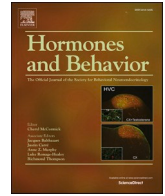


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Invasive alien species as an environmental stressor and its effects on coping style in a native competitor, the Eurasian red squirrel

Francesca Santicchia^{a,*}, Lucas Armand Wauters^{a,b}, Claudia Tranquillo^a, Federica Villa^a, Ben Dantzer^{c,d}, Rupert Palme^e, Damiano Preatoni^a, Adriano Martinoli^a

^a Environment Analysis and Management Unit, Guido Tosi Research Group, Department of Theoretical and Applied Sciences, Università degli Studi dell'Insubria, via J. H. Dunant 3, 21100 Varese, Italy

^b Evolutionary Ecology Group, Department of Biology, University of Antwerp, Campus Drie Eiken Universiteitsplein 1, 2610 Wilrijk, Belgium

^c Department of Psychology, University of Michigan, 530 Church Street, Ann Arbor, MI 48109, USA

^d Department of Ecology and Evolutionary Biology, University of Michigan, 830 North University, Ann Arbor, MI 48109, USA

^e Unit of Physiology, Pathophysiology and Experimental Endocrinology, Department of Biomedical Sciences, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria

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ABSTRACT

Free-living animals cope with environmental stressors through physiological and behavioural responses. According to the unidimensional model, these responses are integrated within a coping style: proactive individuals (bold, active-exploratory and social) have a lower hypothalamic–pituitary–adrenal (HPA) axis reactivity than reactive ones (shy, less active-exploratory, less social). These associations may change when individuals are exposed to human-induced rapid environmental change (HIREC), such as the introduction of invasive alien species (IAS). Here, we studied Eurasian red squirrels to investigate the relationship between personality traits and one integrated measure of HPA axis activity, both in areas uncolonized (natural populations) and colonized by an IAS, the Eastern grey squirrel (invaded populations). We expected an association between physiological and behavioural responses, and that activity, exploration and social tendency would covary, forming a behavioural syndrome in natural populations, while competition with the IAS was predicted to disrupt these associations. We used faecal glucocorticoid metabolites (FGMs) as an integrated measure of adrenocortical activity, and measured the levels of four personality traits (exploration, activity, activity-exploration and social tendency) with an open field test and a mirror image stimulation test. We found no correlation between FGMs and personality traits, neither in natural nor invaded populations. However, we found correlations among personality traits in areas without interspecific competition, indicating a behavioural syndrome, which was disrupted in invaded populations. This is one of the few studies showing that an IAS, acting as an environmental stressor, alters a native species' behavioural syndrome, but does not influence its coping style.

1. Introduction

Vertebrates react to environmental stressors, defined by [Boonstra \(2013\)](#) as unpredictable and/or noxious environmental stimuli which threaten (or are perceived to threaten) survival and/or homeostasis, using a suite of behavioural and physiological responses, which allow them to maintain, or return to homeostasis and reduce fitness loss ([Koolhaas et al., 1999, 2010](#); [Romero, 2004](#); [Clary et al., 2014](#)). Different individuals within a population often show consistent differences in these behavioural responses across a range of contexts and situations, which defines their personality ([Réale et al., 2007](#); [Carter](#)

[et al., 2013](#)). An animal's personality is described by various behavioural (or personality) traits, such as boldness (defined as an individual's propensity for risk-taking), activity, exploration (tendency to explore a new environment or object), aggressiveness, or sociability (towards conspecifics) (e.g. [Réale et al., 2007](#)). When some of these different personality traits are closely associated, they are considered to form a behavioural syndrome ([Réale et al., 2007](#); [Sih et al., 2004, 2012](#)). Similarly, individuals also vary in their physiological reactions to stressors and it has been postulated that behavioural and physiological responses are integrated within a so-called coping style (unidimensional model, [Koolhaas et al., 1999](#)).

* Corresponding author.

E-mail address: f.santicchia@uninsubria.it (F. Santicchia).

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Coping styles have been described to vary along a proactive-reactive continuum. Proactive animals actively deal with environmental stressors, usually have low hypothalamic–pituitary–adrenal (HPA) axis activity and present bold, active-explorative and more aggressive personalities (Koolhaas et al., 2010; Lendvai et al., 2011; Boulton et al., 2015; Santicchia et al., 2020a). In contrast, reactive individuals tend to be more shy, less active and less aggressive and usually have elevated HPA axis reactivity (Carere et al., 2010; Koolhaas et al., 1999, 2010; Costantini et al., 2012). The downstream products of the HPA axis are glucocorticoids (GCs: cortisol and/or corticosterone) released by the adrenal cortex and most studies assessing covariation between behaviour and HPA axis reactivity quantify GCs (e.g. van Kesteren et al., 2019; Westrick et al., 2021), although we emphasize that they are only one component of a complex process and quantify a limited part of physiological response to stressors (Breuner et al., 2013; Romero et al., 2015; MacDougall-Shackleton et al., 2019). According to the above unidimensional model, behavioural and physiological responses should change in an integrated manner with reactive individuals exhibiting increased GC concentrations compared to proactive ones. In other words, there should be strong among-individual covariation between personality types and the physiological stress response (potentially reflected in measures of GCs) and the latter should also be repeatable at the individual's level (Koolhaas et al., 1999; Carere et al., 2010; Boulton et al., 2015; Westrick et al., 2019).

The existence of personality traits and their association in a behavioural syndrome assumes that these traits have limited within-individual plasticity, which might preclude rapid adaptation when environmental changes favour only one of the traits but not the others (Sih et al., 2004; but see Trnka et al., 2018). This can become problematic, and, ultimately, might reduce the fitness of individuals, when new and rapid changes in the environment occur [e.g. Human-Induced Rapid Environmental Change, HIREC (Sih et al., 2012, Sih, 2013)]. For example, when a population of a native species becomes exposed to a novel environmental stressor, such as a competing invasive alien species (IAS), the average expression of certain personality traits might change and their within-species variation can be reduced (Sih, 2013; Wong and Candolin, 2015; Ruland and Jeschke, 2020). Consequently, behavioural syndromes that occurred in populations of the native species might be disrupted after colonization by the IAS. Moreover, recent exposure to an IAS can also alter the physiological stress response, resulting in higher GC concentrations (Narayan et al., 2015; Santicchia et al., 2018), which could influence the association between personality traits and the physiological stress response. A typical example of HIREC affecting native Eurasian red squirrels (*Sciurus vulgaris*) is the introduction and subsequent spread of the Eastern grey squirrel (*Sciurus carolinensis*). The latter is an IAS in Italy and the British Isles, and naturally occurs in North America (Gurnell et al., 2004; Bertolino et al., 2014; Shuttlesworth et al., 2016; McInnes et al., 2020).

Here, we studied six populations of Eurasian red squirrels in N. Italy, three living in areas without the invasive species (red-only sites) and three in areas colonized by grey squirrels, the invasive congener (red-grey sites) to investigate four hypotheses. (1) Under natural conditions (red-only sites), different personality traits, activity, exploration and social tendency (as an indicator of intraspecific interactions) strongly covary forming a behavioural syndrome. (2) In red-only populations, the proactive individuals (high levels of activity, exploration and social tendency) have reduced HPA axis activity resulting in lower production of GCs (unidimensional model, Koolhaas et al., 1999). In contrast, (3) when confronted with grey squirrels, that intensively compete for food resources (Wauters et al., 2002a, 2002b; Gurnell et al., 2004) and thus represent a strong environmental stressor (Dochtermann et al., 2012), all red squirrels, independent of their coping style, will show elevated GC concentrations (Santicchia et al., 2018), that will cause a disruption of the link between physiological and behavioural responses. Finally, (4) since native species may change their behaviour to minimize the impacts from invasive species (Ruland and Jeschke, 2020), we expect a

disruption of the behavioural syndrome in sites colonized by grey squirrels.

We used faecal glucocorticoid metabolite (FGM) concentrations as a non-invasive, integrated measure of both baseline and stress-induced GC levels, which has been used as a proxy of an animal's HPA axis activity over a specific period of time (Dantzer et al., 2016; Palme, 2019; Romeo et al., 2020; Santicchia et al., 2018, 2020a and references therein). FGM concentrations are of course only one measure in response to environmental changes, but they have proven to be a reliable biomarker of the physiological stress response of an individual that corresponds to their circulating GCs over a specific period of time (Sheriff et al., 2011; Palme, 2019). For example, compared to one-off measures of plasma GCs or even GCs in response to exposure to an acute stressor, FGM concentrations are a more repeatable measure of HPA axis activity (Taff et al., 2018). However, they reflect the situation a species-specific time before the sample is collected (Palme, 2019). Estimates of gut passage time from a closely related species (North American red squirrel) where FGMs increased 8–12 h after pharmaceutical stimulation (Dantzer et al., 2010) leads us to believe that our measures of FGM concentrations in this species reflect circulating GCs about 8–12 h before. We then estimated two Bayesian MCMCglmm models, one for each population type (red-only and red-grey), to obtain the population-type specific estimates of the correlations among the behavioural variables and FGMs, necessary to test our hypotheses. In natural (red-only) populations we expect positive correlations among the personality traits activity, exploration, and social tendency, and with FGMs. In invaded (red-grey) populations, we predict a lack of significant correlations among the personality traits and, based on our previous studies (Santicchia et al., 2018), higher FGMs with no association between individual variation in FGMs and the expression of the personality traits.

2. Materials and methods

2.1. Study sites, trapping and handling squirrels

We trapped Eurasian red squirrels in six study sites distributed in Lombardy and Piedmont (Northern Italy) between May 2016 and October 2019. Three of them (Bormio, Cancano and Valfurva) are red-only sites, uncolonized by the Eastern grey squirrel, while the other three (Castelbarco, Vanzago and Passatempo) are red-grey sites, where the native species co-occurs with the introduced species. Details on study sites habitat are reported in Appendix A2 and Table A.1. Capture-mark-recapture (CMR) sessions were carried out one to three times per year, each session lasted from 3 to 5 days and capture sessions were deliberately spaced out to include spring-summer (April–August), autumn (September–November) and winter (December–March). Each CMR session involved the use of 20 to 40 single capture live traps (model 202, Tomahawk Live Trap Co., Hazelhurst, WI, USA), with a fine mesh added underneath to prevent contamination between urine and faeces, placed on the ground or at breast height against tree trunks. Distance among traps arranged in each study site varied between 50 and 150 m. We pre-baited traps with hazelnuts three to four times over a 30 day period, then baited and set for capture session. Traps were activated and checked three times per day to reduce time squirrels were confined in a trap and to minimize time since defecation (max 3 h). The first check was done in late morning, to give the squirrels time to visit the traps, and the last check shortly before sunset to make sure that no animal remained in a trap overnight. Before handling, we completely covered the trap with a cloth to reduce the behavioural response to capture (the animal becomes quiet and stops moving around). After species identification, each captured red squirrel was driven into a cotton handling bag (zipper-tube; Wauters and Dhondt, 1989) to reduce direct contact with the operator and at first capture squirrels were marked using a numbered metal ear-tag (Monel 1005–1L1 ear-tag size 2.3–10 mm, 0.2 g or less than 0.1% of squirrel's body mass; National Band & Tag Co. Newport, KY, USA) putting the tag near the base of the ear to reduce risk

of injury. To reduce stress, only trained researchers handled the squirrels, and handling time was kept as short as possible (< 5 min). Body mass (g) was taken weighing each squirrel to nearest 5 g with a Pesola spring balance (Pesola AG, Baar, Switzerland), and the right hind foot length was measured to the nearest 0.5 mm with a thin ruler. Hind foot length is, a reliable proxy for a squirrel body size (Wauters et al., 2007). Sex and age class were determined from external genitalia and body mass, juvenile red squirrels weighing less than 250 g (Wauters and Dhondt, 1989) were excluded from our dataset. We checked also reproductive condition for each captured squirrel. We defined a female as nonbreeding (anestrous, vulva small, no longitudinal opening, not lactating), post-oestrous and pregnant (vulva partly or strongly swollen with longitudinal opening, enlarged abdomen during late pregnancy), or lactating (nipples large, milk excretion can be stimulated). Male's reproductive condition can be determined observing testes size and position; we classified males as nonbreeding (testes abdominal or semiscrotal with scrotum small) or breeding (testes scrotal and scrotum large) (Dantzer et al., 2016; Santicchia et al., 2018, 2020b). After each capture, the trap, mesh and the ground under the trap were cleaned to remove possible remains of faecal material.

2.2. Arena test personality measures

After checking a red squirrel's identity, we released it inside a portable arena by opening a sliding door and allowing the animal to move from the handling bag into the arena (Mazzamuto et al., 2019; Santicchia et al., 2021). We performed an open field test (OFT; 4 min) which serves to estimate activity, exploration and immobility in a novel environment followed by a mirror image stimulation test (MIS; 3 min) which allowed to determine an 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., 2019; Wauters et al., 2019; Santicchia et al., 2020b, 2021; details in Appendix, A3). At the end of the experiment, the squirrel was released by opening the sliding door and the arena was cleaned with 90% ethylic alcohol to eliminate possible urine or faeces and to avoid that the scent of the previous squirrel affects the behaviour of the next animal. For each experiment we calculated the time that individuals spent in each behavioural state (behaviours defined in Table A.3) using the CowLog 3.0.2 software (Hänninen and Pastell, 2009). To reduce the number of behaviours observed into few personality-linked variables we used the expert-based method (EB; Mazzamuto et al., 2019), which consists in a classification of behaviours into groups, summing the values of the single behaviours to obtain scores for few personality-linked variables, based on researchers' previous knowledge. This method was validated in previous studies on red squirrels (Mazzamuto et al., 2019; Santicchia et al., 2021). To check the assumptions of repeatability of the traits, squirrels were tested multiple times to have repeated measures (individuals tested multiple times: $n = 61$, mean \pm SE = 2.44 ± 0.10 , range = 2–5). Nevertheless, to increase the power of the test, also squirrels with only one estimate were included in the adjusted repeatability estimates (Martin et al., 2011; Santicchia et al., 2021).

2.3. Ethical note

Trapping, marking and handling of red squirrels and arena test experiments were carried out in accordance with the Guidelines for the treatment of animals in behavioural research and teaching (Anonymous, 2020, 159, I-XI; <https://doi.org/10.1016/j.anbehav.2019.11.002>). Approval and legal requirements according to the Italian Wildlife Protection and Hunting Law L.N. 157 from 1992 and authorizations N. 294–34,626 of 12/09/2014 (2014–2016) from the Provincia di Torino and N. 62–3025 (2017–2019) from the Città Metropolitana di Torino, and Decreto N. 11,190 (29/11/2013) and decrees N. 9523 of 15/10/2014 and N. 198 (13/01/2017) from Direzione Generale Agricoltura,

Regione Lombardia; and the permission Protocol N. 414 of 28/02/2014 of the Stelvio National Park.

2.4. Extraction and quantification of FGMs

FGM concentrations reflect the integrated average circulating GCs within an individual animal over some species-specific time period (Sheriff et al., 2011). Although GC are much more than only a stress hormone and FGMs are only one measure of HPA axis activity (MacDougall-Shackleton et al., 2019), they have proven to be a reliable biomarker of exposure of an animal to internal or external stressors. For example, in our study species, trapping and captivity (environmental stressor) causes a significant elevation in FGM concentrations (Dantzer et al., 2016), while another study on grey squirrels (*Sciurus carolinensis*) showed that animals with higher FGMs were responsible for the greatest endoparasite (*Strongyloides robustus*) egg shedding (Romeo et al., 2020). Like most species, the exact window of time that FGM concentrations reflect circulating GCs is not known with great certainty, but based upon our previous study (Dantzer et al., 2016) and a study in a similar species (North American red squirrels: Dantzer et al., 2010), it is likely that FGM concentrations reflect circulating GCs over several hours.

In this study, fresh faecal samples (<3 h) of trapped squirrels that had not previously been trapped or handled within 72 h prior to capture (see below), were collected from underneath the traps, stored dry at -20°C and classified as being taken in the morning (10.00–13.00 h) or in the afternoon (15.00–18.00 h) to account for potential variation in FGMs over the 24-h cycle (Palme, 2019; Romeo et al., 2020; Santicchia et al., 2020a). We used a 5α -pregnane- 3β , 11β , 21 -triol- 20 -one enzyme immunoassay (EIA) to measure FGM concentrations (ng/g dry faeces; Touma et al., 2003). This EIA detects GC metabolites with a 5α - 3β , 11β -diol structure (for cross-reactivity see Touma et al., 2003). Assay validation in this species showed that faecal samples collected from traps represent an integrated measure of glucocorticoids, with peak concentrations occurring 24 to 36 h after initial captivity (Dantzer et al., 2016). Hence, we sampled only squirrels that had not been trapped or handled within 72 h prior to capture to exclude effects of capture stress on FGM concentrations. Details of the EIA procedure and its validation for Eurasian red squirrels can be found elsewhere (Dantzer et al., 2016; Santicchia et al., 2018). We analyzed samples in duplicate. Intra-assay CVs were $8.4\% \pm 7.0\%$ (mean \pm SD). Pools of red squirrel faeces extracts were used as intra-assay controls at dilutions of 1:200 (~30% binding) and 1:1600 (~60% binding). Average inter-assay coefficients of variation (CVs) were 14.6% and 14.9%, respectively, for pools diluted 1:200 and 1:1600.

2.5. Repeatability of personality traits and FGM concentrations

We estimated the repeatability of square-root-transformed values of activity, exploration and immobility from OFT and sociability, avoidance, alert and activity-exploration from MIS (Table 1). Moreover, we also estimated the repeatability of social tendency, a new variable that combined avoidance and sociability scores (Pearson correlation coefficient sociability-avoidance: $r = -0.85$) which measures the tendency to react more or less social or amenable to the mirror image (Santicchia et al., 2020b). We calculated social tendency with the formula:

$$\text{Social tendency} = \log \left[\frac{(\text{sociability score} + (1 - \text{avoidance score}))}{2} \right]$$

Adjusted repeatability (R) was estimated using Bayesian generalized linear mixed effects models based on a Markov Chain Monte Carlo algorithm with the R package MCMCglmm version 2.29 (Hadfield, 2010), calculated as the between-individual variation divided by the sum of the between-individual and residual variation. For each model we used one expert-based personality trait as dependent variable and squirrel identity (ID) as random effect. We included site type (red only or red-grey), sex, year, arena test order (categorical factor: first [coded as 1] vs. subsequent ones [all coded as 0]), based on results from earlier studies

Table 1

Square-root-transformed values of the proportions of time spent (mean and SD) in behaviours related to each of the traits from OFT and MIS. Mean and SD of estimated social tendency (see ‘*Repeatability of personality traits and FGM concentrations*’) and mean and SD of faecal glucocorticoid metabolites concentrations (FGM; ng/g dry faeces). Data are presented for each site-type (red-only or red-grey). Adjusted repeatability (R) and its 95% Credibility Interval based on all data (site-types combined).

| Entire dataset (n = 257) | | Red-only (n = 164, ID = 107) | | Red-grey (n = 93, ID = 62) | | Repeatability | |
|--------------------------|----------------------|------------------------------|--------|----------------------------|--------|---------------|----------------|
| Test | Variable | Mean | SD | Mean | SD | R | 95% CI |
| OFT | Immobility | 0.73 | 0.18 | 0.66 | 0.28 | 0.26 | 0.02 to 0.46 |
| | Exploration | 0.21 | 0.10 | 0.21 | 0.13 | 0.12 | 0.03 to 0.31 |
| | Activity | 0.57 | 0.17 | 0.60 | 0.21 | 0.34 | 0.12 to 0.54 |
| | Sociability | 0.19 | 0.28 | 0.38 | 0.37 | 0.01 | 0.002 to 0.17 |
| | Avoidance | 0.68 | 0.31 | 0.52 | 0.38 | 0.01 | 0.001 to 0.09 |
| MIS | Activity-exploration | 0.37 | 0.20 | 0.41 | 0.21 | 0.40 | 0.15 to 0.60 |
| | Alert | 0.32 | 0.20 | 0.24 | 0.15 | 0.04 | 0.007 to 0.24 |
| | Social tendency | -1.98 | 1.15 | -1.44 | 1.33 | 0.001 | 0.0001 to 0.21 |
| | FGMs | 19,414 | 18,395 | 19,122 | 18,395 | 0.00 | 0.00 to 0.17 |

which showed that scores of arena test personality traits differed between the first arena and all the subsequent ones; Santicchia et al., 2020b, 2021) and number of days since the previous arena test as fixed effects. Arena test order and number of days from the previous test were included to account for habituation and temporal proximity between two tests (number of days since the previous arena test: mean ± SE = 132 ± 11, median = 54, range = 3–966).

We also estimated the adjusted repeatability of FGM concentrations included as a dependent variable in MCMCglmm model with ID as random effect. Site type, sex, squirrel density (calculated on a seasonal basis as minimum number alive, MNA/site size; details in Table A.1), season (Winter [December to March], Spring-Summer [April to August] or Autumn [September to November]) and daytime (faeces collected during the morning or afternoon) were added as fixed effects. Different fixed effects were used in this model compared to the models on personality traits because a different set of environmental factors is known to affect FGM values than those potentially affecting variation in personality traits.

For each model, posterior distributions were based on 1,050,000 iterations with a burn-in of 50,000 iterations and thinning of 40, such that 25,000 iterations were used to obtain point estimates and 95% credibility intervals (CIs). For the random effects and residual variation, an inverse-gamma prior uninformative for the model was used (Wilson et al., 2010). Also squirrels that had only one estimate for the arena test personality traits or FGM concentrations were included in the adjusted repeatability estimates (n = 169, F = 63, M = 106; Martin et al., 2011; Santicchia et al., 2021). Details about repeatability estimates on the restricted dataset (only individuals tested multiple times) are reported in Appendix, Table A.8. We applied the Gelman-Rubin statistic (Gelman and Rubin, 1992) and Geweke diagnostic (Geweke, 1992) which confirmed model consistency and convergence. Full model outputs are provided in Appendix, Tables A.4, A.5, A.6 and A.7.

2.6. Relationship between personality traits and FGM concentrations

Since we expected different relationships between personality and FGMs in relation to the presence/absence of the invasive species, we performed two models, one for each site type (red-only or red-grey), using Bayesian generalized linear mixed effects models based on a Markov Chain Monte Carlo algorithm (Hadfield, 2010). Data used in the models were related to observations with both arena test and FGM concentration measures (sample sizes in Table A.2). We then tested explicitly for any differences in the various correlations between personality traits (activity, exploration, activity-exploration), social tendency and FGM concentrations between the site types (red-only and red-grey) by comparing the slopes of the posterior distributions from the two separate models (Table 2).

Square root transformed values of activity, exploration, activity-exploration personality traits social tendency and FGM concentrations

Table 2

Differences between posterior slopes of the correlation estimates (see Fig. 2) based on the two MCMCglmm models (Differences = red-only – red-grey: mean ± SD and 95% CI). Differences are based on the correlations between the pairs of dependent variables in the first and second column.

| Dependent variables | Difference | |
|----------------------|----------------------|------------------------------|
| Activity | Exploration | 0.69 ± 0.57 (–0.37 to 1.70) |
| | Activity-exploration | 0.16 ± 0.39 (–0.40 to 1.26) |
| | Social tendency | 0.76 ± 0.56 (–0.17 to 1.78) |
| | FGMs | –0.25 ± 0.69 (–1.50 to 1.14) |
| Exploration | Activity-exploration | 0.73 ± 0.60 (–0.46 to 1.75) |
| | Social tendency | 0.67 ± 0.60 (–0.46 to 1.75) |
| | FGMs | –0.47 ± 0.66 (–1.63 to 0.92) |
| Activity-exploration | Social tendency | 0.66 ± 0.54 (–0.12 to 1.76) |
| | FGMs | –0.17 ± 0.68 (–1.46 to 1.23) |
| Social tendency | FGMs | 0.02 ± 0.69 (–1.34 to 1.41) |

were treated as dependent variables after centering and scaling [(x_i – mean x)/SD x] with a Gaussian residual error distribution. Even if not repeatable (see Table 1), social tendency was included in the models to have a measure of how social squirrels behaved during MIS test. As repeated observations were present, and to estimate both among-individual and within-individual variation of the dependent variables, individual (ID) was added as random effect. Sex, body mass (centered and scaled), squirrel density (centered and scaled; details in Table A.1), year, season (as defined above for the FGM repeatability model), daytime, female and male reproductive condition (as detailed above in ‘*Study sites, trapping and handling squirrels*’) were included as fixed effects. Details on seasonal densities are reported in Table A.1. In all models, the effect of arena test order was only estimated for activity, exploration, activity-exploration and social tendency, while the effect of daytime, female and male reproductive condition were estimated only for FGM concentrations. For both the residual and between-individual variation, an unstructured variance-covariance matrix was modelled, allowing the estimation of correlations among the dependent variables (covariance divided by the square root of the product of the variances). Posterior distributions were based on 2,100,000 iterations with a burn-in of 100,000 iterations and thinning of 300, with an effective sample of 6667 iterations. We used non-informative inverse Wishart prior (Tables A.9 e A.10; Houslay and Wilson, 2017). We applied the Gelman-Rubin statistic (Gelman and Rubin, 1992) and Geweke diagnostic (Geweke, 1992) which confirmed model consistency and convergence. Full model outputs are provided in Appendix, Tables A.9 and A.10. All the statistical analyses were carried out using the software R (R Core Team, 2021).

3. Results

We analyzed 164 faecal samples of 107 different squirrels (F = 37, M

= 70) present in red-only sites (Fig. 1) and 93 faecal samples of 62 individuals (F = 26, M = 36) captured in red-grey sites (Fig. 1) from 2016 to 2019 (for details see Table A.2). During OFT, squirrels engaged most of their time in activity or in staying immobile, while less time was spent exploring the arena. During MIS, many animals spend a lot of time avoiding their mirror image or in activity-exploration behaviours. Sociability behaviours were on average more common in the red-grey than in the red-only sites (Table 1), as found in earlier studies (Wauters et al., 2019).

We estimated the adjusted repeatability of all the seven expert-based traits and the new variable, social tendency. Immobility, exploration and activity, measured during the OFT and activity-exploration measured during the MIS had a poor to moderate repeatability (range: 0.12–0.40; see Table 1). Therefore, we considered exploration, activity and activity-exploration as personality traits in our MCMCglmm models (see ‘Relationship between personality traits and FGM concentrations’). Since immobility is strongly and negatively correlated with activity ($r = -0.91$; $n = 257$; $p < 0.0001$), this personality trait was not included in the models. In this dataset, alert, sociability or avoidance traits, or the social tendency score were not repeatable (Table 1). However, to have a variable measuring an animal’s tendency to behave more social, social tendency was included as dependent variable in the MCMCglmm. Finally, the adjusted repeatability of FGM was close to 0, hence FGMs were not repeatable.

3.1. Among- and within individual variance and behavioural syndrome

In the red-only sites ($n = 164$), all the posterior estimates of the correlations among the personality traits and of the traits with social tendency were significant, indicating a behavioural syndrome of active red squirrels being also more explorative and more social and showing

more active-explorative behaviour during MIS (Fig. 2). This was not the case in the red-grey sites ($n = 93$) where none of the personality traits, nor social tendency were correlated (all correlation estimates included 0 in their 95% CIs; Fig. 2). Either in the natural situation (red-only) or co-occurring with the invasive species (red-grey), FGM was not correlated with any of the personality traits or with social tendency (Fig. 2).

The among-individual variances of activity from OFT and of activity-exploration from MIS tended to be higher in the red-only than in the red-grey sites (Table 3a), although the 95% CIs of the estimated differences included 0 (−0.08 to 0.43 and −0.09 to 0.58, respectively). This suggests that more phenotypic variation in activity and activity-exploration is maintained in red squirrel populations without the invasive congener than in populations that co-exist with grey squirrels. In contrast, FGM values showed lower among-individual variation in the red-only than in the red-grey sites (Table 3a), but also here the 95% CIs of estimated difference included 0 (−0.64 to 0.15).

The within-individual variances were lower in red-only than in red-grey sites for exploration and social tendency (Table 3b) suggesting higher plasticity in these behaviours when the invasive species was present. In contrast, the within-individual variance of FGM tended to be higher in red-only than in red-grey sites, but the 95% CIs of estimated difference included 0 (−0.07 to 0.73).

3.2. Red-only sites: fixed effects

We found several significant relationships of fixed effects. Heavier red squirrels were significantly more explorative than individuals with lower body mass ($\beta = 0.22$, 95% CI = 0.06 to 0.36, $p_{\text{MCMC}} = 0.005$), and squirrels were more explorative ($\beta = -0.27$, 95% CI = −0.44 to −0.09, $p_{\text{MCMC}} = 0.003$) and active-explorative ($\beta = -0.32$, 95% CI = −0.54 to −0.12, $p_{\text{MCMC}} = 0.004$) when squirrel density decreased.

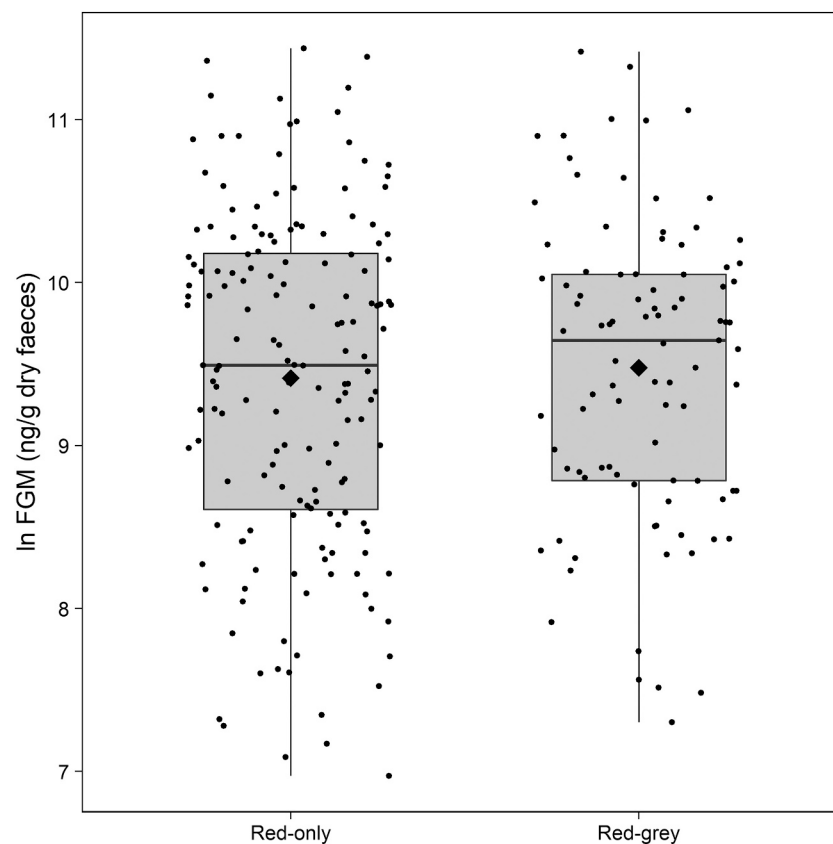


Fig. 1. Concentrations of faecal glucocorticoid metabolites (FGMs) in red-only [$n = 164$; ID = 107 (37 females, 70 males)] and red-grey [$n = 93$, ID = 62 (26 females, 36 males)] study sites. Boxplots show median (solid horizontal line), mean (black diamonds), and 1st (25%) and 3rd (75%) quartiles. Some data points shown represent samples collected from the same individuals.

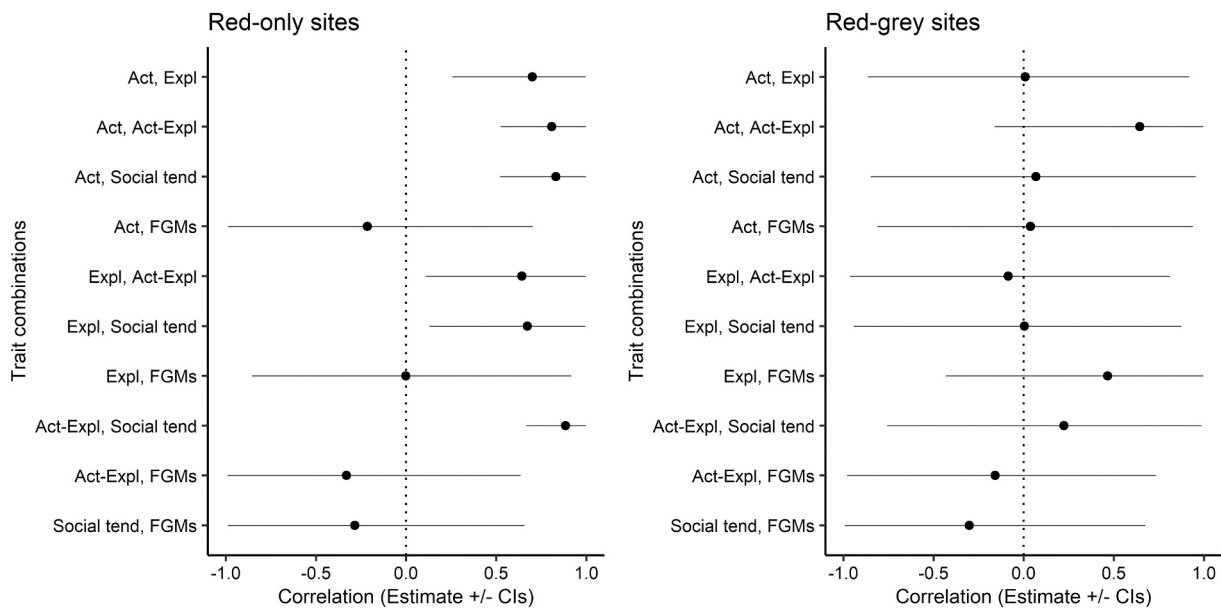


Fig. 2. Correlations (estimate ±95% credibility intervals) between the dependent variables derived from the two MCMCglmm models (by site-type: red-only or red-grey). Significant results (0 not included in the 95% CIs).

Table 3

Mean (95% CI) between-individual variances (a) and within-individual variances (b) and the differences in between-individual and within-individual variances of the dependent variables in the red-only and red-grey site-types (Differences = red-only – red-grey) based on the two MCMCglmm models (see ‘Relationship between personality traits and FGM concentrations’).

| Dependent variables | Red-only | Red-grey | Difference mean ± SD | Difference 95% CI |
|------------------------------|---------------------|---------------------|----------------------|-------------------|
| a) between-individual | | | | |
| Activity | 0.30 (0.11 to 0.52) | 0.11 (0.01 to 0.30) | 0.18 ± 0.13 | -0.08 to 0.43 |
| Exploration | 0.15 (0.03 to 0.32) | 0.17 (0.02 to 0.47) | -0.02 ± 0.14 | -0.34 to 0.22 |
| Activity-exploration | 0.37 (0.10 to 0.68) | 0.13 (0.01 to 0.35) | 0.24 ± 0.17 | -0.09 to 0.58 |
| Social tendency | 0.17 (0.03 to 0.37) | 0.21 (0.02 to 0.60) | -0.04 ± 0.18 | -0.46 to 0.25 |
| FGMs | 0.12 (0.01 to 0.35) | 0.32 (0.05 to 0.73) | -0.20 ± 0.20 | -0.64 to 0.15 |
| b) within-individual | | | | |
| Activity | 0.37 (0.25 to 0.54) | 0.45 (0.28 to 0.66) | -0.08 ± 0.12 | -0.32 to 0.16 |
| Exploration | 0.45 (0.31 to 0.61) | 0.84 (0.56 to 1.20) | -0.39 ± 0.18 | -0.77 to -0.07 |
| Activity-exploration | 0.51 (0.33 to 0.75) | 0.53 (0.33 to 0.76) | -0.02 ± 0.15 | -0.32 to 0.29 |
| Social tendency | 0.76 (0.59 to 0.98) | 0.93 (0.59 to 1.35) | -0.18 ± 0.22 | -0.62 to 0.23 |
| FGMs | 0.95 (0.70 to 1.22) | 0.59 (0.35 to 0.93) | 0.35 ± 0.20 | -0.07 to 0.73 |

During summer-spring, individuals were more explorative than in autumn ($\beta = 0.51$, 95% CI = 0.25 to 0.77, pMCMC = 0.0003). Activity and exploration were higher during the first than during subsequent arena tests (activity $\beta = 0.84$, 95% CI = 0.57 to 1.12, pMCMC < 0.0001; exploration $\beta = 0.29$, 95% CI = 0.01 to 0.54, pMCMC = 0.032). Also, social tendency varied with arena test order, levels of this trait were lower during the first MIS than the following ($\beta = -0.36$, 95% CI = -0.03 to -0.70, pMCMC = 0.034). Moreover, the expression of personality traits varied among years (Appendix, Table A.9). In this dataset (n = 164), there were no significant effects of sex, body mass, year, density, season, daytime and reproductive condition of males or females

on FGMs (Appendix, Table A.9).

3.3. Red-grey sites: fixed effects

In red-grey sites (n = 93), sex had an effect on social tendency, males were less social than females ($\beta = -0.54$, 95% CI = -1.04 to -0.02, pMCMC = 0.036). Similarly as in red-only sites, red squirrels showed higher activity ($\beta = -0.21$, 95% CI = -0.38 to -0.05, pMCMC = 0.014) and engaged more time in active-explorative behaviours ($\beta = -0.22$, 95% CI = -0.39 to -0.04, pMCMC = 0.016) at lower than at higher squirrel densities. A similar tendency was observed for exploration ($\beta = -0.21$, 95% CI = -0.42 to 0.03, pMCMC = 0.064). Furthermore, as for red-only sites, there were significant year effects (Appendix, Table A.10). Squirrels tended to spend more time in activity in the first than in subsequent arena tests ($\beta = 0.42$, 95% CI = -0.01 to 0.84, pMCMC = 0.059).

In contrast with red-only sites, when red squirrels co-occurred with the invasive competitor there were significant relationships between fixed effects and FGMs. Females ($\beta = 1.08$, 95% CI = 0.22 to 1.99, pMCMC = 0.015) and individuals with lower body mass ($\beta = -0.41$, 95% CI = -0.65 to -0.18, pMCMC = 0.0001) had higher levels of FGMs than, respectively, males and heavier squirrels. In addition, FGMs were lower in nonbreeding females than in pregnant and lactating ones ($\beta = -1.30$, 95% CI = -2.29 to -0.32, pMCMC = 0.010).

4. Discussion

According to the unidimensional model, personality traits and physiological stress responses will be integrated within divergent coping styles, which may represent opposite ends of a continuously varying reactive-proactive axis (Koolhaas et al., 1999; Boulton et al., 2015). Moreover, if this model is valid, both behavioural and stress response traits should be repeatable, and they should co-vary along a major axis of among-individual variation. In this study on Eurasian red squirrels, our integrated measure of the physiological stress response, faecal glucocorticoid metabolites (FGMs), was not repeatable and FGMs did not correlate with any of the behavioural and personality traits considered: activity and exploration measured during OFT; or a combined trait of active-explorative behaviour and a tendency to behave more social or amicable towards the mirror image, estimated from MIS

test. This finding is in contrast with our second hypothesis of a negative correlation between a proactive coping style (individuals with higher levels of activity, exploration and social or amicable behaviour towards the mirror image) and the concentration of FGMs under natural conditions, and thus gives no support to the unidimensional model (Koolhaas et al., 1999). Moreover, this lack of covariance between FGMs and personality traits was similar in red-only and in red-grey sites; hence it was not influenced by the occurrence of non-native grey squirrels, an invasive species that is considered an environmental stressor (Santicchia et al., 2018), and tends to outcompete the native red squirrel, causing (local) extinctions (Wauters et al., 2002a, 2002b; Gurnell et al., 2004; Bertolino et al., 2014; Romeo et al., 2021). However, lack of association between individual variation in FGM concentrations and variation in behavioural traits in the presence of the IAS was not caused by an elevation in FGMs in red squirrels co-occurring with the competitor, since, in contrast with our third hypothesis (elevated FGM concentrations will cause a disruption of the link between physiological and behavioural responses), and an earlier study (Santicchia et al., 2018), we did not find higher FGMs in red squirrels from red-grey than from red-only sites. Why we recorded lower average FGMs in red-grey sites in this study than in the earlier one (Santicchia et al., 2018) remains to be investigated. A tentative explanation could be that red squirrels undergo habituation of the stress response after a longer time of coexistence with the IAS (Cyr and Romero, 2009). Alternatively, GC release may be attenuated in time (due to physiological desensitization or to exhaustion), leading to think that the animal is no more chronically stressed. However, although the individual's stress response decreases with the exposure of the stressor, it could still exhibit health effects which demonstrate that there is no true habituation: such effects could be reproductive failure, decreased body condition, reduced survival, increased parasite load, or behavioural alterations (Cyr and Romero, 2009). In order to understand what in our study system caused the lower FGMs in 2016–2019 than those measured previously, more detailed analyses are needed.

Finally, in accordance with our first and fourth hypothesis, where we predicted that under natural conditions (red-only sites) different personality traits will strongly covary forming a behavioural syndrome, and that this behavioural syndrome will be disrupted in sites colonized by grey squirrels, there was a strong correlation among the personality traits in the red-only sites, but no such correlation was found where red squirrels had to compete with the invasive congener. Where effects of HIREC on the expression of personality traits and the occurrence of behavioural syndromes have been demonstrated for environmental changes such as urbanisation (Scales et al., 2011; Sol et al., 2013) and pollution (Royauté et al., 2014), there are few empirical studies showing that interspecific competition by an introduced species disrupts a behavioural syndrome in the native species (reviewed by Ruland and Jeschke, 2020).

4.1. Personality traits and FGM concentrations relationships

Contrary to many studies on free-living and captive animals (Raulo and Dantzer, 2018; Westrick et al., 2019), we did not find any association between red squirrels' FGM concentrations and their behavioural responses, independently from the presence of the invasive congener. Moreover, unlike a previous meta-analysis (Taff et al., 2018) and study in captive red squirrels (Dantzer et al., 2016), we found no significant repeatability of FGM concentrations in wild red squirrels, suggesting that the FGMs in individual red squirrels can vary strongly over time (see also Dantzer et al., 2016). In fact, our models showed that, at least in the red-grey sites, variation in FGMs was affected by a squirrel's sex and body mass, and, for females, by reproductive condition, and the within-individual variance in FGMs was large in both site-types (see Table 3). Hence, in red squirrels the expression of personality traits and the FGM concentrations are not consistent with a simple proactive-reactive axis of an integrated stress-coping style, indicating that these relationships

are more complex than previously suggested (Koolhaas et al., 1999; Lendvai et al., 2011; Boulton et al., 2015), and are consistent with a multi-dimensional interpretation of personality and the stress response in natural populations (Koolhaas et al., 2010; Clary et al., 2014; Westrick et al., 2019; Santicchia et al., 2020a).

Indeed, the unidimensional model (Koolhaas et al., 1999) was mainly based on studies of captive-held animals, most of which showed significant correlations between behaviour measures and the GC concentrations, varying along a reactive-proactive continuum. For example, in male wild house mice (*Mus musculus domesticus*), selected for short and long attack latency, the second line had a higher production of corticosterone after the application of the initial stressor (Veenema et al., 2003). In captive great tits (*Parus major*), fast-bold explorers and slow-shy explorers lines had a different HPA axis activation, indicating a correlation between behaviour and the physiological stress response (Baugh et al., 2012). Conversely, in natural populations, the correlation, or lack of it, between behavioural and physiological responses varies among species, and in a review on a wide variety of vertebrates, only 46% of studies on wild animals found an association between proactive coping type and lower GCs secretion (Raulo and Dantzer, 2018). In fact, already a decade ago, Koolhaas and colleagues proposed an alternative two-tier model, where behavioural responses are consistent for an individual and vary along the proactive-reactive continuum, but the physiological stress response is independent and varies along a different axis (two-tier model, Koolhaas et al., 2010). Several empirical studies supported this model. A recent study on territorial North American red squirrel (*Tamiasciurus hudsonicus*) showed that FGM concentrations, used as an integrated measure of the short-term physiological stress response, were not correlated with three personality traits (activity, aggressiveness and docility) measured in the arena test or during handling (Westrick et al., 2019). Also, a study on invasive Eastern grey squirrels (*S. carolinensis*) in Italy found similar results, where three personality traits (activity, sociability and activity-exploration) measured in the arena test were not related to FGM concentrations (Santicchia et al., 2020a). Furthermore, in the alpine marmot (*Marmota marmota*), there was no association between personality traits and plasma cortisol concentrations, a measure of acute physiological stress (Ferrari et al., 2013). In contrast, other studies on natural populations of sciurid rodents found some significant correlations, although the direction of the associations was not always the same (Costantini et al., 2012; Montiglio et al., 2012; Clary et al., 2014; Zhang et al., 2020). For example, in Richardson's ground squirrels (*Urocyon richardsonii*) there was a positive association between the personality trait vigilance and FGMs, in accordance with the unidimensional model (Clary et al., 2014). While, another study on the alpine marmot, found a positive correlation between activity-exploration and blood cortisol levels, but in the opposite direction than predicted by the model (Costantini et al., 2012). In conclusion, the type of stress measure used (e.g. blood cortisol, FGM, hair cortisol; Palme, 2019), the species' ecology, the influences of fluctuating environmental variables, and the occurrence of IAS are all factors that might influence the expression of an individual's behavioural and physiological responses, causing complex and highly variable associations (Killen et al., 2013; Hämäläinen et al., 2021; Westrick et al., 2021). Moreover, it must be underlined that FGM concentrations are an integrated measure of both baseline GCs levels and of short-time changes (increase or decrease) in GCs production due to, respectively, the upregulation or the downregulation of HPA axis dynamics. Therefore, FGMs may not be the best measure to look for an association between behavioural traits and the physiological stress response. Conducting experimental stress challenges that measure the ability of the HPA axis to mount as well as to terminate the GCs release (van Kesteren et al., 2019; Westrick et al., 2021) may have a higher likelihood to reveal associations with behavioural traits.

4.2. Effects of intrinsic and extrinsic factors on expression of personality traits

Heavier squirrels inhabiting red-only sites are more explorative than individuals with lower body mass. A similar positive association, generally with boldness or exploration, was found in different studies on tree squirrels (Eurasian red squirrel: Santicchia et al., 2021; Eastern grey squirrel: Santicchia et al., 2019, 2020a; Eastern chipmunk, *Tamias striatus*: Martin and Réale, 2008). Body mass is an indicator of body condition and is, at least partly, influenced by food availability (Wauters and Dhondt, 1989; Wauters et al., 2007). Hence, a possible explanation is that more explorative squirrels are more likely to find high-quality resources (Wolf et al., 2007; Wauters et al., 2021).

In both site-types, squirrels' behaviour was influenced by population density. At higher densities, squirrels were less explorative and active-explorative in red-only sites, and showed a lower activity, exploration (not significant) and activity-exploration in red-grey sites. Another study on red squirrels on Fota Island, Ireland, showed variation in animals' personality associated with differences in population density. Squirrels inhabiting higher density areas showed lower levels of aggression and activity (Haigh et al., 2017) than those occupying lower density areas. This could again be linked to more active-explorative squirrels being more capable of acquiring resources and may be advantaged in lower density sites in periods and/or areas of poorer habitat quality (Wauters et al., 2021). Similar results were documented for the Natal multimammate mouse (*Mastomys natalensis*) where changes in densities were strongly associated with variation in personality (Borremans et al., 2017; Vanden Broecke et al., 2019). In red-grey sites, females expressed a significantly higher social tendency than males. The new variable social tendency is determined by both sociability and avoidance scores (see social tendency formula). Hence, this result is in accordance with a previous study where female red squirrels exhibit less avoidance and higher sociability than males (Wauters et al., 2019).

4.3. Behavioural syndrome disruption

The study of personality traits and behavioural syndromes is important for evolutionary ecologists because: (i) an individual's personality and the expression of various personality traits linked in a behavioural syndrome can affect its fitness (e.g. Smith and Blumstein, 2008); and (ii) when the costs and benefits of different personality traits vary among different habitats, the existence of a behavioural syndrome can result in suboptimal behaviour in some environments (Sih et al., 2012). In the red-only sites, the behavioural traits activity, exploration (from OFT), a combined active-explorative trait and the tendency to behave more social and amicable towards the mirror image (from MIS), correlated in a behavioural syndrome, suggesting limited plasticity in the expression of these traits by red squirrels. In the red-grey sites, thus when the habitat occupied by the red squirrel population was invaded by the IAS, this syndrome no longer occurred. This was predicted in a conceptual model by Sih (2013) who regarded invasions by alien species a form of human-induced rapid environmental change (HIREC, Table 1 in Sih et al., 2012). In fact, HIREC can become strong and rapidly acting selective forces that challenge animals' ability to respond adaptively to how the environment is changing (Scales et al., 2011; Bókony et al., 2012; Royauté et al., 2014; Lapiedra et al., 2017). Consequently, they can produce a shift in behavioural variation among individuals within populations (Dochtermann and Dingemans, 2013; Royauté et al., 2014; Lapiedra et al., 2017), as well as in hormonal responses, such as the GC concentrations (Wingfield, 2013). Hence, the occurrence of an invasive alien species (IAS) could favour different personality types in a native species than those observed in areas where the invasive competitor is not yet present (Wauters et al., 2019). In fact, it has been demonstrated that, in some cases, IAS influence the behaviour of native species (Peck et al., 2014; Ruland and Jeschke, 2020) and the correlations between

traits, thus the behavioural syndrome (see Royauté et al., 2014 and references therein), (i) because certain traits of the syndrome are more advantageous where the IAS is present, but others not; or (ii) the effect of the IAS on the native species' expression of personality trait(s) is so severe that the syndrome is disrupted. This breakdown of behavioural correlations in the native species can enhance the invasive species' dominance in interspecific competition (Wauters et al., 2002a; Gurnell et al., 2004; Sih et al., 2012). Indeed, in this study the among individual variance in the traits activity and activity-exploration was higher for red squirrels in red-only sites than for those sharing the habitat with grey squirrels. This might result in reduced capacity for red squirrels to respond to changing environmental conditions when co-occurring with the invasive competitor. In contrast, the within-individual variation in exploration behaviour was significantly higher in red-grey than in red-only sites. Hence red squirrels that had to compete with grey squirrels had a more flexible expression of exploration in the open field test, which could be an advantage when searching for energy-rich food resources and/or high-quality patches in habitats shared with the dominant competitor.

Alternatively, lack of a previously existing behavioural syndrome in the sites with also grey squirrels, might allow these red squirrel populations to take other evolutionary trajectories than those available to allopatric red squirrels (Adriaenssens and Johnsson, 2013; Dochtermann and Dingemans, 2013; Lapiedra et al., 2017). However, since the outcome of competition between the two species is the replacement of native red by invasive grey squirrels in large parts of Great Britain and in North Italy (Gurnell et al., 2004, 2015; Bertolino et al., 2014), it is unlikely that behavioural adaptations in co-occurring red squirrels have any fitness benefits (see also Wauters and Gurnell, 1999; Gurnell et al., 2004; Wauters et al., 2019).

For the MCMCglmm model in red-grey sites, the 95% CIs of the posterior correlation estimates among the behavioural traits were very wide. This can be attributed to two aspects. No doubt, because correlations are much more difficult to estimate accurately, the smaller sample size in the red-grey than in the red-only sites caused the intervals to become relatively wide. In addition, the between-individual variation in the personality traits is, inherently, not very high. The estimated correlations are based on this between-individual variation and not the total variation, again leading to more wide intervals. In a previous study using a larger dataset for the same populations ($n = 152$ in Santicchia et al., 2020b, against the $n = 93$ in this study), a similar lack of association between the traits activity and social tendency and between activity-exploration and social tendency was found, and these estimates had narrower 95% CIs. Thus, our results are in line with previous findings.

5. Conclusions

This is one of the few studies investigating the impacts of an IAS, acting as an environmental stressor through interspecific competition, on changes in the expression of personality traits and the related behavioural syndrome in a native species (Sih, 2013; Ruland and Jeschke, 2020). Behavioural syndrome emergence and persistence has been highly debated, but there is still no clear understanding how they are maintained by natural selection (e.g. Adriaenssens and Johnsson, 2013; Dochtermann and Dingemans, 2013). Studying covariation among behavioural or personality traits and how it varies with environmental context (Killen et al., 2013), in this case the absence or presence of an IAS, could inform about the native species' potential for adaptation under the global widespread problem of IAS introductions. Furthermore, investigating physiological responses in wild populations may be more complex as previously identified. Future studies using multiple stress response measures and their integration with behavioural traits will enhance our understanding of the mechanisms involved in the interspecific competition between invasive and native vertebrate species.

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CRediT authorship contribution statement

FS and LAW developed the hypotheses and study design; CT, FV, LAW and FS collected data. BD carried out laboratory analyses, supplied laboratory space, equipment, and coordinated laboratory analyses. RP produced and supplied reagents for lab analyses. FS carried out statistical analyses with the contribution of CT. The manuscript was drafted by FS, LAW, CT and FV. AM, DP, BD and RP contributed critically to the drafts. All authors gave final approval for publication.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

- Adriaenssens, B., Johnsson, J.I., 2013. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. *Ecol. Lett.* 16, 47–55. <https://doi.org/10.1111/ele.12011>.
- Baugh, A.T., Schaper, S.V., Hau, M., Cockrem, J.F., de Goede, P., van Oers, K., 2012. Corticosterone responses differ between lines of great tits (*Parus major*) selected for divergent personalities. *Gen. Comp. Endocrinol.* 175, 488–494. <https://doi.org/10.1016/j.ygcen.2011.12.012>.
- Bertolino, S., di Montezemolo, N.C., Preatoni, D.G., Wauters, L.A., Martinoli, A., 2014. A grey future for Europe: *Sciurus carolinensis* is replacing native red squirrels in Italy. *Biol. Invasions* 16, 53–62. <https://doi.org/10.1007/s10530-013-0502-3>.
- Bókony, V., Kulcsár, A., Tóth, Z., Líker, A., 2012. Personality traits and behavioral syndromes in differently urbanized populations of house sparrows (*Passer domesticus*). *PLoS ONE* 7, e36639. <https://doi.org/10.1371/journal.pone.0036639>.
- Boonstra, R., 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Funct. Ecol.* 27, 11–23. <https://doi.org/10.1111/1365-2435.12008>.
- Borremans, B., Reijnders, J., Hughes, N.K., Godfrey, S.S., Gryseels, S., Makundi, R.H., Leirs, H., 2017. Nonlinear scaling of foraging contacts with rodent population density. *Oikos* 126, 792–800. <https://doi.org/10.1111/oik.03623>.
- Boulton, K., Couto, E., Grimmer, A.J., Earley, R.L., Canario, A.V.M., Wilson, A.J., Walling, C.A., 2015. How integrated are behavioral and endocrine stress response traits? A repeated measures approach to testing the stress-coping style model. *Ecol. Evol.* 5, 618–633. <https://doi.org/10.1002/ece3.1395>.
- Breuner, C.W., Delehanty, B., Boonstra, R., 2013. Evaluating stress in natural populations of vertebrates: total CORT is not good enough. *Funct. Ecol.* 27, 24–36. <https://doi.org/10.1111/1365-2435.12016>.
- Carere, C., Caramaschi, D., Fawcett, T.W., 2010. Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses. *Curr. Zool.* 56, 728–740. <https://doi.org/10.1093/czoolo/56.6.728>.
- Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlishaw, G., Heinsohn, R., 2013. Animal personality: what are behavioural ecologists measuring? *Biol. Rev.* 88, 465–475. <https://doi.org/10.1111/brv.12007>.
- Clary, D., Skyner, L.J., Ryan, C.P., Gardiner, L.E., Anderson, W.G., Hare, J.F., 2014. Shyness-boldness, but not exploration, predicts glucocorticoid stress response in Richardson's ground squirrels (*Urociellus richardsonii*). *Ethology* 120, 1101–1109. <https://doi.org/10.1111/eth.12283>.
- Costantini, D., Ferrari, C., Pasquarea, C., Cavallone, E., Carere, C., von Hardenberg, A., Reale, D., 2012. Interplay between plasma oxidative status, cortisol and coping styles in wild alpine marmots, *Marmota marmota*. *J. Exp. Biol.* 215, 374–383. <https://doi.org/10.1242/jeb.062034>.
- Cyr, N.E., Romero, L.M., 2009. Identifying hormonal habituation in field studies of stress. *Gen. Comp. Endocrinol.* 161, 295–303. <https://doi.org/10.1016/j.ygcen.2009.02.001>.
- Dantzer, B., McAdam, A.G., Palme, R., Fletcher, Q.E., Boutin, S., Humphries, M.M., Boonstra, R., 2010. Fecal cortisol metabolite levels in free-ranging north american red squirrels: assay validation and the effects of reproductive condition. *Gen. Comp. Endocrinol.* 167, 279–286. <https://doi.org/10.1016/j.ygcen.2010.03.024>.
- Dantzer, B., Santicchia, F., van Kesteren, F., Palme, R., Martinoli, A., Wauters, L.A., 2016. Measurement of fecal glucocorticoid metabolite levels in eurasian red squirrels (*Sciurus vulgaris*): effects of captivity, sex, reproductive condition, and season. *J. Mammal.* 97, 1385–1398. <https://doi.org/10.1093/jmammal/gyw095>.
- Dochtermann, N.A., Dingemans, N.J., 2013. Behavioral syndromes as evolutionary constraints. *Behav. Ecol.* 24, 806–811. <https://doi.org/10.1093/beheco/art002>.
- Dochtermann, N.A., Jenkins, S.H., Swartz, M.J., Hargett, A.C., 2012. The roles of competition and environmental heterogeneity in the maintenance of behavioral variation and covariation. *Ecology* 93, 1330–1339. <https://doi.org/10.1890/11-1025.1>.
- Ferrari, C., Pasquarea, C., Carere, C., Cavallone, E., von Hardenberg, A., Réale, D., 2013. Testing for the presence of coping styles in a wild mammal. *Anim. Behav.* 85, 1385–1396. <https://doi.org/10.1016/j.anbehav.2013.03.030>.
- Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences. *Stat. Sci.* 7, 457–472. <https://doi.org/10.1214/ss/1177011136>.
- Geweke, J., 1992. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. In: Bernardo, J.M., Berger, A.P., Dawid, A.P., Smith, A.F.M. (Eds.), *Bayesian Statistics*. Oxford University Press, Oxford, pp. 169–193. <https://doi.org/10.21034/sr.148>.
- Gurnell, J., Wauters, L.A., Lurz, P.W.W., Tosi, G., 2004. Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. *J. Anim. Ecol.* 73, 26–35. <https://doi.org/10.1111/j.1365-2656.2004.00791.x>.
- Gurnell, J., Lurz, P.W.W., Wauters, L.A., 2015. Years of interactions and conflict in Europe: competition between Eurasian red squirrels and North American grey squirrels. In: Shuttleworth, C.R., Lurz, P.W.W., Hayward, M.W. (Eds.), *Red Squirrels: Ecology, Conservation & Management in Europe*. ESI, UK, pp. 19–37.
- Hadfield, J.D., 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Soft.* 33, 1–22. <http://www.jstatsoft.org/v33/i02/>.
- Haigh, A., O'Riordan, R., Butler, F., 2017. Variations in aggression and activity levels amongst squirrels inhabiting low and high density areas. *Ecol. Res.* 32, 931–941. <https://doi.org/10.1007/s11284-017-1506-8>.
- Hämäläinen, A.M., Guenther, A., Patrick, S.C., Schuett, W., 2021. Environmental effects on the covariation among pace-of-life traits. *Ethology* 127, 32–44. <https://doi.org/10.1111/eth.13098>.
- Hänninen, L., Pastell, M., 2009. CowLog: open-source software for coding behaviors from digital video. *Behav. Res. Methods* 41, 472–476. <https://doi.org/10.3758/BRM.41.2.472>.
- Houslay, T.M., Wilson, A.J., 2017. Avoiding the misuse of BLUP in behavioural ecology. *Behav. Ecol.* 28, 948–952. <https://doi.org/10.1093/beheco/axx023>.
- van Kesteren, F., Delehanty, B., Westrick, S.E., Palme, R., Boonstra, R., Lane, J.E., Boutin, S., McAdam, A.G., Dantzer, B., 2019. Experimental increases in glucocorticoids alter function of the HPA axis in wild red squirrels without negatively impacting survival and reproduction. *Physiol. Biochem. Zool.* 92, 445–458. <https://doi.org/10.1086/705121>.
- Killen, S.S., Marras, S., Metcalfe, N.B., McKenzie, D.J., Domenici, P., 2013. Environmental stressors alter relationships between physiology and behaviour. *Trends Ecol. Evol.* 28, 651–658. <https://doi.org/10.1016/j.tree.2013.05.005>.
- Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. *Neurosci. Biobehav. Rev.* 23, 925–935. [https://doi.org/10.1016/S0149-7634\(99\)00026-3](https://doi.org/10.1016/S0149-7634(99)00026-3).
- Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Front. Neuroendocrinol.* 31, 307–321. <https://doi.org/10.1016/j.yfrne.2010.04.001>.
- Lapidra, O., Chejanovski, Z., Kolbe, J.J., 2017. Urbanization and biological invasion shape animal personalities. *Glob. Chang. Biol.* 23, 592–603. <https://doi.org/10.1111/gcb.13395>.
- Lendvai, Á.Z., Bókony, V., Chastel, O., 2011. Coping with novelty and stress in free-living house sparrows. *J. Exp. Biol.* 214, 821–828. <https://doi.org/10.1242/jeb.047712>.
- MacDougall-Shackleton, S.A., Bonier, F., Romero, L.M., Moore, I.T., 2019. Glucocorticoids and "stress" are not synonymous. *Integr. Org. Biol.* 1, obz017. <https://doi.org/10.1093/iob/obz017>.

- Martin, J.G.A., Réale, D., 2008. Animal temperament and human disturbance: implications for the response of wildlife to tourism. *Behav. Process.* 77, 66–72. <https://doi.org/10.1016/j.beproc.2007.06.004>.
- Martin, J.G.A., Nussey, D.H., Wilson, A.J., Réale, D., 2011. Measuring individual differences in reaction norms in field and experimental studies: a power analysis of random regression models. *Methods Ecol. Evol.* 2, 362–374. <https://doi.org/10.1111/j.2041-210X.2010.00084.x>.
- Mazzamuto, M.V., Cremonesi, G., Santicchia, F., Preatoni, D., Martinoli, A., Wauters, L.A., 2019. Rodents in the arena: a critical evaluation of methods measuring personality traits. *Ethol. Ecol. Evol.* 31, 38–58. <https://doi.org/10.1080/03949370.2018.1488768>.
- McInnes, C.J., Shuttleworth, C.M., Larsen, K.W., Everest, D.J., Bruemmer, C., Carroll, B., Romeo, C., Sainsbury, T., Crawshaw, G., Dubois, S., Gillis, L., Gilray, J., Percival, A., 2020. Introduced Canadian eastern grey squirrels: squirrelpox virus surveillance and why nothing matters. *Hystrix It. J. Mamm.* 31, 95–98. <https://doi.org/10.4404/hystrix-00331-2020>.
- Montiglio, P.-O., Garant, D., Pelletier, F., Réale, D., 2012. Personality differences are related to long-term stress reactivity in a population of wild eastern chipmunks, *Tamias striatus*. *Anim. Behav.* 84, 1071–1079. <https://doi.org/10.1016/j.anbehav.2012.08.010>.
- Narayan, E.J., Jessop, T.S., Hero, J.-M., 2015. Invasive cane toad triggers chronic physiological stress and decreased reproductive success in an island endemic. *Funct. Ecol.* 29, 1435–1444. <https://doi.org/10.1111/1365-2435.12446>.
- Palme, R., 2019. Non-invasive measurement of glucocorticoids: advances and problems. *Physiol. Behav.* 199, 229–243. <https://doi.org/10.1016/j.physbeh.2018.11.021>.
- Peck, H.L., Pringle, H.E., Marshall, H.H., Owens, I.P.F., Lord, A.M., 2014. Experimental evidence of impacts of an invasive parakeet on foraging behavior of native birds. *Behav. Ecol.* 25, 582–590. <https://doi.org/10.1093/beheco/aru025>.
- R Core Team, 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Raulo, A., Dantzer, B., 2018. Associations between glucocorticoids and sociality across a continuum of vertebrate social behavior. *Ecol. Evol.* 8, 7697–7716. <https://doi.org/10.1002/ece3.4059>.
- Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. *Biol. Rev.* 82, 291–318. <https://doi.org/10.1111/j.1469-185X.2007.00010.x>.
- Romeo, C., Wauters, L.A., Santicchia, F., Dantzer, B., Palme, R., Martinoli, A., Ferrari, N., 2020. Complex relationships between physiological stress and endoparasite infections in natural populations. *Curr. Zool.* 66, 449–457. <https://doi.org/10.1093/cz/zoa029>.
- Romeo, C., Piscitelli, A.P., Santicchia, F., Martinoli, A., Ferrari, N., Wauters, L.A., 2021. Invading parasites: spillover of an alien nematode reduces survival in a native species. *Biol. Invasions.* <https://doi.org/10.1007/s10530-021-02611-7>.
- Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol. Evol.* 19, 249–255. <https://doi.org/10.1016/j.tree.2004.03.008>.
- Romero, L.M., Platts, S.H., Schoech, S.J., Wada, H., Crespi, E., Martin, L.B., Buck, C.L., 2015. Understanding stress in the healthy animal – potential paths for progress. *Stress* 18, 491–497. <https://doi.org/10.3109/10253890.2015.1073255>.
- Royauté, R., Buddle, C.M., Vincent, C., 2014. Interpopulation variations in behavioral syndromes of a jumping spider from insecticide-treated and insecticide-free orchards. *Ethology* 120, 127–139. <https://doi.org/10.1111/eth.12185>.
- Ruland, F., Jeschke, J.M., 2020. How biological invasions affect animal behaviour: a global, cross-taxonomic analysis. *J. Anim. Ecol.* 89, 2531–2541. <https://doi.org/10.1111/1365-2656.13306>.
- Santicchia, F., Dantzer, B., van Kesteren, F., Palme, R., Martinoli, A., Ferrari, N., Wauters, L.A., 2018. Stress in biological invasions: introduced invasive grey squirrels increase physiological stress in native Eurasian red squirrels. *J. Anim. Ecol.* 87, 1342–1352. <https://doi.org/10.1111/1365-2656.12853>.
- Santicchia, F., Romeo, C., Ferrari, N., Matthysen, E., Vanlauwe, L., Wauters, L.A., Martinoli, A., 2019. The price of being bold? Relationship between personality and endoparasitic infection in a tree squirrel. *Mamm. Biol.* 97, 1–8. <https://doi.org/10.1016/j.mambio.2019.04.007>.
- Santicchia, F., Wauters, L.A., Dantzer, B., Westrick, S.E., Ferrari, N., Romeo, C., Palme, R., Preatoni, D.G., Martinoli, A., 2020a. Relationships between personality traits and the physiological stress response in a wild mammal. *Curr. Zool.* 66, 197–204. <https://doi.org/10.1093/cz/zoz040>.
- Santicchia, F., Wauters, L.A., Piscitelli, A.P., Van Dongen, S., Martinoli, A., Preatoni, D., Romeo, C., Ferrari, N., 2020b. Spillover of an alien parasite reduces expression of costly behaviour in native host species. *J. Anim. Ecol.* 89, 1559–1569. <https://doi.org/10.1111/1365-2656.13219>.
- Santicchia, F., Van Dongen, S., Martinoli, A., Preatoni, D., Wauters, L.A., 2021. Measuring personality traits in Eurasian red squirrels: a critical comparison of different methods. *Ethology* 127, 187–201. <https://doi.org/10.1111/eth.13117>.
- Scales, J., Hyman, J., Hughes, M., 2011. Behavioral syndromes break down in urban song sparrow populations. *Ethology* 117, 887–895. <https://doi.org/10.1111/j.1439-0310.2011.01943.x>.
- Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166, 869–887. <https://doi.org/10.1007/s00442-011-1943-y>.
- Shuttleworth, C.M., Lurz, P.W.W., Gurnell, J., 2016. The Grey Squirrel: Ecology and Management of an Invasive Species in Europe. European Squirrel Initiative, Stoneleigh Park, Warwickshire, UK.
- Sih, A., 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. *Anim. Behav.* 85, 1077–1088. <https://doi.org/10.1016/j.anbehav.2013.02.017>.
- Sih, A., Bell, A., Johnson, J.C., 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol. Evol.* 19, 372–378. <https://doi.org/10.1016/j.tree.2004.04.009>.
- Sih, A., Cote, J., Evans, M., Fogarty, S., Pruitt, J., 2012. Ecological implications of behavioural syndromes: ecological implications of behavioural syndromes. *Ecol. Lett.* 15, 278–289. <https://doi.org/10.1111/j.1461-0248.2011.01731.x>.
- Smith, B.R., Blumstein, D.T., 2008. Fitness consequences of personality: a meta-analysis. *Behav. Ecol.* 19, 448–455. <https://doi.org/10.1093/beheco/arm144>.
- Sol, D., Lapedra, O., González-Lagos, C., 2013. Behavioural adjustments for a life in the city. *Anim. Behav.* 85, 1101–1112. <https://doi.org/10.1016/j.anbehav.2013.01.023>.
- Taff, C.C., Schoenle, L.A., Vitousek, M.N., 2018. The repeatability of glucocorticoids: a review and meta-analysis. *Gen. Comp. Endocrinol.* 260, 136–145. <https://doi.org/10.1016/j.ygcen.2018.01.011>.
- Touma, C., Sachser, N., Möstl, E., Palme, R., 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *Gen. Comp. Endocrinol.* 130, 267–278. [https://doi.org/10.1016/S0016-6480\(02\)00620-2](https://doi.org/10.1016/S0016-6480(02)00620-2).
- Trnka, A., Smaš, P., Grim, T., 2018. Stability of a behavioural syndrome vs. plasticity in individual behaviours over the breeding cycle: ultimate and proximate explanations. *Behav. Process.* 153, 100–106. <https://doi.org/10.1016/j.beproc.2018.06.003>.
- Vanden Broecke, B., Mariën, J., Sabuni, C.A., Mnyone, L., Massawe, A.W., Matthysen, E., Leirs, H., 2019. Relationship between population density and viral infection: a role for personality? *Ecol. Evol.* 9, 10213–10224. <https://doi.org/10.1002/ece3.5541>.
- Veenema, A.H., Meijer, O.C., de Kloet, E.R., Koolhaas, J.M., Bohus, B.G., 2003. Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. *Horm. Behav.* 43, 197–204. [https://doi.org/10.1016/S0018-506X\(02\)00013-2](https://doi.org/10.1016/S0018-506X(02)00013-2).
- Wauters, L., Dhondt, A.A., 1989. Body weight, longevity and reproductive success in red squirrels (*Sciurus vulgaris*). *J. Anim. Ecol.* 58, 637–651. <https://doi.org/10.2307/4853>.
- Wauters, L.A., Gurnell, J., 1999. The mechanism of replacement of red squirrels by grey squirrels: a test of the interference competition hypothesis. *Ethology* 105, 1053–1071. <https://doi.org/10.1046/j.1439-0310.1999.10512488.x>.
- Wauters, L., Tosi, G., Gurnell, J., 2002a. Interspecific competition in tree squirrels: do introduced grey squirrels (*Sciurus carolinensis*) deplete tree seeds hoarded by red squirrels (*S. vulgaris*)? *Behav. Ecol. Sociobiol.* 51, 360–367. <https://doi.org/10.1007/s00265-001-0446-y>.
- Wauters, L., Gurnell, J., Martinoli, A., Tosi, G., 2002b. Interspecific competition between native Eurasian red squirrels and alien grey squirrels: does resource partitioning occur? *Behav. Ecol. Sociobiol.* 52, 332–341. <https://doi.org/10.1007/s00265-002-0516-9>.
- Wauters, L.A., Vermeulen, M., Van Dongen, S., Bertolino, S., Molinari, A., Tosi, G., Matthysen, E., 2007. Effects of spatio-temporal variation in food supply on red squirrel *Sciurus vulgaris* body size and body mass and its consequences for some fitness components. *Ecography* 30, 51–65. <https://doi.org/10.1111/j.0906-7590.2007.04646.x>.
- Wauters, L.A., Mazzamuto, M.V., Santicchia, F., Van Dongen, S., Preatoni, D.G., Martinoli, A., 2019. Interspecific competition affects the expression of personality-traits in natural populations. *Sci. Rep.* 9. <https://doi.org/10.1038/s41598-019-47694-4>.
- Wauters, L.A., Mazzamuto, M.V., Santicchia, F., Martinoli, A., Preatoni, D.G., Lurz, P.W.W., Bertolino, S., Romeo, C., 2021. Personality traits, sex and food abundance shape space use in an arboreal mammal. *Oecologia.* <https://doi.org/10.1007/s00442-021-04901-2>.
- Westrick, S.E., van Kesteren, F., Palme, R., Boonstra, R., Lane, J.E., Boutin, S., McAdam, A.G., Dantzer, B., 2019. Stress activity is not predictive of coping style in north American red squirrels. *Behav. Ecol. Sociobiol.* 73, 113. <https://doi.org/10.1007/s00265-019-2728-2>.
- Westrick, S.E., van Kesteren, F., Boutin, S., Lane, J.E., McAdam, A.G., Dantzer, B., 2021. Maternal glucocorticoids have minimal effects on HPA axis activity and behavior of juvenile wild north American red squirrels. *J. Exp. Biol.* <https://doi.org/10.1242/jeb.236620>. In press.
- Wilson, A.J., Réale, D., Clements, M.N., Morrissey, M.M., Postma, E., Walling, C.A., Kruuk, L.E.B., Nussey, D.H., 2010. An ecologist's guide to the animal model. *J. Anim. Ecol.* 79, 13–26. <https://doi.org/10.1111/j.1365-2656.2009.01639.x>.
- Wingfield, J.C., 2013. The comparative biology of environmental stress: behavioural endocrinology and variation in ability to cope with novel, changing environments. *Anim. Behav.* 85, 1127–1133. <https://doi.org/10.1016/j.anbehav.2013.02.018>.
- Wolf, M., van Doorn, G.S., Leimar, O., Weissing, F.J., 2007. Life-history trade-offs favour the evolution of animal personalities. *Nature* 447, 581–584. <https://doi.org/10.1038/nature05835>.
- Wong, B.B.M., Candolin, U., 2015. Behavioral responses to changing environments. *Behav. Ecol.* 26, 665–673. <https://doi.org/10.1093/beheco/aru183>.
- Zhang, V.Y., Williams, C.T., Palme, R., Buck, C.L., 2020. Glucocorticoids and activity in free-living arctic ground squirrels: interrelationships between weather, body condition, and reproduction. *Horm. Behav.* 125, 104818. <https://doi.org/10.1016/j.yhbeh.2020.104818>.