Relationships between personality traits and the physiological stress response in a wild mammal

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Running title: personality and physiological stress in grey squirrels

Abstract

Glucocorticoids are involved in regulation of an animal's energetic state. Under stressful situations, they are part of the neuroendocrine response to cope with environmental challenges. Animals react to aversive stimuli also through behavioural responses, defined as coping styles. Both in captive and wild populations, individuals differ in their behaviour along a proactive – reactive continuum. Proactive animals exhibit a bold, active-explorative and social personality, while reactive ones are shy, less active-explorative and less social. Here we test the hypothesis that personality traits and physiological responses to stressors co-vary, with more proactive individuals having a less pronounced glucocorticoid stress response. In wild populations of invasive grey squirrels (*Sciurus carolinensis*), we measured faecal glucocorticoid metabolites (FGMs), an integrated measure of circulating glucocorticoids, and three personality traits (activity, sociability, exploration) derived from open field test (OFT) and mirror image stimulation (MIS) test. Grey squirrels had higher FGMs in autumn than in winter and males with scrotal testes had higher FGMs than non-breeding males. Personality varied with body mass and population density. Squirrels expressed more activity-exploration at higher than at lower density and heavier squirrels had higher scores for activity-exploration than animals that weighed less. Variation in FGM concentrations was not correlated with the expression of the three personality traits. Hence, our results do not support a strong association between the behavioural and physiological stress responses but show that in wild populations, where animals experience varying environmental conditions, the glucocorticoid endocrine response and the expression of personality are uncorrelated traits among individuals.

Key words: glucocorticoids, HPA axis reactivity, *Sciurus carolinensis*, personality-traits, MCMCglmm, FGM concentration.

Introduction

Wild animals interact continuously with the environment and many of these interactions are mediated in part by the action of the hypothalamic–pituitary–adrenal (HPA) axis and by the autonomic nervous system. These neuro-endocrine responses allow animals to cope with a variety of environmental stimuli through the influence of its downstream products (e.g., glucocorticoids, catecholamines) on behaviour, reproduction, growth and energy allocation (Jansen et al. 1995; Sapolsky et al. 2000; Ricklefs and Wikelski 2002; Koolhaas et al. 2010). Glucocorticoids (GCs), in terms of baseline activity of the HPA axis, affect the animal's energetic state through the regulation of circulating glucose levels related to broad changes in activity patterns (e.g. seasons, life-history stages), and by influencing circadian patterns of appetite and foraging behaviours (Sapolsky et al. 2000; Crespi et al. 2013). GCs also play a mediatory role in processes that require energetic expenditure and resource allocation trade-offs, to cope with unpredictable or predictable environmental stressors, hence in the animal's physiological stress response (Wingfield and Sapolsky 2003; Crespi et al. 2013; Wingfield and Romero 2015).

Animals can react to environmental stimuli also through a set of behavioural responses that are consistent within individuals over time, independently from their life-history state, sex and motivational state, referred to as coping styles (Koolhaas et al. 1999; Pfeffer et al. 2002; Réale et al. 2007). Based upon previous studies of farm or laboratory animals under controlled conditions, which demonstrated how the activity of the HPA axis is associated with certain coping styles, it was proposed that behavioural reactions to stressors are mediated by hormones exerting pleiotropic actions on both the animal's behaviour and physiology (Koolhaas et al. 1999; Carere et al. 2005; Dantzer and Swanson 2017), and that these relationships are conserved throughout the vertebrate lineage (Øverli et al. 2007).

Both in captive and wild populations, individuals differ in behaviours related to the animal's personality, hence their tendency to behave consistently over time and in different contexts (Réale et al. 2007; Carere et al. 2010), along a proactive – reactive continuum (Koolhaas et al. 1999, 2010). In mammals, proactive animals tend to exhibit a bold, active-explorative and social personality, while reactive ones are shy, less active-explorative and less social (Carere et al. 2010; Koolhaas et al. 2010). Proactive individuals are predicted to exhibit high levels of catecholamines but low levels of GCs in response to an environmental challenge, whereas reactive animals are predicted to exhibit low levels of catecholamines and high levels of

GCs in response to an adverse stimulus (Carere et al. 2005; Cockrem 2007; Koolhaas et al. 2010; Pusch et al. 2018; Raulo and Dantzer 2018). Despite several studies on laboratory animals finding these associations, the causal relationship between physiological responses and behavioural reactions is still debated (Carere et al. 2010; Koolhaas et al. 2010). In fact, in their review Koolhaas et al (2010) proposed that individual variation can have several dimensions and that the behavioural response to a challenging condition (measured as the expression of single personality traits or of coping styles), is partly independent from the physiological response (stress reactivity). This hypothesis is known as the two-tier model. According to the two-tier model, individuals may show stable trait-like variation on two independent axes, a qualitative coping style axis and a quantitative stress reactivity axis. Measurements of traits along each axis (reactive – proactive traits along the behavioural axis, and low – high reactivity along the stress axis) do not necessarily need to be correlated.

In wild mammals, the measurement of faecal glucocorticoid metabolites (FGMs; Sheriff et al. 2011; Dantzer et al. 2014), which represents a combination of both baseline and stress-induced GC levels, has been used as an integrated measure of the animal's HPA axis activity and reactivity over a specific period of time (Touma and Palme 2005; Sheriff et al., 2011; Palme 2019). Here we measured FGM concentrations in different wild populations of invasive Eastern grey squirrel (*Sciurus carolinensis*) in Italy, to investigate the relationship between individual personality traits and hypothalamic-pituitary-adrenal (HPA) axis activity. Although catecholamines, also released from the adrenals, may further influence behavioural responses to environmental stimuli, we only measured the steroid hormone response in terms of changes in GCs. FGM was determined from fresh (< 3 hours old) faeces taken from trapped animals to ensure they reflected typical GC levels from about 12-24 hours before and were not influenced by trapping and handling (Bosson et al. 2013). We characterised the personality of squirrels along the proactive-reactive continuum through the open field test (OFT) and mirror image stimulation (MIS) test (Mazzamuto et al. 2018) and measured FGMs of individuals under different intrinsic (sex, reproductive condition, body mass) and environmental conditions (season, squirrel density). First, we determined which behavioural groups of those that we measured [activity, exploration, shyness (from OFT), sociability, avoidance, activity-exploration and alert (from MIS); Mazzamuto et al. 2018] were repeatable within an individual and thus could be used as personality traits to describe a proactive or reactive coping style. Based on the two-tier model (Koolhaas et al. 2010), we predicted that the personality traits would have significant repeatability (consistent differences among individuals) and would be correlated forming coping styles. Moreover, if FGM measures are a good indicator of individual variation in the GC stress response, they should also have some degree of repeatability, as previous studies have shown (Fanson and Biro 2018; Taff et al. 2018). We further tested whether squirrels that exhibit a reactive coping style (less active, exploratory, and sociable) also have higher FGMs, whereas those with a more proactive coping style (more active, exploratory, and sociable) have lower FGMs (Raulo and Dantzer 2018).

Materials and Methods

Study species

The Eastern grey squirrel (*Sciurus carolinensis*) is a North American tree squirrel species which has been introduced in Britain, Ireland and Italy (O'Teangana et al. 2000; Bertolino et al. 2014; Gurnell et al. 2015) where it negatively impacts native biodiversity, through interspecific competition for resources, disease-mediated competition, and damage to forestry (Gurnell et al. 2015; Romeo et al. 2015). Grey squirrels also increase levels of physiological stress (Santicchia et al. 2018a) and affect the expression of personality traits (Wauters et al. 2019) in co-occurring native red squirrels. Densities in natural habitats range from < 1 to > 5 squirrels/ha, but can be much higher in parks and urban woodlands (Koprowski 1994). Home ranges overlap extensively between males and females, and home range size tends to be negatively related with squirrel density (Koprowski 1994; Shuttleworth et al. 2016). The social structure of grey squirrel populations is stable and hierarchical, principally determined by the sex, age and relatedness, with older heavier animals dominant over smaller adults and subadults (Shuttleworth et al. 2016). This structure is mainly composed by kinship groups, which consist of mothers and their female offspring, and where agonistic behaviour are minimal and amicable behaviours between related individuals are common (Koprowski et al. 1994; Gurnell et al. 2001). Alien grey squirrels in Italy have a poor macroparasite fauna (parasite-release, Romeo et al. 2014), and the probability of infection by the dominant gastro-intestinal helminth and intensity of infection (worms/infected host) are related to the squirrel's personality (boldness-exploration tendency) and its body mass, respectively (Santicchia et al. 2019).

Study areas, trapping and handling squirrels

We trapped grey squirrels in five study areas in Piedmont, Northern Italy, between November 2014 and December 2016: BER (4.9 ha); PIO (2.6 ha); RS (5.9 ha); MOR (37 ha) and COM (3.2 ha). All areas are woodlands or parks with mature broadleaf trees, mainly oaks (*Quercus robur*, *Quercus petraea*), hornbeam (*Carpinus betulus*), lime (*Tilia cordata*) and black walnut (*Juglans nigra*), and few ornamental conifers, surrounded by agricultural landscapes. In each area, we carried out two (COM) to three (other areas) capture-mark-recapture (CMR) sessions, once every two months between Autumn and early Spring (November/December – March/April), lasting 4 to 5 days. Number of traps used varied slightly between sessions and/or study areas. A trapping session involved the use of 16 (PIO), 16 (RS), 17 (BER), 30 (COM), 48 (MOR) ground-placed Tomahawk traps (model 202, Tomahawk Live Trap Co., WI, USA) evenly spaced throughout the areas, with a fine mesh added underneath traps to prevent contamination between urine and faeces. Traps were checked two to three times/day to minimise time in trap and time since defecation (maximum 3 h). Each captured squirrel was individually marked using ear-tags, weighed (Pesola spring balance, ± 5g), measured (length of the right hind foot, ± 0.5 mm) and sexed (Wauters and Dhondt 1995; Gurnell et al. 2001). Reproductive status was defined as non-breeding (Nbr), post-oestrus and pregnant (Preg) or lactating (Lact), for females, and non-breeding with abdominal testes (Abd) or breeding with scrotal testes (Scr), for males. Details of the methods used to estimate grey squirrel population size are available in Supplementary Material (SM1, Table S1). Trapping and handling squirrels complied with current laws on animal research in Italy and were carried out with permit of the authorities for wildlife research and management of Turin and Cuneo Provinces (Respectively, D.D. 294-34626 of 2014 and Prot. n. 0002624 of 13/01/2014) and of the Italian Institute for Environmental Protection and Research (ISPRA). All of these procedures abided by ASM guidelines (Sikes and Gannon 2011).

Faeces collection, extraction of hormone metabolites and enzyme immunoassay

Faecal samples of trapped squirrels were collected from underneath the traps and stored in vials (for details see Dantzer et al. 2010; Santicchia et al. 2018a). We only used fresh faecal samples (< 3 hours) from squirrels that had not previously been trapped or handled within 72 h prior to capture to minimise effects of capture stress on FGMs (Dantzer et al. 2010; Bosson et al. 2013). Each faecal sample was classified as being taken in the morning (10.00 – 13.00h) or in the afternoon (15.00 – 18.00h) to account for potential variation in FGMs over the 24 h cycle (Palme 2019).

We used a 5α-pregnane-3β, 11β, 21-triol-20-one enzyme immunoassay (EIA) to measure FGMs (ng/g dry faeces; Touma et al. 2003; Dantzer et al. 2010; Santicchia et al. 2018a). This EIA detects glucocorticoid metabolites with a 5α-3β, 11β-diol structure (for cross-reactivity see Touma et al. 2003). Assay validation in this species showed how faecal samples collected from traps represent an integrated measure of cortisol produced ~16 hours before defecation (FGMs peak between 12 and 24 h after ACTH challenge, Bosson et al. 2013). Details of the EIA procedure and its validation for Eastern grey squirrels can be found elsewhere (Bosson et al. 2013). Samples were analysed in duplicate. We assayed 342 faecal samples of grey squirrels. Pools of grey squirrel faecal extracts were used as intra-assay controls at dilutions of 1:50 (~30% binding) and 1:400 (~70% binding). Average intra-assay coefficients of variation (CVs) were 8.7% and 14.8% respectively for pools diluted 1:50 and 1:400. Inter-assay CVs were estimated from standards of known concentration with a high (n = 25 plates, 12.4% binding) and low (n = 25 plates, 80.9% binding) concentration that had inter-assay CVs of 15.2% and 9.1%, respectively.

Personality measured with arena test

We performed 128 arena tests on a restricted sample of 83 individuals, from COM (n = 35, 14 males, 21 females) and MOR (n = 48, 22 males, 26 females). In 96 cases (41 males, 55 females) we also had FGM measures. Arena tests consisted of an open field test (OFT) to measure the expression of the personality traits activity, exploration and shyness in a novel environment, followed by a mirror image stimulation (MIS) test to determine the animal’s degree of sociability or avoidance, aggressiveness, and being alert towards a conspecific, as well as its tendency for expressing behaviours that define a combined activity-exploration trait (Mazzamuto et al. 2018; for details see Supplementary Material, SM2, Table S2). In this study, the OFT lasted 6 min and MIS 4 min.

Repeatability estimates of personality traits

On 37 individuals tested more than once (32 two times, 5 three times, *n* = 79 tests), we estimated repeatability of the expert-based personality traits (Mazzamuto et al. 2018) as the between-individual variation divided by the sum of the between-individual and residual variation, using Bayesian generalized linear mixed effects models based on a Markov Chain Monte Carlo algorithm with the R package *MCMCglmm* version 2.26 (Hadfield 2010). The personality-trait scores were square root transformed before analysis. Each model had a personality trait as dependent variable and study area, sex, and experiment order (first, second or third test) as fixed effects, and squirrel identity as random effect (SM3, Supplementary Material). Posterior distributions were based on 1050000 iterations with a burnin of 50000 iterations and thinning of 40, such that 25000 iterations were used to obtain point estimates and 95% credibility intervals. For the random effects and residual variation an inverse-gamma prior uninformative for the model was used (Wilson et al. 2010). We found moderate repeatabilities (R > 0.20; see also Bell et al. 2009) for activity from OFT and for sociability and activity-exploration tendency (referred to trait "other" in Mazzamuto et al. 2018) from MIS (details in Table S2 and in SM3, Supplementary Material), which were further used as personality traits in the MCMCglmm model below.

Analysis of personality-stress relationships

We applied a multivariate mixed model fitted in a Bayesian framework using the package *MCMCglmm* in R (Hadfield 2010). The three retained expert-based personality traits [activity from OFT, sociability and "other" (activity-exploration) from MIS] and FGM concentrations were used as dependent variables after standardisation (with zero mean and variance equal to 1), using a Gaussian residual error distribution. As repeated observations were present, individual was added as a random effect. For both the residual and between-individual variation, an unstructured variance-covariance matrix was modelled, allowing the estimation of correlations among the response variables (covariance divided by the square root of the product of the variances). Sex, arena test order, daytime (animal sampled in morning or afternoon), season (winter [December to March], spring-summer [April to August], or autumn [September to November]) were included as fixed effects (factors) and also the standardised continuous variables body mass and population density were added as fixed effects. Reproductive condition was added as a fixed effect for males (with two levels: non-breeding and breeding) and as a separate factor for females (with three levels: non-breeding, pregnant, lactating). Daytime and reproductive condition were added as fixed effect to account for potential differences in FGMs due to diel rhythm and reproductive activity in males and females (Goymann 2012; Dantzer et al. 2016; Palme 2019). The effects of daytime, reproductive condition and season were set to zero for the dependent variables activity, sociability and activity-exploration (hence estimating their relationship only with FGM concentrations) and the effect of arena test order was set to zero for FGM. Posterior distributions were based on 1050000 iterations with a burnin of 50000 iterations and thinning of 40, such that 25000 iterations were used to obtain point estimates and 95% credibility intervals. For the random effects and residual variation a parameter-expanded prior uninformative for the model was used (Houslay and Wilson 2017). Also, we applied the Gelman-Rubin statistic (Gelman and Rubin 1992) and Geweke diagnostic (Geweke 1992) which confirmed model consistency and convergence. Details of model script and output are provided in Supplementary Material, SM4. There were three samples with very high FGMs (66550, 68090 and 79675 ng/g dry faeces) but eliminating them from the dataset did not change model outputs (results not shown).

Results

Patterns of variation in FGM concentrations in grey squirrels

We used 340 samples of 193 different animals (mean samples/id = 1.76; range 1 to 4). FGM concentrations in grey squirrels were highly variable (mean ± SD = 12610 ± 10749; range: 1226 – 79675 ng/g faeces). Samples collected during the morning had lower FGM concentrations than samples collected during the afternoon (estimate $\beta = -0.28$, 95% CI = -0.49 to -0.06, pMCMC = 0.01). FGM varied seasonally, with higher values in autumn than in winter ($\beta = 0.52$, 95% CI = 0.15 to 0.88, pMCMC = 0.005), but no difference between autumn and spring-summer (spring-summer versus autumn $\beta = -0.40$, 95% CI = -0.88 to 0.10, pMCMC = 0.11; Fig. 1). There was no effect of grey squirrel population density on FGM ($\beta = -0.01$, 95% CI = -0.18 to 0.17, pMCMC = 0.92). Among females, changes in reproductive condition did not influence variation in FGM (see SM4), while among males, animals with abdominal testes (non-breeding) tended to have lower FGM concentrations than males with scrotal testes (breeding; $\beta = -0.32$, 95% CI = -0.64 to 0.00, pMCMC = 0.051; Fig. 2).

Relationship between FGM concentrations and personality

Activity measured during OFT (R = 0.43, 95% CI = 0.15 – 0.67) and activity-exploration tendency measured during MIS (R = 0.42, 95% CI = 0.17 – 0.71) were repeatable among multiple measures within the same individual. Also sociability (MIS) had moderate repeatability (R = 0.29, 95%CI = 0.004 – 0.54) (more details in Table S2). Hence, we retained these three behavioural groups as personality traits in our MCMCglmm model (SM4). Heavier grey squirrels were more active explorers than individuals with lower body mass ($\beta = 0.28$, 95% CI = 0.07 to 0.47, pMCMC = 0.006; SM4; Fig. 3) and squirrels expressed more activity-exploration during the MIS test when densities were higher ($\beta = 0.16$, 95% CI = 0.005 to 0.31, pMCMC = 0.047; SM4). Estimates of correlations between the three personality traits suggested that active individuals tended to be also more sociable and more explorative, although the credible intervals overlapped zero (Table 1 and SM4). The correlations of the three personality traits with FGMs were close to zero (Table 1) indicating the lack of an association between the physiological and behavioural stress response in this species. Activity had the largest between-individual variance and the smallest within-individual variance, indicating that an individual squirrel is consistent in its activity in the OFT but there is broad variation in activity among individuals in the populations (Table 1). In contrast, FGMs had the smallest between-individual and the largest within-individual variance (repeatability R = 0.05, 95% CI = 0.00 – 0.14), suggesting it fluctuates within a limited species-specific range, but each individual's FGM can vary strongly over most of that range (Table 1). The within-individual covariance estimates among the personality traits and FGM were small and their 95% CIs included 0 (Table 1).

Discussion

We showed that in free-ranging grey squirrels, open field test (OFT) and mirror image stimulation (MIS) test in an arena returned moderate within-individual repeatability for three personality traits: activity (OFT), sociability (MIS) and activity-exploration (MIS) tendency. Although active squirrels also tended to be more social and more explorative, suggesting a proactive coping style, correlations from the MCMglmm model did not exclude 0 from the 95% credibility intervals. Furthermore, we found that neither of the three personality traits co-varied with one measure (FGMs) of the physiological stress response in a wild mammal. This main result confirmed the findings from an earlier study on free-ranging North American red squirrels (*Tamiasciurus hudsonicus*) that variation in the behavioural response and variation in the physiological stress response are independent and not correlated (Westrick et al. 2019). Hence, our results do not fully support the two-tier hypothesis (Koolhaas et al. 2010), but suggest that under variable natural conditions individuals can express consistent behavioural responses that are independent from their physiological stress response. In other words, whether animals exhibit a more proactive (high activity, exploration, sociability) or a more reactive (low activity and/or exploration and less sociable) personality is not functionally related to low or high HPA axis reactivity.

General pattern of FGM concentration variation

FGM concentrations were significantly higher in autumn than in winter, with a non-significant difference between autumn and spring-summer. During the autumn grey squirrels are subject to an increase in intraspecific interactions due to competition for feeding and caching of high-energy tree seeds (Koprowski 1994). Moreover, autumn is also the period of juvenile/subadult dispersal, which may force resident adult males and female kin group to defend their core-areas to limit immigration (Koprowski 1994; Gurnell et al. 2001). This increase in social pressure and foraging activity could explain the observed seasonal differences in FGM concentrations, that were also reported in previous studies on grey squirrels (Bosson et al. 2013) and other tree squirrel species (*Sciurus vulgaris*, Dantzer et al. 2016).

Patterns of variation in FGMs with reproductive condition in grey squirrels were, albeit only partly, in agreement with findings of previous studies on sciurids (Montiglio et al. 2015; Dantzer et al. 2016). Males with scrotal testes (reproductively active) had on average higher FGMs than those with abdominal testes (no reproductive activity). Males with scrotal testes will engage in mating chases and compete intensively with other males for access to the oestrus female (Koprowski 1994). This high intra-specific contact and the many aggressive interactions among the competing males may result in the observed increase in FGMs (see also Santicchia et al. 2018a for *Sciurus vulgaris*). Among females there were no marked differences in FGMs between pregnant, lactating or non-breeding individuals, in contrast with findings on Eurasian red squirrels (Dantzer et al. 2016). Overall, differences in FGMs depending on reproductive condition could match a change in circulating hormones or, alternatively, metabolism or gut passage time modifications (Goymann 2012). Although these factors could also lead to sex differences in glucocorticoid levels (Touma et al. 2003; Palme 2019), we found no difference in mean FGMs between males and females, in agreement with previous studies on tree squirrels (Dantzer et al. 2010, 2016; Bosson et al. 2013; Santicchia et al. 2018a).

FGM concentrations of grey squirrels did not co-vary with changes in squirrel density. This result is in contrast with findings reported in studies on other rodents (deer mice, *Peromyscus maniculatus*, southern redbacked voles, *Clethrionomys gapperi*: Harper and Austad 2004; root vole, *Microtus oeconomus*: Bian et al. 2011; North American red squirrel, *Tamiasciurus hudsonicus*: Dantzer et al. 2013; Algerian mice, *Mus spretus*: Navarro-Castilla et al. 2017). We did not measure other potential sources of physiological stress that could differ among the study areas, such as anthropogenic disturbance (Wingfield 2013; Dantzer et al. 2014; Rehnus et al. 2014), differential predation pressure (Clinchy et al. 2013) or differences in parasite load (Raouf et al. 2006).

Lack of repeatability in FGM

In a recent meta-analysis on the repeatability of GC measures in vertebrates, Schoenemann and Bonier (2018) found 12 studies that used FGM as in integrated measure of GCs. Five of these were on wild mammals (4 sciurid species and 1 deer species). A previous study in our study population of Eurasian red squirrels reported high and significant repeatability for wild-caught but captive held animals on a 48-hour time span (n = 17, R = 0.52, 95% CI = 0.25 – 0.69). However, this significant repeatability was not confirmed in a larger dataset of wild-caught animals trapped over a much longer sampling interval (n = 82, R = 0.12, 95% CI = 0 – 0.45; Dantzer et al. 2016). The other four studies from Schoenemann and Bonier (2018) reported repeatabilities of GC measures ranging from 0.12 to 0.57, with the latter value over a short sampling interval (0 – 7 days). Hence, the repeatability in our study (R = 0.05, 95% CI = 0 – 0.14) was very low and comparable with those found by Dantzer et al (2016) for wild-caught Eurasian red squirrels and reported by Schoenemann and Bonier (2018) for yellow-bellied marmots (*Marmota flaviventris* R = 0.12).

Another meta-analysis by Fanson and Biro (2018) reported FGM repeatabilities from 16 studies on wild-caught mammals (13 species), with values ranging from 0 to 0.67.

In our study, the low repeatability was likely due to a combination of relatively low among-individual and high within-individual variation in FGM. If high within-individual variance is a concern, the study should control for as many sources of environmental and life-cycle variation as possible (Schoenemann and Bonier 2018). We addressed this by adding the effects of squirrel density, daytime, season, sex, reproductive condition and the animal's body mass in our model. Nevertheless, we must admit that the number of samples per individual was low, which tends to increase estimates of within-individual variance.

Relationship between physiological stress and personality

We did not find significant correlations between FGMs and the three personality traits that describe the proactive-reactive continuum. In a recent review on a wide variety of vertebrate species, only 46% of studies that measured personality and GCs found a negative relationship between stress responsiveness or glucocorticoid levels and personality as defined by the proactive and reactive profiles (Raulo and Dantzer 2018). Fifteen studies reported a lack of any correlation, as we found here. Also other studies on sciurid species reported a similar result that certain personality traits, mainly activity and exploration, were not related to glucocorticoid levels (Ferrari et al. 2013; Clary et al. 2014; Dosmann et al. 2015; Montiglio et al. 2015; Westrick et al. 2019). Also for sociability, measured as the individual's tendency to slowly approach or sit close to its mirror image, hence its willingness to engage in social contact, there was no relationship between FGMs and the expression of sociability. This can be explained by diverse factors that can influence how the tendency for an individual to behave more socially and their levels of GCs interact with one another (Creel et al. 2013). For example, behaviours related to the acquisition and maintenance of social status (rank) are likely to affect the degree of social stress and GC levels associated with that social status (Goymann and Wingfield 2004). Moreover, environmental factors like changes in resource availability or predator pressure might have different effects on low-ranked than on high-ranked individuals, affecting the social status – GC relationships (Creel et al. 2013; Dantzer et al. 2017). The grey squirrel has a social system intermediate between solitary and social group-living species (Koprowski 1994; Gurnell et al. 2001): males are solitary but with overlapping home ranges, while adult females tend to form female kin-groups (philopatric daughters). Females from a kin-group do not forage together but have strongly overlapping core-areas, they rarely interact aggressively and may share dreys or dens (Gurnell et al. 2001). However, so far it is unknown to what degree differences in social status in this species are correlated with the expression of sociability measured during MIS test; a relationship that should be investigated in future studies.

In this study, more active and exploratory squirrels had a higher body mass than less active/exploring animals. A similar positive association was found between a boldness-exploration score estimated with an indirect method (PCA score derived from trappability and trap-diversity indices) and body mass of grey squirrels in our study areas (Santicchia et al. 2019). Less active-explorative animals may be less efficient foragers and/or may be less likely to find high-quality food patches than more active-explorative ones, and this could produce a fitness advantage for phenotypes with high body mass and strong active-exploration tendency, at least under certain environmental conditions (Le Coeur et al. 2015; but see Santicchia et al. 2018b). In fact, a recent study on wild great tits (*Parus major*) demonstrated that an individual's morphological (body size, body condition) and behavioural traits represent an expression of an integrated phenotype and suggested that phenotypic integration can play a role in generating animal personalities (Moiron et al. 2019).

In conclusion, using FGMs as an integrated measure of physiological stress, we showed there was no significant association between the expression of personality traits and a physiological stress response in wild grey squirrels that live under (spatio-temporal) variable environmental conditions. However, it should be noted that the quantification of faecal glucocorticoid metabolite concentrations in wild mammals represents a mix of basal circulating GCs and stress-induced GCs and may not allow for direct measurements of the reactivity of the HPA axis, which may correlate more strongly with behavioural stress responses (Baugh et al. 2013; Westrick et al. 2019). Despite this caveat, our results confirm the findings of a growing number of studies that tested for co-varying behavioural and physiological stress responses in natural populations of free-ranging fish, birds and mammals (reviewed in Raulo and Dantzer 2018; and Table 1 in Westrick et al. 2019), but did not find a positive relationship between personality traits representing a reactive profile and high HPA axis reactivity. However, it must be noted that in some study species conflicting results have been found, depending on which particular personality trait was used (e.g. Clary et al. 2014) but also on the type of physiological measurement or on sample size (e.g. Baugh et al. 2013; Ferrari et al. 2013). For example, in Richardson's ground squirrels (*Urocitellus richardsonii*), there was a positive association between vigilance and FGMs, but no association between exploration and FGMs (Clary et al. 2014). In alpine marmots (*Marmota marmota*) there was a positive relationship between activity/exploration and blood cortisol in a small sample (n = 28), thus in the opposite direction than predicted by the two-tier model. However, in a larger sample (n = 146) there was no association between three personality traits (activity, impulsivity, docility) and blood cortisol (Ferrari et al. 2013). In great tits, there was a positive association between exploration and true baseline blood corticosterone, but a negative association between exploration and stress-induced blood corticosterone (measured after a 90 minutes handling-restraint, Baugh et al. 2013). To overcome some of these problems, we suggest that studies on wild animals exploring relationships between personality/behaviour and the physiological stress response should measure both FGM concentrations and the expression of several personality traits multiple times over a seasonal and/or annual time-period and determine the degree of between-individual as well as that of the within-individual variation. Combining measures of an individual's average HPA axis reactivity and its variability over time and relating those with measures of behavioural consistency and plasticity may allow us to discover associations between personality and stress response not documented so far.

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Author contributions

FS, LAW and NF designed the study and the analyses, and AM supervised the project. Fieldwork and data collection were done by FS, CR and LAW. FS carried out laboratory analyses and BD supplied laboratory space, equipment, and coordinated laboratory analyses. RP produced and supplied reagents for lab analyses. FS and SEW carried out statistical analyses with the contribution of CR, DGP and BD. The manuscript was drafted by FS and LAW; all other authors contributed to improve the manuscript and gave approval for publication.

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Table 1. Results of the multivariate MCMCglmm model. Between-individual and within-individual variances are listed on the diagonal (within-individual in italics), covariances below the diagonal (within-individual in italics), and correlations in bold above the diagonal (lower and upper bounds of 95% Credibility Intervals in brackets).

Parameter	Activity	Sociability	Activity/exploration	FGM
Activity	0.66 (0.29 – 1.06)	**0.40**	**0.19**	**-0.15**
	0.30 (0.12 – 0.54)	**(-0.12 – 0.88)**	**(-0.31 – 0.68)**	**(-0.88 – 0.63)**
Sociability	0.18 (-0.05 – 0.45)	0.33 (0.85^{-8} – 0.67)	**0.30**	**-0.16**
	0.03 (-0.17 – 0.27)	*0.78 (0.41 – 1.18)*	**(-0.32 – 0.88)**	**(-0.88 – 0.70)**
Activity/exploration	0.11 (-0.14 – 0.35)	0.12 (-0.08 – 0.36)	0.45 (0.36^{-7} – 0.79)	**0.02**
	0.09 (-0.09 – 0.30)	*0.12 (-0.11 – 0.37)*	*0.48 (0.20 – 0.84)*	**(-0.74 – 0.78)**
FGM	-0.03 (-0.18 – 0.10)	-0.02 (-0.15 – 0.08)	0.003 (-0.11 – 0.12)	0.04 (0.26^{-9} – 0.14)
	0.04 (-0.19 – 0.26)	*-0.14 (-0.42 – 0.13)*	*-0.02 (-0.27 – 0.22)*	*0.91 (0.75 – 1.07)*

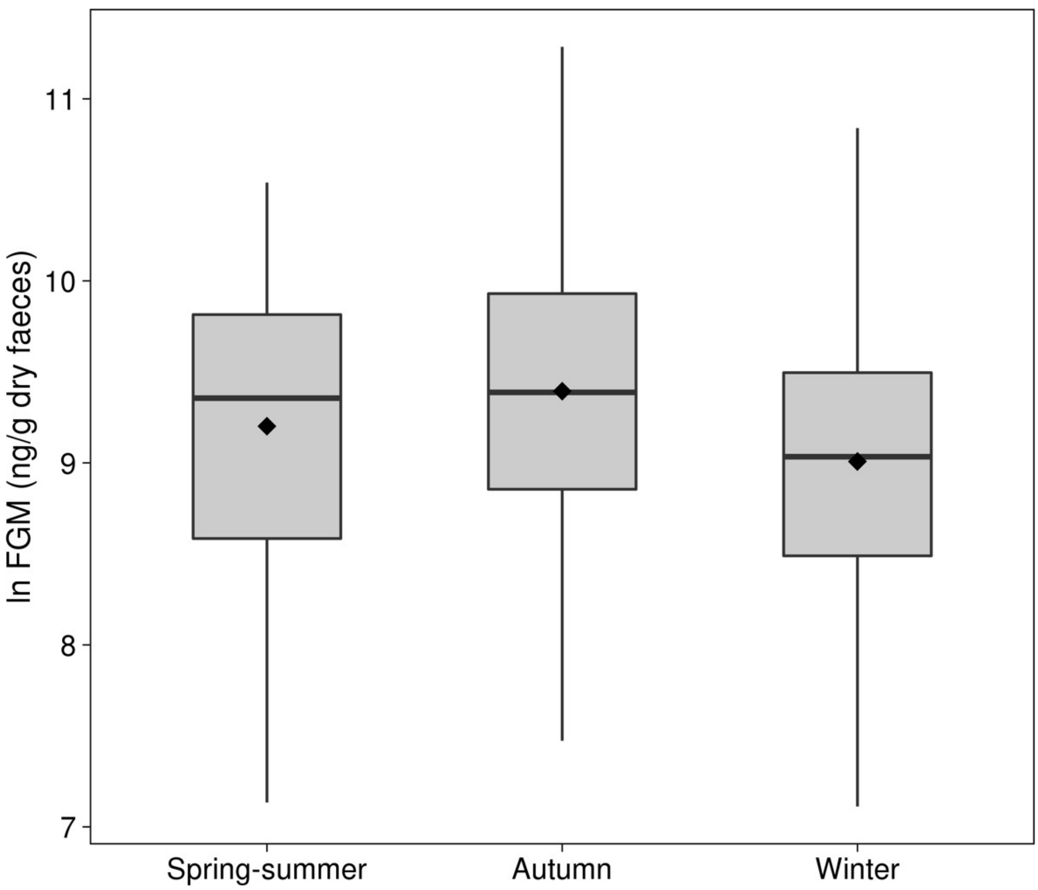

Figure 1. Faecal glucocorticoid metabolite (FGM) concentrations (ln transformed) in grey squirrels captured in spring-summer (*n* = 61), autumn (*n* = 91) or winter (*n* = 188). Boxplots show median (solid horizontal line), mean (black diamond), and 1st (25%) and 3rd (75%) quartiles.

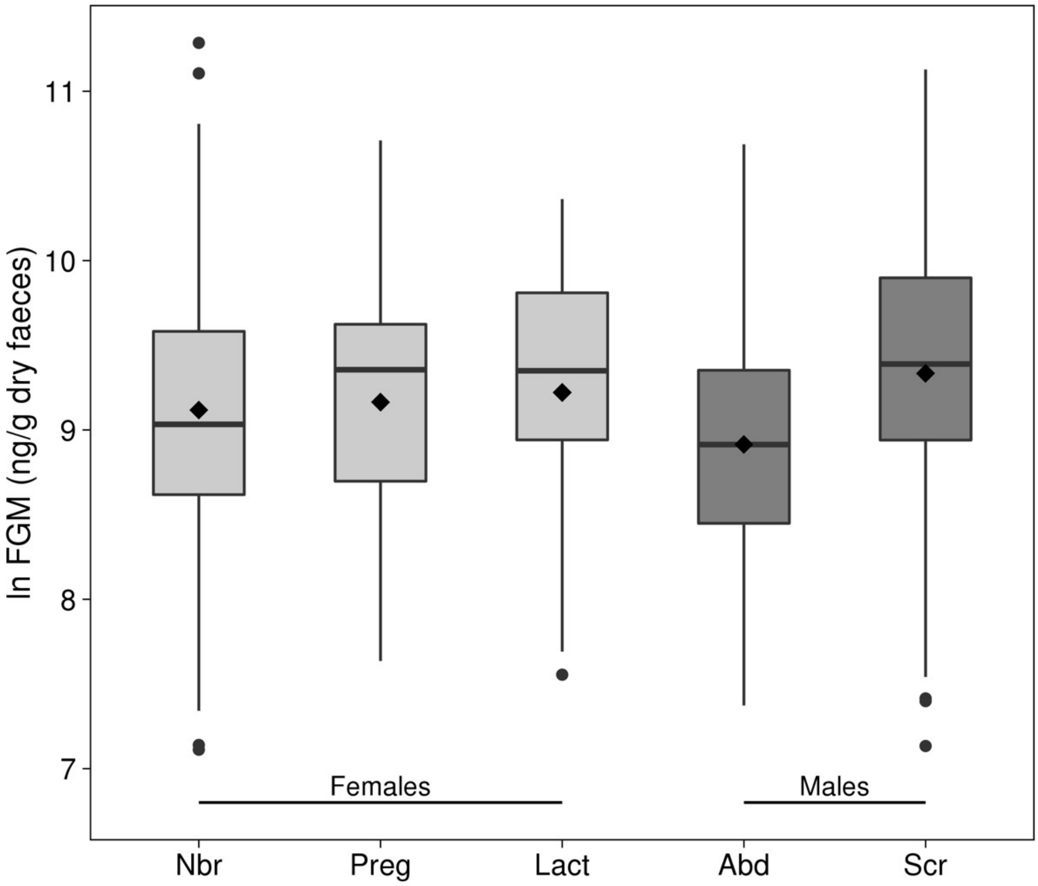

Figure 2. Faecal glucocorticoid metabolite (FGM) concentrations (ln transformed) in female and male grey squirrels in relationship to reproductive condition, defined as non-breeding (Nbr, *n* = 116), post-oestrus and pregnant (Preg, *n* = 39) or lactating (Lact, *n* = 26), for females; and non-breeding with abdominal testes (Abd, *n* = 70) or breeding with scrotal testes (Scr, *n* = 89), for males. Boxplots show median (solid horizontal line), mean (black diamond), and 1st (25%) and 3rd (75%) quartiles.

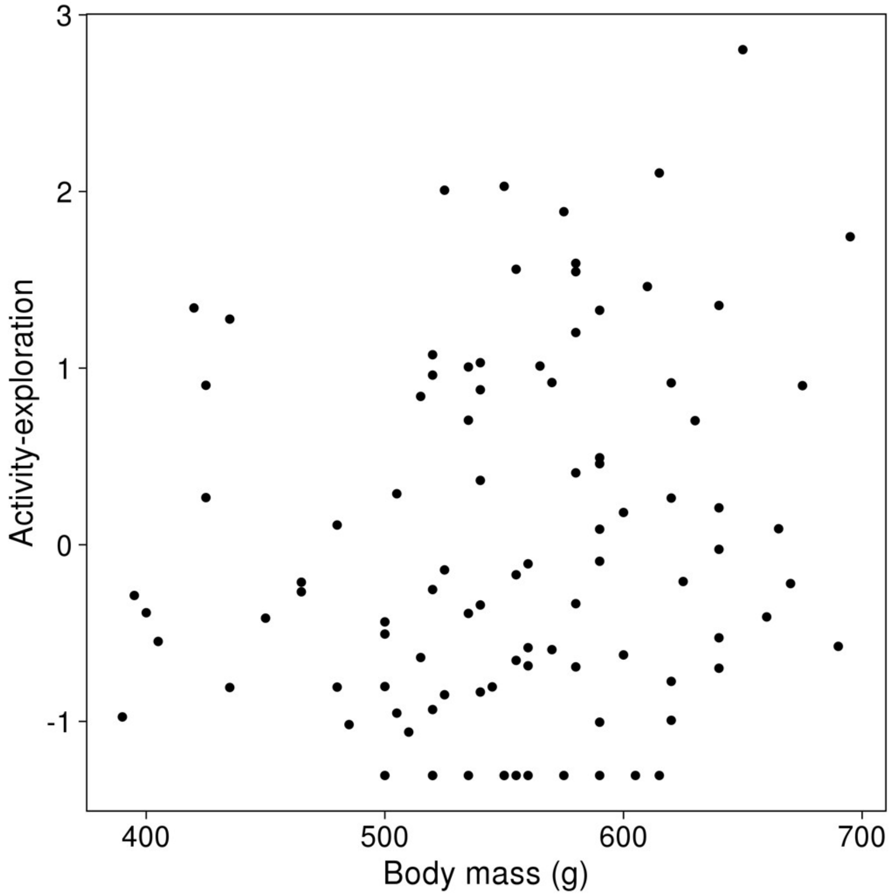

Figure 3. Association between grey squirrel body mass at time of capture and the standardised score of the personality trait activity-exploration measured during mirror image stimulation (MIS) test carried out after the capture event (*n* = 128, for details, see results MCMCglmm model).