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## RESEARCH ARTICLE

# Oxidative profile varies with personality in European greenfinches

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**SUMMARY**

**Where behavioural responses differ consistently between individuals, this is termed 'personality'. There is the suggestion, but with little supporting data, that personality traits reflect underlying variation in physiology. Here, we tested whether greenfinches *Carduelis chloris* differing in personality traits differed in various plasma indices of oxidative profile: antioxidant capacity (OXY), pro-oxidant status (reactive oxygen metabolites, ROMs), oxidative stress (OS) and an end-product of oxidative damage: malondialdehyde (MDA). We measured two personality traits: neophobia (latency to approach food near novel objects) and object exploration (latency to approach novel objects). These traits were uncorrelated. ROMs, OXY, OS and MDA were also uncorrelated with each other. Highly neophobic birds had lower OXY, higher ROMs and higher OS than less neophobic birds. Fast exploring birds had higher OXY than slow explorers, but did not differ in ROMs or OS. Variation in MDA was described by a quadratic relationship with neophobia: birds with extremely high or low neophobia had lower MDA than birds with intermediate neophobia, despite highly neophobic birds exhibiting lower OS than intermediately neophobic birds. Additively in that model, fast explorers had lower MDA than slower explorers. To conclude: first, personality types can differ in oxidative profile. Second, although physiological differences (e.g. hormonal stress responsiveness) between personality types generally range along a linear continuum, physiological costs may not. Finally, relationships with oxidative profile differed between neophobia and object exploration. Understanding how oxidative profile and thus physiological costs vary within and between personality traits may explain how differences in personality traits can predict fitness.**

Key words: neophobia, exploration, behavioural syndromes, oxidative stress, antioxidant capacity, lipid peroxidation.

## INTRODUCTION

Oxidative stress occurs when pro-oxidants, chemicals produced during normal metabolism that can induce reactive oxygen species and/or inhibit antioxidant systems, exceed the body's antioxidant capacity (Finkel and Holbrook, 2000). Costs, in terms of tissue damage and also investment in cellular repair and replacement, accrue under oxidative stress. As such, intraspecific variation in 'oxidative profile' (antioxidants, pro-oxidant capacity, oxidative damage and oxidative stress) often predicts variation in health and longevity (Costantini et al., 2008a; Harman, 1956; Hulbert et al., 2007). An individual's rate of pro-oxidant production is context dependent (Alonso-Alvarez et al., 2004; Monaghan et al., 2009). For example, pro-oxidant production and/or oxidative damage have been shown to increase during physically demanding periods such as migration (Costantini et al., 2008b) and reproduction (Wiersma et al., 2004). Such increases may be the by-product, and indeed physiological cost, of for example raising metabolism to cope with the increased energy demands of such periods (Monaghan et al., 2009). However, within the same context, individuals of the same mass are expected to have the same metabolic rate yet often differ, and thus presumably differ also in the rate of pro-oxidant production and/or requirements for antioxidant defences (Ferguson et al., 2008; Careau et al., 2009; Careau et al., 2008; Krol and Speakman, 2003). Such context-independent variation in metabolic rate may be explained by a phenomenon widely observed across animal taxa: 'personality' (Careau et al., 2008).

Personality traits are differences in behaviour between conspecifics that are consistent across time or contexts (Wilson et al., 1994; Gosling, 2001). Common axes of variation include a tendency towards aggression (e.g. Huntingford, 1976), neophobia (e.g. Herborn et al., 2010; Mettke-Hofmann et al., 2002) and exploration (e.g. Dingemanse and de Goede, 2004). Personality traits are often highly correlated within individuals. For example, a commonly described trait correlation is the 'proactive–reactive' or 'fast–slow' syndrome, which encompasses boldness or neophobia, aggression and exploration (Koolhaas et al., 1999; Carere et al., 2005). To respond quickly and actively, 'fast' (i.e. aggressive, bold, fast exploring) personality types may have a generally higher metabolic rate than 'slow' (i.e. passive, neophobic, slow exploring) types (e.g. Drent and Daan, 1980). Alternatively, they may channel more energy towards the activities described by these traits from a limited energy budget (e.g. Cutts et al., 2002). Given they differ systematically in metabolic rate, we predicted that personality types would also differ in their rate of pro-oxidant production, and hence oxidative profile.

Few studies have investigated the relationship between personality and oxidative profile explicitly. An exception is recent work by Costantini and colleagues (Costantini et al., 2008a), which showed that mice from strains characterised by a long attack latency ('LAL',

i.e. passive personality types) had higher antioxidant capacity than mice from strains with relatively short attack latencies ('SAL', i.e. aggressive personality types). However, several lines of evidence support this relationship indirectly. First, longevity varies with personality (Cavigelli and McClintock, 2003; Cavigelli et al., 2009; Dingemanse et al., 2004; Ewalds-Kwist and Selander, 1996), suggesting a cumulative cost to personality. In LAL–SAL mice for example, young LAL mice have a higher antioxidant capacity than SAL mice yet their oxidative stress levels are not lower, and ultimately they have shorter life spans (Costantini et al., 2008a; Ewalds-Kwist and Selander, 1996). To achieve the same level of oxidative stress, therefore, young LAL mice may up-regulate their antioxidant system, an additional investment that may be costly in later life (Costantini et al., 2008a). Cumulative effects of personality are also observed in the 'activity' personality trait, where the most active (hence the most metabolically active) individuals have the shortest lifespan, a finding consistent across a broad taxonomic range (Biro and Stamps, 2008). Specifically, we could therefore predict that fast personality types, or at least individuals on the fast end of the exploration spectrum, suffer higher oxidative damage than slow personality types.

Conversely, we might make the opposite prediction if we focus on the relationships between behavioural and hormonal stress responsiveness. In species with well-defined personality types, individuals that are fast to engage with novel or threatening stimuli often have lower glucocorticoid (stress hormone) levels than their slower counterparts, including for example SAL *versus* LAL mice (*Mus musculus*) (Veenema et al., 2003), 'proactive' or 'fast' (neophilic, aggressive, fast exploring) *versus* 'reactive' or 'slow' great tits *Parus major* (Carere et al., 2003), docile *versus* non-docile chipmunks *Tamias striatus* (Martin and Reale, 2008), neophilic *versus* neophobic rats *Rattus norvegicus* (Cavigelli and McClintock, 2003) and zebra finches *Taeniopygia guttata* (Martins et al., 2007), and less *versus* more environmentally sensitive rainbow trout *Oncorhynchus mykiss* (Hoglund et al., 2008). Glucocorticoids stimulate the metabolism to facilitate rapid behavioural responses, such as the fight or flight response (Cockrem, 2007). Consequently, slow personality types, which have consistently higher or more reactive stress responses, may be expected to suffer higher oxidative damage than fast types, diverting more energy away from other activities into stress responses. Interestingly, dietary supplementation of poultry over a time period of days to achieve chronic levels of the glucocorticoid corticosterone (Cort, the avian stress hormone) raises lipid peroxidation (a measure of oxidative damage) (Lin et al., 2004a), but acute exposure to Cort, *via* injection, does not (Lin et al., 2004b). With chronic exposure perhaps akin to persistent differences in stress physiology as predicted by personality, and a single acute exposure analogous to short-term fluctuations in stress that may be experienced by any individual (for a review, see Cockrem, 2007), this provides experimental support for stress responsiveness as a mechanism for personality differences in oxidative stress. However, it should also be noted that personality traits are not always related to either baseline or stress-induced Cort, and the relationship is not always in the predicted direction (K.E.A., L.A. and K.A.H., unpublished data) (Muller et al., 2006; Martins et al., 2007); thus, much still needs to be explored.

With two inter-related potential mechanisms linking personality to oxidative profile – metabolic rate and stress responsiveness – it is perhaps surprising that relationships between these traits have not been explored more extensively. In this study, we investigated the relationship between personality and oxidative profile in captive-bred European greenfinches (*Carduelis chloris*). We made no *a priori* predictions on the direction of the relationship between these traits. As discussed above, it is possible to make opposite predictions on whether fast or slow extremes endure higher oxidative costs, dependent on the mechanism one refers to as the source of increased pro-oxidant production. Indeed, it is important to note that not all personality traits may be linked to hormonal stress responsiveness: some 'stressors' presented in personality assays elicit an immediate increase in Cort [e.g. sight of a predator (Cockrem and Silverin, 2002); novel object by food (Richard et al., 2008)], but others do not [e.g. novel object by food (Apfelbeck and Raess, 2008), novel object alone (Mettke-Hofmann et al., 2006)]. Where personality traits are correlated in a proactive–reactive behavioural syndrome, therefore, it is possible that both scenarios may operate but in opposite directions. Moreover, in some species (e.g. Herborn et al., 2010; Mettke-Hofmann et al., 2002; Coleman and Wilson, 1998), personality traits such as neophobia and exploration are not correlated at all. In failing to account for the relationship between traits, therefore, we may be confronted with non-intuitive patterns. Hence, to identify relationships and infer the mechanisms linking oxidative profile and personality, it is crucial to measure multiple personality traits and consider also the relationships between these traits.

For our personality traits, we measured two commonly described responses to novelty. First, 'neophobia': latency for hungry individuals to approach novel objects placed near food. Here, the object may generate a motivational conflict between hunger and the desire to avoid the unknown (potentially risky) object (Herborn et al., 2010; Mettke-Hofmann et al., 2002). The latency to approach in these trials may also be motivated by the novel object itself, however, for information gathering. Therefore, second, to distinguish the affects of neophobia and information gathering on oxidative profile, we measured 'object exploration': the latency to approach novel objects in the absence of food (Mettke-Hofmann et al., 2002). Responses to novelty such as these appear to reflect variation in the tendency to find or use novel foraging opportunities more generally, both across species, correlating positively with niche breadth (Mettke-Hofmann et al., 2002), and between individuals foraging in the wild (Herborn et al., 2010). We were interested in examining possible physiological costs of these ecologically relevant traits.

Our measures of pro-oxidant status and oxidative damage were a propagator and end product of the lipid peroxidation cascade, respectively: reactive oxygen metabolites (ROMs) and malondialdehyde (MDA). ROMs are primarily hydroperoxides (ROOH). On reaction with circulating metal ions, ROMs cleave to produce highly reactive alkoyxl (R-O•) and alkylperoxyl (R-OO•) free radicals that instigate an oxidative cascade culminating in tissue damage, primarily lipid peroxidation, a by-product of which is MDA (see Kohen and Nyska, 2002). We measured antioxidant capacity (OXY) as the capacity of the plasma to resist oxidation by another pro-oxidant, hypochlorous acid (HOCl). Oxidative stress (OS) was then defined as the ratio of ROMs to OXY (Costantini et al., 2006). Concentrations of plasma MDA, ROMs and OXY measured *in vitro* have been correlated with various measures of individual health (Trotti et al., 2001; Dotan et al., 2004) and condition (e.g. Costantini et al., 2007; Costantini and Bonadonna, 2010; Larcombe et al., 2008). Across individuals (regardless of personality), body mass may also affect pro-oxidant production, as very high and very low mass are both associated with increased oxidative stress (Costantini et al., 2007; Larcombe et al., 2010a; Wiersma et al., 2004). Therefore, we also investigated the effects of body mass on oxidative profile. Specifically, we had three aims. First: to determine whether differences between individuals in neophobia and exploration were consistent

and repeatable, and hence constituted personality traits in greenfinches. We also tested whether neophobia and exploration were correlated within individuals. Such correlations can imply proximate links between traits, *via* genetic linkage or shared physiology (Van Oers et al., 2005). However, correlations can also occur when two traits are not mechanistically connected but, rather, are subject to the same selection pressures (Bell and Sih, 2007; Dingemanse et al., 2007). Second: to investigate how measures of oxidative profile related to one another. Finally: to determine whether neophobia or exploration co-varied with oxidative profile, and consequently whether personality types may differ in their oxidative costs.

## MATERIALS AND METHODS

This study utilised 22 birds from a colony of captive bred greenfinches, *C. chloris* (L.) (13 males and 9 females). Birds were sourced from seven private breeders, so individuals were from a diverse genetic background. Relatedness and early life conditions were unknown, but all were aged between 15 and 17 months and had been in the colony for at least 7 months at the start of the trial. Birds were kept singly, in 120×50×50 cm cages, but in auditory and visual contact. Outside of trials, birds had *ad libitum* access to Haith's™ greenfinch mix and water, and were provided with 10 defrosted frozen garden peas per day. During personality trials, screens were positioned to shield the focal individual from visual contact with other birds. All work was carried out under licence from the UK Home Office and in accordance with ASAB/ABS's guidelines for the treatment of animals in research, and was subject to ethical review by the Ethics Committees of the WALTHAM® Centre for Pet Nutrition and the University of Glasgow. No birds became ill or died during this experiment. Neophobia trials were conducted between 26 August 2008 and 4 September 2008 and exploration trials between 5 September 2008 and 8 September 2008.

### Personality trials

#### Neophobia

Each bird took part in four neophobia trials across an 8 day period. Each trial had two phases: a novel object phase and a disturbance phase. Prior to a phase, the food bowl and any spilt food were removed from the cage to motivate birds towards foraging activity. After 30 min, the water bowl was also removed. After a further 30 min (1 h in total without food), the food bowl was returned to the cage and the latency to land on the food bowl recorded. In the disturbance phase, just the food bowl was returned. In the novel object phase, the food bowl also contained one of four novel objects: a red, blue, green or yellow plastic biscuit cutter of approximately 3×2×1 cm. Birds that did not approach within 30 min were given a maximum latency of 1800 s. We measured latency to approach the food bowl and not latency to feed because of ceiling effects in the novel object phase: birds approached the bowl at a variety of intervals but generally did not feed until near 30 min or at all in that phase. Phases were alternated each day; the first phase a bird received was randomised. Independent of response to the novel object, individuals may also differ in their motivation to feed or tolerance of disturbance by the observer returning the food bowl. This is why we conducted the disturbance phases: for each object we regressed novel object phase latencies against disturbance phase latencies (Boogert et al., 2006), after first log-transforming both latencies to meet the assumptions of normality and homogeneity of variance. The residuals of these models, converted to *z*-scores, provided four measures of neophobia for each bird, one per object.

#### Object exploration

Each bird took part in two object exploration trials, conducted on consecutive days. The home cage contained four, evenly spaced perches. Object exploration may relate to information gathering on cryptic food types (Mettke-Hofmann et al., 2002). Prior to a test, therefore, the food and water bowls were removed as per the neophobia trial to motivate birds towards foraging behaviour. To start the trial, the observer placed one of two novel objects onto the centre of the furthest left perch, stepped behind a screen, and observed the focal bird through a small hole for 30 min. In this position, there were 1.5 body lengths to either side of the object on the perch and 2 body lengths between the object perch and the next nearest perch. The novel objects were a bundle of white cotton bud sticks tied together with white string, and two interlocking transparent colourless rings. The order of the objects was randomised per bird. The latency to first land on the object perch was recorded. We measured latency to land on the perch and not latency to touch the object because of ceiling effects: most birds did not touch the objects within 30 min. After 30 min, the object was removed and the food and water bowls returned. Therefore, each individual had two object exploration latencies.

### Oxidative profile

Oxidative profile measures were derived from a blood sample of up to 300 µl collected on 30 October 2008, taken within 3 min of capture from the home cage by venepuncture of the wing vein. The plasma was immediately separated from the red blood cells by centrifuging for 5 min at 14,000 ***g***, and was then frozen at –80°C until analysis. Body mass (g) was recorded immediately after blood sampling.

#### OXY

Using the Oxy-Adsorbent test (Diacron, Grosseto, Italy), OXY was measured as the capacity of the plasma to withstand oxidation by HOCl (see Costantini et al., 2007). The plasma sample was defrosted at room temperature and 2 µl of the sample or 2 µl of a calibrator was diluted 1:99 with distilled water ($dH_2O$). Then, 200 µl HOCl solution was combined with 5 µl of the diluted plasma, calibrator or $dH_2O$ (control), and incubated at 37°C for 10 min. Finally, 2 µl of a chromogen solution of 0.01 mol $l^{-1}$ acetic acid/sodium acetate buffer (pH 4.8) and *N*,*N*-diethylphenylenediamine was added to each sample. Alkyl-substituted aromatic amine in the chromogen solution is oxidised by HOCl remaining in the sample (i.e. not quenched by plasma OXY), and produces a pink derivative, the intensity of which was measured at 490 mm using a microplate spectrometer (Multiskan Spectrum, Thermo Fisher Scientific, Basingstoke, UK). OXY concentration is inversely proportional to the intensity of the pink, and was expressed as µmol HOCl $ml^{-1}$ of sample.

#### ROMs

Using the d-ROMs test (Diacron), ROMs were measured as the pro-oxidant capacity of the plasma equivalent to mmol $l^{-1}$ hydrogen peroxide ($H_2O_2$). ROMs are expressed as Carratelli units (CARR U), with 1 CARR U equivalent to the pro-oxidant capacity of 0.08 mg $H_2O_2$. The above chromagen solution was combined in a 1:50 ratio with 4 µl of the diluted plasma, calibrator or $dH_2O$ and incubated at 37°C for 75 min. Colour intensity was measured at 490 nm using the Multiskan Spectrum microplate spectrophotometer). The process cleaves hydrogen peroxides in the sample into two free radicals. These free radicals then react with alkyl-substituted aromatic amine in the chromagen solution and produce a pink colour with an intensity directly proportional to the hydroperoxide (pro-oxidant) content of the sample (see Costantini et al., 2007).

#### OS

An index of OS was calculated as the ratio of ROMs to OXY (×1000) (Costantini et al., 2006).

#### MDA

MDA, an indicator of lipid peroxidation and oxidative stress, was measured by reaction with thiobarbituric acid, following Young and Trimble (Young and Trimble, 1991) with modifications as described by Larcombe and colleagues (Larcombe et al., 2008). Briefly, a solution of thiobarbituric acid (0.44 mol $l^{-1}$, 100 µl) and phosphoric acid (1.22 mol $l^{-1}$, 100 µl) was added to 50 µl plasma, 50 µl of a malonaldehyde bis(dimethyl acetyl) standard (Sigma Aldrich, St Louis, MO, USA) or 50 µl of $dH_2O$ (control). For one bird where only 45 µl of plasma was available, 5 µl of distilled water was added for consistent volume, and MDA concentration was later proportionally scaled to the sample size. Under a nitrogen blanket, test tubes were sealed, vortexed and then incubated for 1 h at 70°C. After cooling in a water bath at room temperature, 200 µl of the mixture was pipetted into a centrifuge tube that contained sodium hydroxide (1 mol $l^{-1}$, 100 µl). Methanol (500 µl) was added and samples were vortexed then centrifuged (10 min, 1800 ***g***). We used a Summit HPLC system (Dionex, Idstein, Germany) with Chromeleon software (Dionex) and an acclaim 120 C18 5 µl column (Dionex) and guard to measure fluorescence (excitation 532 nm and emission 553 nm) of this supernatant. The mobile phase (40:60 methanol:phosphate buffer; 40 mmol $l^{-1}$, pH 6.5) had a flow rate of 1 ml $min^{-1}$.

### Statistical methods

Analyses were carried out using R version 2.9.1 (R Development Core Team, 2009). There were no significant sex differences in ROMs (ANOVA: $F_{1,20}$=0.32, $P$=0.58), OXY ($F_{1,20}$=0.17, $P$=0.68), MDA ($F_{1,20}$=0.87, $P$=0.36), neophobia ($F_{1,20}$=0.77, $P$=0.39) or object exploration ($F_{1,20}$=3.56, $P$=0.08). There were also no differences between birds sourced from different breeders, a proxy of unknown pedigree and early life conditions (ANOVA: ROMs $F_{6,15}$=0.58, $P$=0.74; OXY $F_{6,15}$=2.18, $P$=0.1; MDA $F_{1,20}$=0.62, $P$=0.71; neophobia $F_{6,15}$=0.39, $P$=0.88; object exploration $F_{1,20}$=1.84, $P$=0.16). Therefore data were pooled across sexes and breeders.

Relationships within and between oxidative profile measurements and personality traits were then analysed using general linear models (GLMs). To determine whether individual neophobia or object exploration was consistent over trial replicates, and hence constituted personality traits, repeatability was calculated using the mean squares from an analysis of variance, following Lessells and Boag (Lessells and Boag, 1987). To identify relationships between oxidative profile and personality we constructed GLMs with each measure of oxidative profile as the dependent variable and all two-way interactions between linear and quadratic expressions of both personality traits as the independent variables. We specified both linear and quadratic expressions of the personality traits to examine whether oxidative profile differed between the linear ends of the trait continua (low *versus* high neophobia or fast *versus* slow exploration) or instead between intermediate and extreme (low and high neophobia or fast and slow exploring) personality types. These models were simplified by backwards stepwise regression, removing first non-significant ($P$>0.05) quadratic terms (interactions or main effects) and then linear terms in turn until only significant ($P$<0.05) or no independent variables remained. The significance of removing each term was assessed by comparing models with a likelihood ratio test (LRT). All oxidative profile measurements and body mass were log transformed to normalise the residuals of these models. Means and s.e.m. and/or ranges are presented.

## RESULTS

### Individual variation in behaviour

In the neophobia trials, we measured mean latency to approach a familiar food bowl in the presence of a novel object. Latency was significantly greater when a novel object was present than when it was absent (paired Wilcoxon rank-sum test: $V$=351, $N_1$=$N_2$=22, $P$=0.0001); thus, the presence of the object induced neophobia. Bird identity explained a marginally significant proportion of the variation in approach latency during disturbance phases (linear mixed-effects model, LME, with trial order as random factor: $F_{1,63}$=1.71, $P$=0.05) and a significant proportion of variation in the novel object phases (LME, with object identity nested in trial order as a random factor: $F_{1,51}$=6.76, $P$<0.0001). Therefore, birds were consistently fast or slow within phases. Individual neophobia was significantly repeatable across the four trials (ANOVA: $r$=0.57, $F_{21,66}$=3.6, $P$<0.0001), so individuals differed consistently in their latency to approach food near novel objects. Therefore, we used a mean $z$-value per individual as the neophobia score for remaining analyses. Neophobia was independent of body mass ($F_{1,20}$=1.09, $P$=0.31).

In the object exploration trials, we measured latency to approach novel objects in the absence of food. Controlling for trial order and object identity, individual identity explained a significant proportion of variation in the exploration trials (LME with object identity nested within trial order as random factors: $F_{22,18}$=3.26, $P$=0.007), so individuals were consistently fast or slow to approach independent of learning effects between trials or the order in which objects were encountered. Individual latency was significantly repeatable over two trials (ANOVA: $r$=0.47, $F_{21,22}$=2.69, $P$=0.013). Therefore, we used a mean latency per individual as the object exploration score for remaining analyses. Object exploration was independent of body mass (GLM: $F_{1,20}$=1.39, $P$=0.25). There were no linear (GLM: $F_{1,20}$=0.57, $P$=0.46) or quadratic relationships (GLM: $F_{2,19}$=0.29, $P$=0.76) between individual neophobia and object exploration. Thus, neophobia and object exploration were personality traits but did not constitute a proactive–reactive behavioural syndrome.

### Individual variation in oxidative profile

The mean ROM concentration was 85.6 CARR U (range 40.2–205.1 CARR U) and mean OXY concentration was 97.4 mmol $l^{-1}$ neutralised HOCl (range 42.2–93.3 mmol $l^{-1}$). The mean OS value was 0.001 (range 0.0003–0.004). The mean MDA concentration was 0.66 nmol $ml^{-1}$ (range 0.26–1.09 nmol $ml^{-1}$).

Variation in the time of blood sampling (10:00 h–16:00 h) did not affect OXY (GLM: $t_{1,20}$=0.66, $P$=0.51), ROMs ($t_{1,20}$=0.56, $P$=0.58) or MDA ($t_{1,20}$=–1.46, $P$=0.16), nor did the duration of handling at capture prior to blood sampling (mean=136 s, range=50–178 s; OXY $t_{1,20}$=0.32, $P$=0.75; ROMs $t_{1,20}$=0.22, $P$=0.83; MDA $t_{1,20}$=1.52, $P$=0.15). ROMs were independent of OXY (GLM: $t_{1,20}$=1.3, $P$=0.22). MDA was independent of ROMs ($t_{1,20}$=0.35, $P$=0.73), OXY ($t_{1,20}$=0.51, $P$=0.62) and OS ($t_{1,20}$=0.23, $P$=0.82). ROMs (GLM: $F_{1,20}$=2.12, $P$=0.12), OXY ($F_{1,20}$=2.68, $P$=0.12), OS ($F_{1,20}$=0.03, $P$=0.87) and MDA ($F_{1,20}$=0, $P$=0.95) were all independent of body mass.

### Relationships between personality and oxidative profile

#### ROMs

In models with ROMs as the dependent variable, only neophobia explained variation in ROMs: birds with low neophobia had significantly lower ROMs than birds with high neophobia ($t_{1,20}$=2.57, $P$=0.018; Fig. 1A). There were no significant effects of removing quadratic interactions or main effects (LRT between increasingly

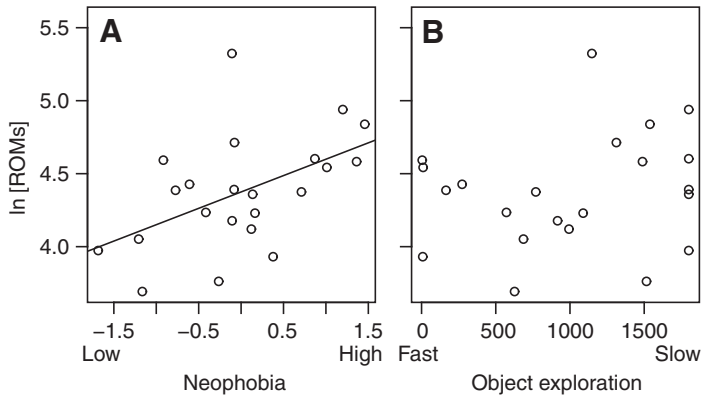


Fig. 1. Relationships between reactive oxygen metabolites (ROMs; in CARR U) and personality traits. (A) Highly neophobic birds had higher ROMs than less neophobic birds. (B) No relationship was identified between ROMs and object exploration.

simplified models: all $P>0.52$). There was also no significant interaction between the linear expressions of neophobia and object exploration (LRT: deviance=–0.18, $P=0.22$), and object exploration did not contribute significantly as a main effect (LRT: deviance=0.01, $P=0.75$; Fig. 1B).

### OXY

However, in another GLM, after removing all non-significant quadratic terms and the linear interaction term (LRT: all $P>0.14$), we identified additive relationships between object neophobia (Fig. 2A) and object exploration (Fig. 2B) in explaining variation in OXY ($F_{2,19}=4.5$, $P=0.025$). Both traits had significant, independent positive relationships with OXY.

### OS

In the model investigating OS levels, only neophobia explained variation in OS: birds with low neophobia had lower OS than birds with high neophobia ($t_{1,20}=3.17$, $P=0.005$; Fig. 3A). There were no significant quadratic terms (LRT: all $P>0.36$), and both the interaction between novel exploration and neophobia (LRT: deviance=–0.22, $P=0.36$) and object exploration as a main effect (LRT: deviance=0.27, $P=0.31$; Fig. 3B) were non-significant.

### MDA

Finally, variation in MDA was explained by an additive relationship between the quadratic expression of neophobia ($F_{2,19}=4.06$, $P=0.034$; Fig. 4A) and the linear expression of object exploration (Fig. 4B): birds that were fast exploring or showed very high or low neophobia had lower MDA than birds that were slow exploring or were intermediate in their neophobia scores. In this GLM investigating variation in MDA, there was no significant contribution of the quadratic expression of object exploration, either as an interaction term or as a main effect (LRT: all $P>0.07$).

## DISCUSSION

Neophobia and object exploration were consistent within individuals across days, and thus constitute personality traits in the greenfinch. Both traits were related to oxidative profile. Most relationships were linear, suggesting higher oxidative costs at the ‘slow’ (highly neophobic or slow exploring) than at the ‘fast’ extreme: highly neophobic birds had lower OXY, higher ROMs and consequently higher OS than less neophobic birds; fast explorers had higher OXY and lower MDA than slow explorers. However, there was also a quadratic relationship between MDA and personality: birds with extremely high or low neophobia had lower MDA than intermediate responders. There was no correlation between the personality traits, and they contributed additively to variation in OXY and MDA. Therefore, neophobia and object exploration were independent, and oxidative profile differed both within and between personality traits. We found no relationship between body mass and oxidative profile. Interestingly, we found no direct relationship between ROMs and OXY or, though ROMs are one of the steps in the lipid peroxidation chain that produces MDA, between ROMs, OS and MDA. Finally, the time of blood sampling and the duration of handling prior to blood sampling did not affect blood parameters. These findings are consistent with previous studies showing that acute increases in corticosterone (Lin et al., 2004b), or stressors such as handling (Costantini et al., 2007) or containment (Costantini and Lipp, 2008), do not induce immediate, short-term changes in MDA, ROMs and/or OXY. As such, we suggest the measures of oxidative profile presented in this study represent long-term, background differences in oxidative profile between individuals.

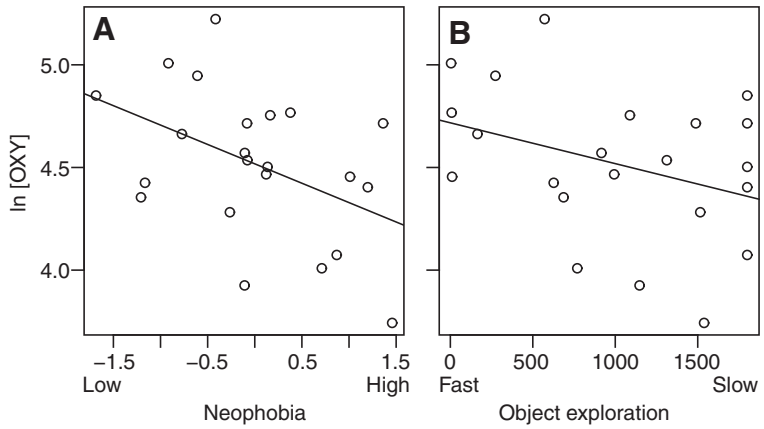


Fig. 2. Relationships between antioxidant capacity (OXY; in mmol $l^{-1}$ of neutralised HOCl) and personality traits. (A) Highly neophobic birds had lower OXY than less neophobic birds. (B) Slow exploring birds had lower OXY than fast exploring birds.

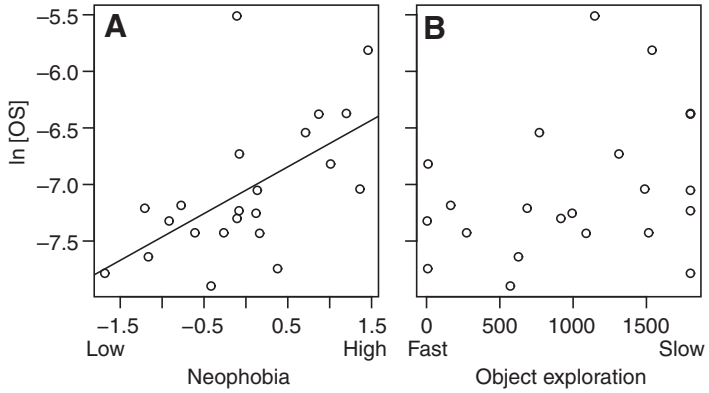


Fig. 3. Relationships between oxidative stress (OS; ROMs/OXY×1000) and personality traits. (A) Highly neophobic birds had higher OS than less neophobic birds. (B) No relationship was identified between OS and object exploration.

The results of this study confirm our prediction that personality types would differ in oxidative profile. Measuring multiple aspects of oxidative profile, as we have done here, is crucial for drawing conclusions on variation in oxidative profile (Costantini and Verhulst, 2009; Monaghan et al., 2009). For example, the fast ends of both trait axes (birds with low neophobia and fast exploration) had higher OXY than the slow extremes. Alone, this would suggest superior or up-regulated plasma antioxidant capacity in 'fast' personality types. With ROMs, however, it is apparent that, whilst the least neophobic birds do have lower OS than the most neophobic birds, fast explorers achieve only the same plasma oxidative stress as slow explorers. This pattern of high OXY but equivalent OS has also been observed by Costantini and colleagues (Constantini et al., 2008a) when considering aggression in mice, but at the slow (passive) extreme of that trait. Passive mice also have shorter life spans (Ewalds-Kwist and Selander, 1996) and show greater hormonal stress responsiveness than aggressive mice (Veenema et al., 2003). Unifying these studies, Costantini and colleagues suggest that the higher antioxidant capacity in stress-responsive LAL mice may in fact be a surplus, buffering against short-term, stress-induced increases in pro-oxidant production (Constantini et al., 2008a). Accordingly, a short life span is suggested as the cumulative cost of this up-regulation. Life span has also been shown to vary with personality in wild animals (Dingemanse et al., 2004), and direct behavioural mechanisms for this variation, such as risk-taking propensity (e.g. Bell and Sih, 2007) and ability to control sparse resources (Dingemanse and de Goede, 2004), have been suggested. Less common studies on potential cumulative, physiological costs, through variation in oxidative profile (e.g. Costantini et al., 2008a) or physiological stress responsiveness (e.g. Cavigelli et al., 2009), are an intriguing new angle on the survival costs to personality.

Interestingly, despite equivalent OS amongst exploration types, we found that fast explorers had lower MDA than slow explorers. Similarly, we found a positive relationship between neophobia and OS but a quadratic relationship with MDA, such that highly neophobic birds with the highest OS levels in fact had lower MDA than intermediate responders. These apparent discrepancies illustrate both the complexity of the antioxidant systems and, again, the importance of combining multiple measures of oxidative profile in their interpretation. Assays of 'total antioxidant capacity', such as the OXY-Adsorbent test, are often conducted on plasma samples, and in aqueous solution. As such, important lipid-soluble antioxidants such as α-tocopherol (vitamin E) and ubiquinol (co-enzyme Q) that occur mostly in the cell membranes can be underestimated by these methods (Bartosz, 2010), although some studies find correlations between lipophilic antioxidants and antioxidant capacity (e.g. Cohen et al., 2007). Comparing our results on OS with those for MDA, a possible explanation for the discrepancy at the highly neophobic extreme may be further personality variation in cell membrane antioxidant capacity; specifically, greater cell membrane antioxidant defences in the high extreme of the neophobia trait than in intermediately neophobic birds.

The neophobia results with MDA raise a further interesting point: physiological differences between personality types generally range along a linear continuum. For example, in several species, stress responsiveness ranges from low to high (baseline and/or elevated glucocorticoid level) with increasing neophobia (for a review, see Cockrem, 2007). However, our results demonstrate that the physiological costs of personality may not be linear:

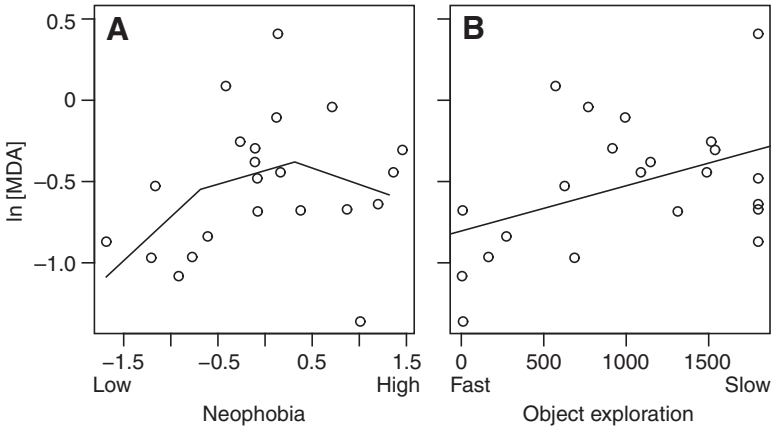


Fig. 4. Relationships between malondialdehyde (MDA; in nmol $ml^{-1}$) and personality traits. (A) Birds with extremely high or low neophobia had lower MDA than intermediately neophobic birds. (B) Slow exploring birds had higher MDA than fast exploring birds.

intermediately neophobic birds had higher MDA than those at the low and high neophobia extremes. Indeed, neophobia may constitute a two-dimensional continuum in greenfinches, with general 'responsiveness' to novel objects (whether high or low neophobia) being a shared mechanism differentiating oxidative profile (i.e. antioxidant status) between extremes and (less responsive) intermediates. It is a common assumption of personality research that responsiveness to stimuli falls along a one-dimensional continuum, with fast and slow individuals at each end of the trait axes. Comparing the same trait across contexts, however, variance in behaviour is often lower in intermediates than in extremes, suggesting lower responsiveness to environmental stimuli (Coleman and Wilson, 1998; Magnhagen and Staffan, 2005; Vas et al., 2008). Indeed, in wild great tits (*Parus major*), survival and reproductive success also vary less with environmental variation in intermediates than in extremes (Dingemanse et al., 2004). Whilst the physiology of fast and slow personality types is often well characterised by selection line studies (e.g. Carere et al., 2003; Cavigelli and McClintock, 2003; Martins et al., 2007; Veenema et al., 2003), our results suggest that the physiology of intermediate personality types warrants further investigation.

That neophobia and object exploration, the latency to approach novel objects in the presence and the absence of food, respectively, were not correlated was surprising given the similarity of the two behavioural assays. However, comparing responses to novel objects in feeding and neutral contexts in a broad range of parrot species, Mettke-Hofmann and colleagues found no general correlation between neophobia and object exploration (Mettke-Hofmann et al., 2002). Moreover, trait expression correlated with species ecology: exploration was fastest in species that appeared to benefit most from information gathering – those that inhabited relatively changeable (e.g. forest edge) *versus* homogeneous habitats (see also Tebbich et al., 2009) and consumed cryptic *versus* conspicuous prey (e.g. buds *versus* fruit/flowers). Conversely, neophobia appeared to be related to dietary risk: novel insects are potentially noxious, and insectivorous species were more neophobic than leaf-eating species (Mettke-Hofmann et al., 2002). In physiological studies too, neophobia appears to be related to risk sensitivity. In the few studies that have compared the level of Cort before and after presentation of a novel object, presenting a novel object with food appears to stimulate a Cort response (Richard et al., 2008) whilst presenting the novel object in a neutral location does not (Mettke-Hofmann et al., 2006) (but see Apfelbeck and Raess, 2008). Given the differences in oxidative profile between the neophobia and object exploration traits, we predict that whilst both trials presented an opportunity for information gathering, only the neophobia trial assayed individuals for stress responsiveness. Overall though, we found that oxidative profile related differently to different personality traits. It is also important to note that there were additive relationships between traits in explaining oxidative profile; for example, slow exploring intermediately neophobic birds had higher MDA than birds that were slow exploring but showed extremely high or low neophobia. Correlations between different personality traits, or 'behavioural syndromes', often vary across wild populations of the same species (Sih et al., 2004). This variation may be produced by differences in selection pressures on combinations of traits, such as predation risk, between populations (Bell, 2005; Bell and Sih, 2007; Dingemanse et al., 2007). Understanding how oxidative profile, and thus physiological cost, varies within and between personality traits may therefore provide new insight into the selection mechanisms differentiating behavioural syndromes between populations.

In line with a number of within-species studies on metabolic rate (for a review, see Careau et al., 2008), we found no relationship between body size and our measurements of ROM, MDA and OS. However, the metabolic demands on the study animals were low: temperature was ambient, food abundant and activity (in cages) limited. It is notable that MDA levels in these captive birds were around half those found in wild-caught greenfinches (0.66±0.33 *versus* 1.23±0.68 nmol MDA $ml^{-1}$ plasma) (Hõrak et al., 2006). Wild birds may differ substantially from caged birds in the demands on their antioxidant systems. For example, one prolonged flight in homing pigeons can immediately deplete serum antioxidants (Costantini et al., 2008b). Wild (active) birds may also differ in their efficiency at meeting such demands. For example, previously 'unfit' captive budgerigars (*Melopsittacus undulatus*) showed reduced MDA following weeks of regular flight training (Larcombe et al., 2010a). The lack of direct correlation between OXY, ROMs and MDA may similarly be explained by undemanding living conditions: in humans, lipid peroxidation and plasma antioxidant levels are often uncorrelated in healthy subjects, but correlated in subjects under heightened physiological demands; for example, negatively in individuals with pathological diseases but increasingly positively in subjects in exercise studies (for a review, see Dotan et al., 2004). However, variation in oxidative profile between personality types suggests that, even within benign, homogeneous captive environments, physiological demands may differ between personality types. Wild animals face many physiologically taxing periods when oxidative stress is enhanced, such as growth (Alonso-Alvarez et al., 2006; Larcombe et al., 2010b), migration (Costantini et al., 2008b) and reproduction (Wiersma et al., 2004). If variation in oxidative profile between personality types is apparent in wild animals too, personality types may differ in the extent or manner in which they respond to these challenges.

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