). Here, we have to point out that yearlings in this flag experiment had a quite small sample size ( $N=8$  birds) compared with adults ( $N=16$ ), so that this inconsistency perhaps reflects the small sample size of yearling birds in that particular experiment.

**Table 3. GLZ (Poisson distribution) on the number of hissing calls uttered by the incubating female in the 15 s period during handling in relation to age (yearling and adult) and *SERT* SNP290 alleles (CC and CT) of the birds**

	Wald statistic	d.f.	<i>P</i>
N eggs	2.42	1	0.12
Laying date	0.73	1	0.39
Age	42.45	1	<0.001
SNP290	31.23	1	<0.001

The interaction Age×SNP290 was not significant and thus was removed from the analysis.  $N=34$  individuals.



**Fig. 3. Hissing call rate (adjusted means) of incubating female great tits in relation to the *SERT* SNP290 genotypes (CC versus CT).** Sample size was 23 individuals for CC birds and 11 for CT birds.

More data are hence needed to better ascertain the effect of age in these experiments.

In spite of the fact that CT birds seemed bolder than CC birds across the different experiments, which entails a consistent role of the different behaviors, the different behavioral responses were not correlated among them. According to theoretical developments on the topic, this would suggest that we are not facing a personality syndrome (Dingemanse and Wolf, 2010). However, a recent meta-analysis has found that effect sizes to detect a syndrome are generally quite small, which implies that a huge sample size would be required to demonstrate a correlation between behaviors with sufficient statistical power (Garamszegi et al., 2012), which was not the case in our study. Hence, larger sample sizes would be required to test for the inter-correlation between the different behavioral variables we have used in this study.

Because results from *SERT* SNP290 are consistent across three measures of coping style, we suggest that this SNP has a crucial role in adjusting the risk-taking choices of great tits and may be a standard genetic landmark of coping style. Additionally, this single SNP can change the methylation level of the *SERT* promoter, plus it may alter a transcription factor binding site that regulates expression levels; it all adds to the interest of SNP290.

Measuring gene expression of individuals with different genotypes of *SERT* SNP290 in the brain tissue (using qRT-PCR) can validate the role of this SNP in *SERT* gene expression and, consequently, the behavior of great tits. Owing to animal rights of wild species, we were not willing to kill the birds only for this purpose, but future studies can shed further light on *SERT* gene expression.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: S.R., F.C., J.C.S.; Methodology: S.R., J.G.C.-O., F.U., F.C., J.C.S.; Formal analysis: S.R., F.C., J.C.S.; Investigation: S.R., J.G.C.-O., F.U., F.C., J.C.S.; Resources: J.C.S.; Data curation: S.R., J.G.C.-O., F.U.; Writing - original

draft: S.R., J.C.S.; Writing - review & editing: S.R., J.G.C.-O., F.U., F.C., J.C.S.; Supervision: J.C.S.; Funding acquisition: J.C.S.

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#### Data availability

Data from this paper are available in Dryad (Riyahi et al., 2022): <https://doi.org/10.5061/dryad.ns1rn8pvz>.

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