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VARIATION IN RESPONSE TO ENVIRONMENTAL CUES WHEN FORAGING

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B.A. M.RES

THIS THESIS IS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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ABSTRACT

Animals often respond differently to the same environmental cues. Where behavioural responses differ consistently between individuals over time or contexts, this is "personality". In wild animals, personality is linked to variation in fitness and survival. Predictions on the behavioural mechanisms underlying this variation come from captive studies, on the often untested assumption that captive behaviour reveals how animals would behave in the wild. In chapter 2, using blue tits (*Cyanistes caeruleus*) I tested first whether behaviour in captivity predicted foraging behaviour in the wild. I measured the personality traits neophobia (latency to feed in novel scenarios) and exploratory tendency, first by relatively standard captive protocols and second, using an electronic monitoring system at feeding stations, by novel wild methods. As predicted, analogous traits correlated across contexts. Moreover, neophobia and exploratory tendency were uncorrelated within individuals in both contexts, in contrast to many other species. In captive studies, personality types also respond differently to changing environmental cues, or "environmental sensitivity": neophobic and non-exploratory types adjust behaviour whilst neophilic and exploratory types maintain foraging routines. In chapter 3, I tested this second captive prediction in the wild, defining environmental sensitivity in the wild by changes in feeder use with varying air temperature or food supply. Neophobic and, contrary to expectation, exploratory blue tits were most environmentally sensitive. By contrast, neophilic and non-exploratory birds visited feeders at a fixed level independent of temperature and continued to visit feeders for a prolonged period even after they were emptied. Age and body size also influenced environmental sensitivity, suggesting learning and dominance interactions modify the expression of personality in the wild. From potential behavioural costs, in chapter 4 I turned to the physiological costs of personality. Variation in metabolic rate and stress metabolism may be proximate mechanisms for personality. Whilst these physiological traits are linked to oxidative stress directly, with pro-oxidants that damage body tissue a by-product of metabolism, few studies link personality to oxidative stress. I found that oxidative profile (pro-oxidants, antioxidants, oxidative stress and oxidative damage) and hence physiological costs differed not

only within traits but also related differently to neophobia and object exploration in captive-bred greenfinches (*Carduelis chloris*). Finally, variation in response to environmental cues may reflect differences in learning between individuals, as perhaps illustrated by age differences in environmental sensitivity (Chapter 3). In chapters 5 and 6, I investigated whether learning that a feeding site is temporally stable could cause changes in response to food appearance ("local cues") when foraging. I predicted that birds would re-find food by spatial rather than local cues in these scenarios, as appearance can change hence local cues become unreliable over time. In chapter 5, I carried out an associative learning test to test this prediction in captive-bred greenfinches. Within a simple foraging scenario, the prediction was upheld: greenfinches favoured local cues in situations where the temporal stability of food was unknown, but switched to spatial cues when temporal stability was learnt through repeated encounters. In chapter 6 though, four of five wild bird species foraging at temporally stable bird feeders continued to respond to local cues, selecting feeders on the basis of colour. Most species were biased toward red feeders, and also responded to social cues when finding feeders: foraging strategies better suited to finding ephemeral food than re-finding temporally stable feeding sites. I suggest that wild birds use information on temporal stability from the broader environment (i.e. natural ephemeral food beyond temporally stable artificial feeders). This illustrates how animals may not necessarily forage in the wild as we would expect within specific contexts. Throughout this thesis therefore, my findings illustrate the importance of testing predictions generated from captive behaviour in the wild. Moreover, identifying variation in both the foraging strategies and physiological costs to individual variation in behaviour, this thesis provides new insight into the adaptive significance of animal personality.

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CANDIDATE'S DECLARATION

I declare that the work recorded in this thesis is entirely my own composition and that research described herein was carried out by me unless otherwise stated or acknowledged. No part of this thesis has been submitted for another degree.

Katherine Herborn

May 2010

CHAPTER 1

GENERAL INTRODUCTION

1.1 PRIMER

The question of how animals adapt to an environment that is constantly changing is at the heart of evolutionary theory (Darwin, 1859; MacAuthur and Pianka, 1966; Maynard Smith, 1982). Describing life in the intertidal zone, perhaps the archetype of variability, Scapini (1988) observed that "*stable, predictable environments tend to produce homogenous behaviour while rapidly changing, unpredictable environments tend to induce learning and plasticity*". Thus, animals that are more behaviourally plastic and learn may be expected to cope better in a highly variable environment than animals that are behaviourally fixed and do not learn (Klopfer and MacArthur, 1960). In a more stable environment though, where a stereotyped response will suffice, behavioural plasticity and learning may be a "luxury" (Dall and Johnstone, 2002) or even maladaptive (DeWitt et al., 1998). It is predicted therefore that species evolve a level of behavioural plasticity and consequently learning that corresponds positively to the variability of their environment (DeWitt and Scheiner, 2004). Equipped with a range of potential responses, to then respond adaptively to particular environmental cues animals must make a trade-off between the current costs and benefits of responding in alternate ways (Maynard Smith, 1982).

Response to an environmental cue may therefore be limited both by phylogenetic constraints (i.e. a species' innate plasticity) and also an individual's past opportunity to learn about that particular cue (Caro and Bateson, 1986). We can infer the selective pressures favouring plasticity by comparing the response of different species to the same environmental cues (see Fig. 1-1a). For example generalist species such as the song sparrow (*Melospiza melodia*) must constantly find new food types as they move through environments and seasons: this may explain why song sparrows respond less

"neophobically" toward novel foods or feeding environments than specialist swamp sparrows whose diet and habitat is constant *Melospiza georgiana*; Greenberg, 1989).

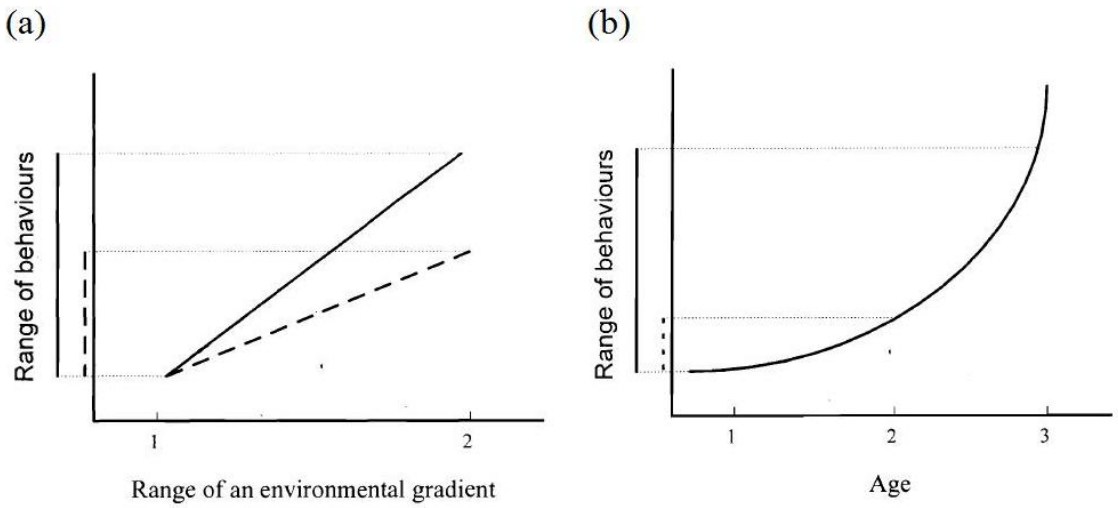


Figure 1-1 Behavioural reaction norm plots to illustrate sources of variation in behavioural plasticity, modified from Komers (1997). Reaction norms are linear functions relating the change in a trait to an environmental gradient (Via et al., 1995). (a) The degree of behavioural plasticity (i.e. range of behaviours exhibited) as a function of environmental variability, with 1 low and 2 high variability. The steeper the line, the greater the behavioural variation, hence the line is most plastic and dashed line most stereotyped. The lines may represent different species (e.g. the line a generalist and dashed line a specialist; Greenberg, 1990), conspecifics differing in experience (e.g. the line predator-naive sticklebacks, *Gasterosteus aculeatus*, and dashed line predator-experienced sticklebacks, Bell and Sih 2007) or conspecifics differing in genotype (e.g. the line slow-exploring great tits, *Parus major*, and the dashed line fast-exploring great tits; Marchetti and Drent 2000). (b) Behavioural plasticity as a function of age. In this scenario, the costs of plasticity (or benefits of stereotypy) may be lower in adults and/or certain responses may be acquired by learning (Caro and Bateson, 1986). If the cost-benefit trade-off favours lower plasticity though (Bell and Sih 2007), by learning not to express certain responses the inverse curve is possible. In chapter 3, I compare individuals' behavioural reactions norms in response to variation in air temperature.

Populations subject to different selection pressures may also be compared. For example, populations of house sparrow (*Passer domesticus*) in the act of invading new environments have lower neophobia than settled populations (Martin and Fitzgerald, 1995). However, variation between house sparrows may also reflect learning: invaders may be adapting by learning new food types and residents may have learnt to recognise what food is good. To further partition variation into innate and learnt components, we can compare naive individuals before and after experiencing environmental cues (Bell and Sih, 2007) or juveniles to adults (Exnerova et al., 2010; see Fig 1-1b). Finally, by comparing responses within individuals as contexts (functional categories, e.g. foraging, anti-predator) or situations (time points or gradients - e.g. temperature, predation risk - within contexts) change, I can infer the motivation or experience equated in a given trade-

off. For example, on encountering a competitor the variety of aggressive responses in sticklebacks and, on encountering a potential mate, courting behaviours in pipefish (*Sygnathus typhle*) are reduced in the presence of predators (Bell and Sih, 2007) or as predation risk increases (Berglund 1993). Therefore a cost of aggression and courtship respectively is increased predation risk.

A striking observation from such studies though, is that a proportion of behavioural variation between individuals cannot be explained by an individual's species, experience, context or situation. Moreover, differences between otherwise similar conspecifics, in for example aggression (aggressive - passive; Huntingford, 1976), activity level (active-inactive; Sih et al., 1992), sociality (sociable - antisocial; Cote and Clobert, 2007), exploratory tendency (fast - slow or high - low; Verbeek et al., 1994) and "boldness" (latency to feed in novel or risky environments; Clark and Ehlinger, 1987, Wilson et al., 1993, van Oers et al., 2004) often persist across situations or contexts (for review: Sih et al., 2004). This "intra-individual consistency and inter-individual variation" (Schuett and Dall, 2009) is often referred to as "personality" (Gosling, 2001). Heritability in personality traits (Dingemanse et al., 2002, Drent et al., 2003, van Oers et al., 2004) and differences in fitness or survival between personality types (Biro and Stamps, 2008) suggest that personality may be substrate from which innate behavioural plasticity evolves.

In this thesis, I will examine within and between-individual variation in response to environmental cues, with two key aims. First: to identify and assess the consequences of behavioural consistency, i.e. personality, for animals within captive and wild environments. And second: to examine the role of learning and memory in individual response to a temporally stable foraging situation.

1.2 Personality: within-individual consistency and between-individual variation in response to environmental cues

Personality is defined as between-individual differences but within-individual consistency in behaviour across situations or contexts. It constitutes limited behavioural plasticity, such that behaviour may be relatively adaptive within some contexts but suboptimal in others (see Fig. 1-2). Personality research represents a break with tradition: animals are compared not by their proximity to a postulated behavioural optimum but instead by their differences in behaviour (Sih et al., 2004). Such differences, falling into five general categories: activity, exploration, boldness, aggression and sociability (Reale,

2007) appear ubiquitous to animal life, described in animals as diverse as humans (Nettle, 2006) and invertebrates (reviewed in: Gosling and John, 1999).

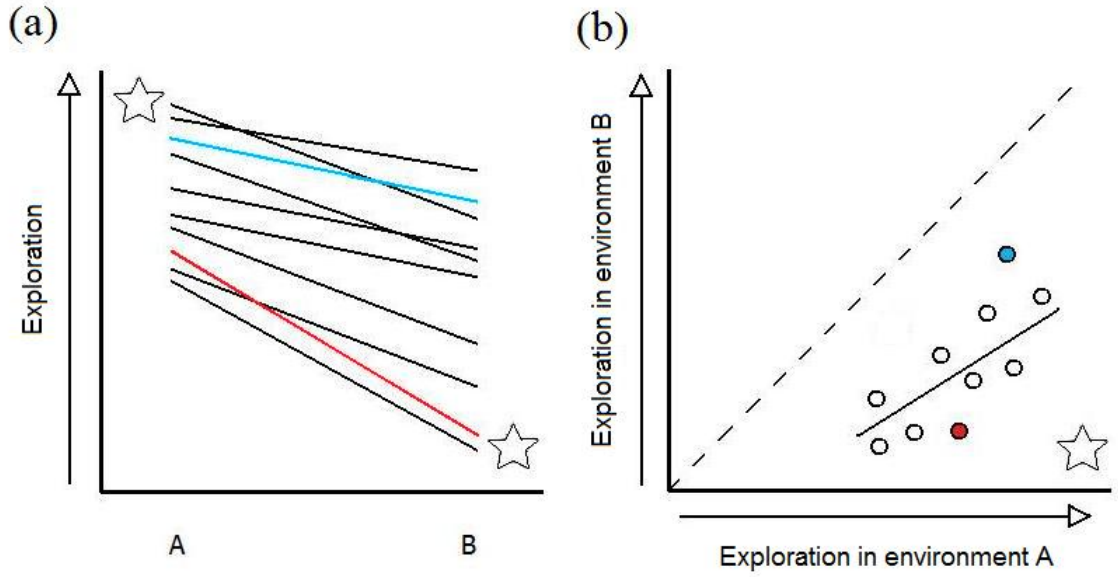


Figure 1-2 Plots describing the personality trait exploratory tendency (modified from Sih et al., 2004). (a) A plasticity plot representing individual change in exploration level across environments. Each line represents an individual, with the intercept at each environment (A or B) their exploration level within that environment. The optimal level of exploration is indicated by stars (high in A, low in B). Individuals alter their level of exploration according to environment, but not to the optimum level. Rank-order differences in exploratory tendency are largely maintained across environments (e.g. the blue individual has higher exploratory tendency than the red individual in both environments). Often, individuals at the "slow" end of personality traits (i.e. passive, inactive, shy or, here, less exploratory types) change behaviour more or faster in response to environmental change than fast (aggressive, active, bold, exploratory) types, i.e. they are more "environmentally sensitive" . For example, the less exploratory red individual changes exploration level more (has a steeper slope) than the more exploratory blue individual. (b) Personality types adjust their behaviour in accordance with environment: the points represent individuals, the dashed line a scenario where exploration level is independent of the environment, and the line the actual relationship between exploration levels across environments. Again, the red individual is more environmentally sensitive than the blue individual, so has closer to the optimum level of exploration (indicated by the star) in each environment.

Changing predation risk (Bell and Sih, 2007, Fraser et al., 2001, Reale and Festa-Bianchet, 2003), food availability (Dingemanse et al., 2004) and habitat quality for breeding (Quinn et al., 2009) have all been shown to differentially affect survival or reproduction between personality types in the wild. Furthermore, an increasing number of studies show that personality traits may be heritable (Benus et al., 1991, Dingemanse et al., 2002, van Oers et al., 2004b, Brown et al., 2007, Quinn et al., 2009) and linked to particular genes (Fidler et al., 2007, Korsten et al., 2010). Personality variation may therefore reflect ecologically relevant variation within populations, maintained by differential selection across environments or time (Dingemanse et al., 2007, Dingemanse

et al., 2004, Bell, 2005). Moreover, the existence of alternative phenotypes that differ in their performance of ecological functions such as foraging are a route by which allopatric or indeed, via niche specialisation, sympatric speciation may occur (Darwin, 1859; Maynard-Smith, 1966; West-Eberhart, 1989, 2003).

1.2.1 Proactive-reactive personality

As a description of consistent behaviour differences across contexts or situations, the term personality is often used interchangeably with temperament (Fairbanks, 1993, Gosling, 1998), coping style (Benus et al., 1991, Verbeek et al., 1996, Koolhaas et al., 1999) or behavioural syndrome (Sih et al., 2004). Whilst these are largely analogous, the definition of behavioural syndromes and coping styles place particular emphasis on the existence of "suites of correlated behaviours", i.e. correlations between different personality traits across contexts or situations (Sih et al., 2004). Personality traits are often highly correlated within individuals, for example activity with exploration (Dingemanse et al., 2007, Martin and Reale, 2008), exploration with sociability (Nomakuchi et al., 2009, Pike et al., 2008) or boldness with aggression (Bourne and Sammons, 2008, Bell, 2005, Johnson and Sih, 2005). A commonly described trait correlation is the "proactive-reactive" syndrome, which encompasses boldness, aggression and exploration (Koolhaas et al., 1999, Carere et al., 2005).

Such correlations imply proximate links between traits, via genetic linkage or shared physiology (Verbeek et al., 1994). However, correlations can also occur when two traits are not mechanistically connected but rather subject to the same selection pressures (Bell and Sih, 2007, Dingemanse et al., 2007). This is evident when selection pressures are removed or altered and traits become uncoupled (Bell and Sih, 2007; Ruiz-Gomez et al., 2008; see also chapter 4).

Two traits which are often correlated are neophobia and exploration. Neophobia and exploration are both responses to novelty. In birds, neophobia is measured as the latency to return to a desired resource, for example food, in the presence of a novel object (Greenberg, 1983; van Oers et al., 2004, van Oers et al., 2005b). Literally "fear of the new", the object is assumed to generate a trade-off between desires to avoid unknown risks associated with the object but to obtain the resource (Richard et al., 2008). Hence: an individual's position on the neophobia axis reflects their trade-off between these costs and benefits. Exploration is the tendency to engage with novelty *per se*. Novelty may be introduced in the form of a new environment ("spatial exploration", Verbeek et al., 1994)

or a novel object within a familiar environment ("object exploration", Mettke-Hofmann et al., 2002). Food is not presented in exploration trials, so the motivation is assumed to be information gathering (Mettke-Hofmann et al., 2002). The costs of exploration may be the time, energy and attention diverted from other activities or risks, or indeed the potential (unknown) dangers of the novel object or environment itself (Johnston, 1982). The latency to enter (Mettke-Hofmann et al., 2009) or explore all parts of a novel environment (e.g. Verbeek et al., 1994), activity (e.g. Dingemanse et al., 2002) or space use within novel environments (e.g. Minderman et al., 2009), and latency to approach or time spent in contact with novel objects (e.g. Mettke-Hofmann et al., 2002) are all assays of exploratory tendency.

In the great tit, a positive correlation between neophobia and exploration appears to be under genetic control (van Oers et al., 2004a). Indeed in a range of species “novelty seeking” behaviour may be linked to polymorphisms within a single gene: the dopamine receptor D4 (DRD4), referred to colloquially as the “curiosity gene” (Schinka et al., 2002, Korsten et al., 2010, Fidler et al., 2007). As such, the personality traits neophobia and exploration may be alternate measures of a single proactive-reactive syndrome, measured in different (familiar versus novel) environments. However two lines of evidence suggest that these are distinct personality traits subject to different motivations and selection processes. First, comparing neophobia and object exploration in a broad range of parrot species Mettke-Hofmann and colleagues (2002) found different ecological correlates to the two traits. Exploration was fastest in species that may benefit most from information gathering, for example those inhabiting relatively changeable (e.g. forest edge) versus homogeneous habitats (see also Tebbich et al. 2009). Conversely, neophobia appeared related to dietary risk: novel insects are potentially noxious, and insectivorous species were more neophobic than leaf-eating species. Similarly, amongst tit species innate neophobia, measured in naive hand-raised juveniles, correlates to body size: it is suggested that this reflects the proportional risks of ingesting toxins (Exnerova et al., 2010).

Second, in physiological studies neophobia appears related to physiological stress responsiveness, i.e. reactivity of the hypothalamic-pituitary-adrenal axis ("HPA" axis, hypothalamic-pituitary-interrenal axis in fish; Koolhaas et al., 1999, Cockrem, 2007), whilst exploration does not. An animal’s HPA axis is activated in response to environmental stressors (Cockrem and Silverin, 2002). This causes an increase in circulating gluccocorticoids (stress hormones), which in turn cause secretion of glucose into the plasma, stimulating the metabolism to allow rapid behavioural reactions to environmental stressors (Cockrem, 2007, von Holst, 1998). In birds for example, the

main gluccocorticoid stress hormone is corticosterone ("CORT", Cockrem, 2007), and elevated CORT is associated with stressors such as sight of a predator (Cockrem and Silverin, 2002) and low food availability (Muller et al., 2007). Accordingly, CORT stimulates behavioural responses such as the fleeing (Cockrem, 2007) or increased foraging effort (Rich and Romero, 2005). However a behavioural response does not necessarily indicate a CORT response (Muller et al., 2006). Encountering novel objects either with or without food nearby can both elicit avoidance behaviours in birds. In the few studies that have compared the level of CORT before and after presentation of a novel object though, with food approaching the novel object appears to be physiologically stressful (Richard et al., 2008) but in a neutral location it is not (Mettke-Hofmann et al., 2006, but see Apfelbeck and Raess, 2008). Therefore it appears that only neophobia elicits a physiological stress response.

An aim of this thesis was to test whether neophobia and exploration in blue tits (*Cyanistes caeruleus*; chapter 2) and greenfinches (*Carduelis chloris*; chapter 4) are distinct personality traits or part of a single proactive-reactive trait. In chapter 4, I investigated also whether these traits correlated together or were independent predictors of individual oxidative stress or oxidative damage, potential physiological costs of personality (see 1.2.3).

1.2.2 Ecological relevance of personality

The field of behavioural ecology has traditionally drawn inspiration from the behaviour of animals in the wild (for review: Krebs and Davies, 1997). Cognitive ecologists for example, who examine adaptation at the level of psychology and neurology (Real, 1993; Healy et al., 2005), have been inspired by observations of the remarkable abilities of storing animals such as the coal tit (*Periparus ater*) and marsh tit (*Parus palustris*), which hide and then are able to retrieve often hundreds of food items about their environment days or even months later (Clayton, 1995). How they perform this feat has been the subject of captive study for over twenty years, with these food storers and non-storing congeners compared in memory, brain morphology and cue selection (for review see: Healy et al., 2005).

In contrast, the field of animal personality has stemmed from side observations on captive animals. The existence of consistent behavioural differences between individuals was recognized in fields such as animal husbandry (Metcalfe et al., 1989; Cutts et al.,

1998, Koolhaas et al., 1999) and behavioural genetics (Benus et al., 1987, Benus et al., 1991) long before the term "animal personality" (Buss, 1991) was coined. It was seemingly counterintuitive examples of consistency however, for example aggression in bold sticklebacks (*Gasterosteus aculeatus*, Huntingford, 1976), persistent foraging under predation risk in salamander larvae (*Ambystoma barbouri*; Sih et al., 1988), or correlations between aggression and sexual cannibalism in the fishing spider (*Dolomides triton*; Arnqvist and Henriksson, 1997) that first sparked interest in behavioural ecologists, and the beginnings of this new field.

Until recently, the emphasis of animal personality research has been on establishing the existence of personality in non-human animals (for review: Gosling and John, 1999), with some work on the proximate mechanisms underlying personality variation (for review: Dingemanse and Reale, 2005). By comparison, studies on ultimate, adaptive explanation for personality variation are relatively few (Dingemanse and Reale, 2005). A collection of recent theoretical (Wolf et al., 2007, Wolf et al., 2008, Dall et al., 2004, Stamps, 2003, McNamara et al., 2009) and field studies (Dingemanse et al., 2004, Dingemanse and Reale, 2005, Fraser et al., 2001, Boon et al., 2008, Reale and Festa-Bianchet, 2003) on fitness and survival have begun to redress this balance. However the behavioural mechanisms which manifest in fitness and survival differences are still predicted from captive studies, on the assumption that behaviour in captivity will reflect behaviour in the wild (e.g. Dingemanse et al., 2004). In the few studies that compare behaviour across captive and wild contexts in a like-for-like manner, support for this assumption is mixed (Wilson and McLaughlin, 2007, Dingemanse and de Goede, 2004, van Overveld and Matthysen, 2010, Briffa et al., 2008). Moreover, as correlations between personality traits are often highly context specific (Dingemanse et al., 2007, Bell and Sih, 2007), without examining behaviour in the wild we cannot establish whether or indeed which of correlated traits explain variation in fitness or survival (Hollander et al., 2008).

More recent studies have sought to give ecological relevance to behaviours in captivity. Captive studies relating personality to changes in perceived predation risk for example are an important step in linking captive to wild behaviour. By introducing a pike (*Esox lucius*) to predator-naive captive sticklebacks (*Gasterosteus aculeatus*), Bell and Sih (2007) were able to examine the effects of both predation and predation experience on population level variation in personality in sticklebacks, and found differences pre- and post-predator experience that mirrored variation between wild predator-experienced and predator-naive populations (Bell, 2005, Dingemanse et al., 2007). Qualitative

responses to predators too are important, and the captive environment enables controlled study of changes in behaviour (Jones et al., 2008). Quinn and Cresswell (2005) for example, utilised the captive environment to subtly manipulate predator exposure in wild caught chaffinches (*Fringilla coelebs*). Hawk attack was simulated by gliding a model hawk over the caged bird, either to one side (low predation risk) or directly above (high predation risk). A general switch from freeze to flee responses with increasing predator proximity could be explained by the value of these responses in each context. At the individual level though, personality types differed in the optimality of their response across contexts, illustrating a mechanism by which behavioural consistency may result in differential selection on animals living under different levels of predation risk.

However in the wild, animals are subject to and learn about different and constantly changing environmental conditions. This diversity of conditions can never truly be replicated in captivity. A collection of recent studies investigating both behaviour and survival in the wild are an important step toward understanding the ecological relevance of personality (Boon et al., 2008, Reale and Festa-Bianchet, 2003, Fraser et al., 2001). An aim of this thesis (chapter 2) was to test first the assumption that neophobia and exploratory tendency in captivity corresponds to neophobia and exploratory tendency in the wild.

1.2.3 Physiological costs of personality

Oxidative stress is an imbalance between pro-oxidants, which react with and damage body tissue, and antioxidants, which neutralize pro-oxidants, in favour of pro-oxidants (Finkel and Holbrook, 2000, Costantini and Verhulst, 2009). As pro-oxidants are primarily a metabolism by-product, a physiological cost of increases in activity or physiological stress, which both stimulate the metabolism, can be increased oxidative stress or damage (Finkel and Holbrook, 2000). Animals face many taxing periods in life when stress and activity levels and consequently oxidative stress are enhanced, such as migration (Costantini et al., 2007) and reproduction (Wiersma et al., 2004). However, research on laboratory animals (Careau et al., 2009), particularly lines selected for specific personality types (Groothuis et al., 2008, Veenema et al., 2003, Martins et al., 2007, Overli et al., 2007, Richard et al., 2008, Saint-Dizier et al., 2008, Costantini et al., 2008), and also farm animals selected for certain behavioural tendencies (Cutts et al., 2001, Cutts et al., 2002b, Cutts et al., 1998, Fraisse and Cockrem, 2006, Koolhaas et al.,

1999) suggest personality may represent consistent differences in metabolic rate or stress responsiveness, and hence possibly also oxidative costs (Costantini et al., 2008).

First, personality may relate to systematic variation in basal or standard metabolic rate ("BMR" or "SMR", Careau et al., 2008). The trait aggression for example is positively associated with SMR in salmonid fish species, with aggressive types shown to have higher SMR than passive types (Cutts et al., 2002a, Cutts et al., 2002b, Cutts et al., 1998). In this case, a higher SMR explains aggression by allowing greater energetic expenditure in agonistic encounters. With the trait exploration though, within muroid rodents it appears that exploratory types have lower BMR than less exploratory types (Careau et al., 2009). Muroid rodents with low BMR also have delayed reproduction: apportioning energy into exploration in lieu of reproduction, for these animals exploratory tendency may be selected in food-poor environments where it is beneficial to acquire resources prior to breeding (Careau et al., 2009). With metabolic rate therefore, the physiological costs of personality may differ between personality traits or species, and are contingent on the environment in which the animal lives (Stamps, 2007, Biro and Stamps, 2008). In general, in food-rich environments, personality types with high metabolic rates may thrive and out-compete others by monopolizing food (Cutts et al., 2002a, Dingemanse et al., 2004) or investing more into reproduction (Careau et al., 2009). In food-poor environments though, these energetically costly behaviours cannot be sustained, and personality types with lower metabolic rates may cope best (Stamps, 2007, Dingemanse et al., 2004).

Second, the personality traits boldness and aggression are linked to variation in physiological stress responsiveness, i.e. reactivity of the HPA axis (Koolhaas et al., 1999, Cockrem, 2007, von Holst, 1998). HPA axis reactivity has been shown to be higher in shy or passive than bold or aggressive mammals (Veenema et al., 2004, Ruis et al., 2000, Cavigelli and McClintock, 2003, Martin & Réale, 2008; Cavigelli et al., 2009) birds (Carere et al., 2003, Martins et al., 2007, Richard et al., 2008, for review: Cockrem, 2007) and fish (Hoglund et al., 2008, Brelin et al., 2008). Eliciting rapid behavioural responses to stressors, stress responses can clearly be beneficial in the short term (Korte et al., 2005). In the long term though, high or chronic stress responsiveness is costly: stress responses are an investment of time and energy and gluccocorticoids themselves suppress immune function (Koolhaas, 2008, Korte et al., 2005). Despite these costs, high stress responsiveness may be beneficial in environments where the dangers of excessive aggression or risk-taking by insensitivity to stressors can outweigh these physiological costs (Korte et al., 2005, Bell and Sih, 2007, Natoli et al., 2005). Like metabolic rate, the

costs of personalities with high versus low stress responsiveness will depend on the environment in which the animal lives.

As pro-oxidants are mostly a metabolism by-product, personality types with higher metabolic rate and/or higher stress responsiveness may be expected to suffer worse oxidative stress. However, whether personality relates to oxidative stress has only been studied once: in a study comparing aggressive strains of mice (*Mus musculus* with short attack latencies, i.e. "SAL" mice) to passive strains (long attack latency, i.e. "LAL" mice), Costantini and colleagues (2008) found higher antioxidant capacity in LAL than SAL mice. Despite greater antioxidant capacity LAL mice did not have lower oxidative stress than SAL mice, so the authors suggest that higher antioxidant capacity may conceal the production (and neutralization) of more pro-oxidants. Whilst no greater oxidative stress was evident therefore, this up-regulation of the antioxidant system may itself be costly and, as noted by the authors, LAL mice have generally a shorter lifespan than SAL mice (Ewalds-Kwist and Selander, 1996). Whilst stress responsiveness is the most likely explanation for the oxidative profile variation in this case (SAL mice would be expected to have higher pro-oxidant than LAL mice if metabolic rate was the cause), in other studies there is indirect evidence of a cumulative oxidative cost to personality types with higher metabolic rate. For example, within the "activity" personality trait, active (hence most metabolically active) types have shortest lifespan (for review: Biro and Stamps, 2008). An aim of this thesis was to explore relationships between oxidative profile and personality in the European greenfinch (*Carduelis chloris*; chapter 4).

1.3 Responding to changing environmental cues

The natural environment is constantly changing. One strategy for coping with uncertainty is to reduce it, by learning about changing environmental cues. Shepard's law states that, with experience of a conditioned stimulus (e.g. food) at two or more points along an environmental gradient (e.g. plant density), an animal will weight its response to a novel point by its expectation of how the conditioned stimulus and environmental gradient relate (Shepard, 1987). Therefore by sampling for food at two points along visual (e.g. food colour), temporal or spatial gradients, a forager may "*be a statistician*" (Pyke, 1984) and predict the appearance of as yet un-encountered foods, rates of turnover and broader spatial distributions (Church and Gibbon, 1982; Cheng et al., 1999; Cheng and Spetch, 2002). By sampling more points along these gradients, an individual's "information state" (Mitchell, 1989) is further refined, and their ability to predict the

environment improved (Dall and Cuthill, 1997). Therefore variation in behaviour between individuals may reflect differences in opportunity for learning, for example experience of particular environmental stimuli or with age, hence total experience.

However, learning comes at a cost (Johnston, 1982). To learn animals must gather information (i.e. sample), the time, energy and attention required for which is diverted from other activities, such as foraging, mating and avoiding predators (Johnston, 1982). Moreover, where information is incomplete, animals may change their behaviour in ways that are not adaptive (i.e. make "mistakes"; Johnston, 1982; West-Eberhart, 2003). It is predicted therefore that behavioural plasticity via learning, and its accompanying sensory and cognitive machinery, is selected only in more variable environments where the benefits of "keeping pace" outweigh these costs (Mangel, 1990; Bergman and Feldman, 1995; Stephens, 1989). For example interspecific variation in exploratory tendency may reflect differential benefits of learning about environmental change and hence information gathering between for example generalist and specialist species (e.g. Greenberg, 1995) or animals in complex (forest edge) versus homogeneous habitats (Mettke-Hofmann et al., 2002, Tebbich et al., 2009).

1.3.1 Environmental sensitivity

The process of responding to environmental change via learning requires behavioural plasticity, i.e. an ability to modify behaviour. Intriguingly, behavioural plasticity may vary not only between but also within species: personality types differ in their response to environmental cues but also their propensity to modify behaviour as environmental cues change (Dingemanse et al., 2010). For example, trained to navigate a maze to find food, passive mice adjusted their behaviour in response to a change in maze structure more quickly than aggressive mice (Benus et al., 1987). This responsiveness to change, termed "environmental sensitivity" (Koolhaas et al., 1999; Boyce and Ellis 2005), is often greater or faster at the "slow" (passive, neophobic, non-exploratory) than the "fast" (aggressive, neophilic, exploratory) extremes of personality traits (Benus et al., 1987, Benus et al., 1988, Benus et al., 1990, Verbeek et al., 1994, Marchetti and Drent, 2000, Koolhaas et al., 1999, Jones and Godin, 2010, see Fig. 1-2). With traits such as exploration therefore, this seems contrary to expectation, as between-species variation in exploratory tendency is predicted to enable greater behavioural flexibility via information gathering. Within species though, "fast" exploration may not necessarily indicate a greater tendency but

rather qualitatively different approach to information gathering and learning (Van Overveld and Matthyssen, 2009).

In captive studies on great tits for example, fast-exploring great tits continued to visit former feeding sites for a prolonged period after food is withdrawn (Marchetti and Drent, 2000). In contrast, slow-explorers quickly shifted their search for food toward new parts of the aviary (Marchetti and Drent, 2000). Therefore slow explorers were defined as more environmentally sensitive (i.e. they responded more quickly), and it appears that this environmental sensitivity is based on a faster learnt association between the feeding site and unprofitability. Similar captive examples of routine-formation in fast types are found in rodents (Benus et al., 1987). From these studies, it is predicted that fast-explorers may form similarly fixed foraging routines in the wild. However, investigating a population of wild great tits, Overveld and Matthyssen (2009) found that the abrupt removal of an artificial feeding site stimulated fast explorers to move to new foraging areas whilst slow explorers remained within the vicinity of the old feeding site (Overveld & Matthysen, 2009). As the great tits in that study did not differ in feeding range size after that manipulation, the authors suggest the movement reflected not differences in propensity toward information gathering but rather variation in the way individuals used information: fast explorers relied on old information, returning to formerly encountered feeding sites (thus explaining the sudden distant movement). In contrast, slow explorers relied on current information, remaining able to forage within the site by having updated their information on alternate food sources despite the availability of food at feeders. This example illustrates two important points. First, again, that the ecological significance of personality variation cannot be fully understood without examining behaviour in the wild. And second, that measuring environmental sensitivity may be equally as important to understanding the adaptive significance of personality as measuring personality traits *per se*. In chapter 5, I investigate the relationship between environmental sensitivity and neophobia and exploratory tendency in wild blue tits.

1.3.1 Cue selection: measuring perception of environmental predictability

Cue selection is the process of recognising, learning or remembering a particular aspect of a multimodal stimulus. Broadly, two types of cue may be used to re-find food in a fixed location: spatial cues and local cues. Spatial cues are geometric relationships between a food location and more permanent landmarks (Cheng 1999; Collett, 1987) or

the broader environment (the "cognitive map"; O'Keefe and Nadel, 1978). Local cues are associatively learnt aspects of the food or feeding site itself, for example its colour, pattern, texture or odour. When the distribution of food is ephemeral, local cues can be learnt to increase foraging efficiency (the 'search image': Tinbergen, 1960; Dawkins 1971; Lawrence 1986). However, local cues generally change over time more quickly than permanent spatial features, for example the appearance, taste and smell of a fruit alter as it ripens but the position of the fruit tree does not. Therefore, spatial cues are more reliable than local cues when relocating temporally stable food sources. This generates a prediction: where animals perceive the environment to be temporally stable, they should favour spatial over local cues.

Preferences for spatial or local cues, and accordingly perception of temporal stability, may be inferred by first training animals to first locate food by a compound cue (e.g. a colour marker in a set location) and then dissociating the local and spatial cues (moving the colour marker to a new location) to see to which an animal attends first. By such dissociation tasks, ecologically pertinent differences between species in cue selection arise. For example food-storing birds, which hide and must relocate food throughout the winter, often hold spatial biases whilst closely related non-storers use both cues equally (Clayton and Krebs, 1994, Brodbeck, 1994, Brodbeck and Shettleworth, 1995, but see Hodgson and Healy, 2005, LaDage et al., 2009). As the location of stored food does not change over time, a stereotyped spatial response may be adaptive in storers (Krebs, 1990).

However, it could equally be inferred that the use of both cues by non-storers represents adaptation toward greater plasticity in cue use. Non-storers (most birds) feed on ephemeral food (e.g. insects, fruit) that may vary widely in distribution from scattered to clumped, i.e. more or less temporally stable. Therefore innate plasticity coupled with an ability to learn about environmental predictability may be advantageous in these species. Odling-Smee and Braithwaite demonstrated innate plasticity coupled with learnt cue prioritization in sticklebacks: sticklebacks from ponds use both cue types to navigate a maze to find food. Conversely, sticklebacks from streams are biased toward spatial cues, perhaps because the turbidity of the water renders local cues less reliable (Odling-Smee and Braithwaite, 2003). Most cue selection research has focused on species such as storers that are expected to have special learning abilities. Whether non-storers equipped with a prior expectation of temporal stability use spatial cues in place of local cues has not been tested. The aim of chapters 5 and 6 was to test this hypothesis, first in a controlled captive foraging scenario (Chapter 5) and then in the wild (Chapter 6).

1.4 AIMS OF THE THESIS

The thesis is divided into two parts. In chapters 2, 3 and 4 I investigate personality, examining variation in foraging behaviour between personality types in wild blue tits (*Cyanistes caeruleus*; chapters 2 and 3) and physiological costs of personality in captive-bred greenfinches (*Carduelis chloris*; chapter 4). In chapters 5 and 6, I then investigate cue selection in temporally stable environments, first in captivity with greenfinches and then in the wild with five common garden passerines.

1.4.1 THE BLUE TIT

For wild studies on personality (chapters 4 and 5), my study species was the blue tit (*Cyanistes caeruleus*, formally *Parus caeruleus*). The blue tit is a small (c. 11g) insectivorous passerine of the Paridae family. Breeding readily in nest boxes, it has become a model species for studies on sexual selection (e.g. Hunt et al., 1998, Hadfield, 2006), breeding phenology (Liedvogel et al., 2009, Sanz et al., 2002) and provisioning behaviour (Tripet et al., 2002, Arnold et al., 2010). Personality research though has generally focused on the blue tit's congener, the great tit (*Parus major*; for review: Groothuis and Carere, 2005). I opted to study personality in blue tit for three reasons. First, blue tits are more numerous than great tits at my study site: they outnumbered great tits by a ratio of 2:1 in the 2007-2008 season (mist-netted individuals at artificial feeders: 113:61) and 3:1 in the 2008-9 season (58:19).

Second, in 37 great tits that I personality tested in captivity, participation in trials was low. In the neophobia trial, 23 great tits could not be classified for neophobia, i.e. failed to approach the food bowl at least once in the presence and once in the absence of a novel object over two attempts. This is compared to just 5 out of 125 blue tits. In the first of two exploration trials, which was used to classify "exploratory tendency", activity was much lower in the great tit (median number of movements and IQR: 12, 163) than the blue tit (182.5, 300.8; see Appendix I). For drawing comparisons between captive and wild behaviour therefore, blue tits were more often successfully classified for neophobia and presented a greater range of exploration types than did great tits.

Finally a collection of recent studies on the genetics and ontogeny of behaviour (Hansen and Slagsvold, 2007, Nilsson et al., 2009, Exnerova et al., 2010, Arnold et al.,

2007, Liedvogel et al., 2009, Exnerova et al., 2003) suggest that blue tits may differ consistently in behaviour within natural contexts. As such, thus are a good study species for personality research. Three studies propose genetic variation in behaviour. First, natural variation in the *Clock* gene, which in humans is associated with the personality trait "agreeableness" (Terraciano et al., 2008), influences timing of reproduction in female blue tits (Johnsen et al., 2007, Liedvogel et al., 2009). Blue tits may therefore vary consistently in their responsiveness to environmental cues such as day length. Second, blue tits have heritable differences in resting metabolic rate (Nilsson et al., 2009). Variation in metabolic rate is associated with personality in a range of species and may be a mechanism underlying personality variation (Careau et al., 2008). Third, blue tits exhibit an innate aversion toward red aposematic (as opposed to brown palatable) insects (Exnerova et al., 2010, Exnerova et al., 2003). If variation in this trait exists, individuals may differ in the personality trait: neophobia.

Two studies imply ontogeny-based variation in behaviour. Within my study population Arnold et al. (2007) have identified affects of nestling nutrition on the development of "bold" personality types (fast object exploration). Specifically, individuals supplemented with taurine, an amino acid found within the nestling diet in spiders (Ramsay & Houston, 2003), developed bolder personality types than controls. As a high proportion of spiders relative to moth larvae, the main constituent of the nestling diet, could represent a poor foraging environment, the authors suggest early nutrition could then predispose individuals toward boldness as an aid to foraging in a poor foraging environment. Second, Hansen and Slagsvold (2004) demonstrated that early social environment predisposed individuals toward later aggressive tendency. When great tit nestlings, which share a similar ecological niche but are competitively dominant over blue tits, were cross-fostered into blue tit nests or vice versa, in adulthood their nest-mate blue tits were less aggressive toward conspecific immigrants (unfamiliar and generally subordinate birds) than controls (Hansen and Slagsvold, 2004).

That personality has not otherwise been studied in blue tits is surprising as, whether a variable of interest or as noise to control, studies on mate or territory quality often demonstrate that their behaviour is repeatable and independent of environmental conditions. For example provisioning rate (Biard et al., 2005), provisioning quality (Banbura et al., 1994), nest building behaviour (Mennerat et al., 2009) and, perhaps consequently, offspring quality (Pryzbylo et al., 2001) have all been shown to be repeatable and independent of habitat (hence food and nest material availability) in blue

tits. Over-winter repeatability in body mass (Payne and Payne, 1989) also hints at behavioural consistency.

I studied a population of blue tits wintering in oak dominated woodland on the east bank of Loch Lomond, UK (56°08'N 4°37'W). Captive behavioural studies on these birds were conducted in aviary facilities at the Scottish Centre for Ecology and the Natural Environment (SCENE). Wild behavioural data was collected using an electronic monitoring system: birds were fitted with leg-ring mounted passive integrated transponders ("PIT tags") and artificial feeding stations were fitted with PIT tag antennae to record feeder use during various manipulations.

1.4.2 THE GREENFINCH

For captive studies on the physiological costs of personality (Chapter 4) and cue selection (chapter 5), my study species was the European greenfinch (*Carduelis chloris*). The greenfinch is a predominantly granivorous finch from the Cardueline family (Newton, 1972). Greenfinches are often used as a physiological model for the costs of behavioural variation. Notable examples are the relative costs of mass gain with social status (Hake et al., 1996) or infection status to social status or predator escape performance (Lindstrom et al., 2003; Lindstrom, 2004). In greenfinches, condition indices such as body mass, metabolic rate and antioxidant capacity often prove consistent within individuals over months (Horak et al., 2002; Sepp et al., 2010). To maintain such differences between individuals, or perhaps as a consequence of them, greenfinches may also differ consistently in behaviour, i.e. exhibit personality. In a pilot study, I demonstrated that the personality trait neophobia was repeatable within individuals and consistent over a four month period (Appendix II). Therefore, greenfinches are a good model for studying the physiological consequences of personality (chapter 4).

In the early literature of interspecific variation in cue selection, the greenfinch was also used as a non-storing, "non-tit" control to demonstrate the special spatial-learning ability of tits (Hilton and Krebs, 1990). Comparative studies on hippocampus size too, the brain region associated with spatial learning, contrast the relatively small hippocampus of greenfinches (amongst other passerines) to the large hippocampuses of storing tits (Krebs et al., 1989). Not adapted to relocate food-stores therefore, the greenfinch has a long tradition of being the model of a species expected not to use spatial cues. Outside of the

breeding season however, the greenfinches naturally ephemeral food varies greatly in abundance: abundant ephemeral food (e.g. ripened crops) may be regarded and hence located, for a period, as temporally stable food sources (Humber et al., 2009). As such, I predicted that greenfinches may be particularly sensitive to temporal stability in feeding opportunities, but use spatial cues only in temporally stable contexts (Chapter 5).

1.4.3 THESIS STRUCTURE

Chapters 2, 3 and 4 investigate the consequences of personality for individuals within captive and wild environments. Chapters 5 and 6 deal with the use of local cues (colour cues) in temporally stable contexts, first in captive greenfinches and second in a five wild passerines. The chapters and their aims are described below.

Chapter 2: Personality in captivity reflects personality in the wild

The aim of chapter 2 was to test whether personality traits measured in captivity reflect variation between individuals in wild behaviour. I used a population of wintering blue tits as the focal species, and investigated the personality traits: neophobia and exploratory tendency.

- Examine effects of environmental conditions, body condition, sex and age on behaviour in captivity
- Test whether neophobia and spatial exploration are personality traits in blue tits
- Test whether blue tits exhibit a proactive-reactive behavioural syndrome
- Test whether personality traits measured in captivity predict analogous wild traits

Chapter 3: Personality predicts environmental sensitivity in the wild

Having validated that captive personality tests assay variation in wild behaviour in chapter 2, in chapter 3 I investigate the key prediction from other captive studies (Benus et al., 1990, Marchetti and Drent, 2000) that personality predicts environmental sensitivity. I measured environmental sensitivity in two ways. First, I measured environmental sensitivity to temperature as the tendency to reduce feeder use in response

to natural increases in temperature. Second, I measured environmental sensitivity to food availability as latency to abandon unrewarding feeding sites.

- Identify population level response to changes in temperature and food supply
- Examine whether average feeder use differs between personality types, ages or sexes or with body size
- Determine whether average feeder use, sex, age or body size affects tendency to respond to changes in temperature or food supply
- Assess whether personality types differ in environmental sensitivity to temperature and food supply

Chapter 4: Personality types differ in oxidative profile

Chapter 4 explores the physiological consequences of different personality traits within a stable environment. In captive bred greenfinches, I measured two personality traits: neophobia and object exploration, which I then related to various measures of oxidative profile. Specifically, I:

- Test whether neophobia and object exploration are personality traits in greenfinches
- Test whether greenfinches exhibit a proactive-reactive behavioural syndrome
- Determine whether oxidative profile measures are correlated within individuals
- Assess whether body mass and sex affects personality and/or oxidative profile measures
- Asssess whether personality affects oxidative profile measures

Chapter 5: Context specific preferences for local or spatial cues in the European greenfinch

In chapter 5, using captive bred greenfinches I test whether individuals respond to perceived changes in the temporal stability of a simple foraging situation by switching from local cues to spatial cues. Specifically, I:

- Examine whether greenfinches exhibit cue biases in temporally unstable contexts
- Determine whether cue switching was a dependent on time or repeated encounters with the foraging situation
- Assess whether greenfinches are able to learn spatial cues in the absence of local cues

Chapter 6: Use of colour cues by wild birds in a stable foraging location

The aim of chapter 6 was to test the prediction generated by the captive study in chapter 5 that birds should disregard local cues when foraging in a temporally stable foraging situation. The temporally stable foraging situations were sets of feeders of different colours installed in urban parks. I compare visitation to feeders by five common garden passerines between colours on the assumption that colour biases would be evident only if the situation was perceived to be temporally unstable.

- Describe affects of time of day and temperature on artificial feeder use
- Infer mechanisms underlying colour biases

CHAPTER 2

PERSONALITY IN CAPTIVITY REFLECTS PERSONALITY IN THE WILD

2.1 ABSTRACT

To investigate the ecological significance of personality, researchers generally measure behavioural traits in captivity. Whether behaviour in captivity is analogous to behaviour in the wild however, is seldom tested. I compared individual behaviour between captivity and the wild in blue tits (*Cyanistes caeruleus*). Over two winters, blue tits (N = 125) were briefly brought into captivity to measure exploratory tendency and neophobia using variants of standard personality assays. Each was then released, fitted with a Passive Integrated Transponder (PIT) tag. Using an electronic monitoring system, individuals' use of feeders was then recorded as they foraged in the wild. I used variation in the discovery of new feeders to score 91 birds for exploratory tendency in the wild. At eight permanent feeding stations, 78 birds were assayed for neophobia in the wild. Behavioural variation between individuals in the captive personality trials was independent of permanent (e.g. sex) and non-permanent (e.g. condition or weather at capture) sources of between-individual variation at capture. Individual behaviour in both captive and wild trials was repeatable, therefore exploratory tendency and neophobia constituted personality traits in the blue tit. Exploratory tendency and neophobia were not correlated with each other, either in the captive or wild context. Therefore they are independent traits in blue tits, in contrast to many species. Finally, exploratory tendency and neophobia measured in captivity positively predicted the analogous traits measured in the wild. Reflecting differences in the use of feeding opportunities, personality in captivity therefore revealed relevant differences in foraging behaviour between individuals.

2.2 INTRODUCTION

Confronted with the same environmental or behavioural stimuli, even within a homogenous captive environment, individuals of the same species often differ markedly in their behaviour (Verbeek et al., 1996, Gosling, 2001). Notable axes of variation are aggression (aggressive - passive; Huntingford, 1976), activity (active-inactive; Sih et al., 1992), sociality (sociable - antisocial; Cote and Clobert, 2007), exploratory tendency (fast - slow explorer; Verbeek et al., 1994) and risk-responsiveness (risk-prone-risk-averse, neophobic-neophilic or bold-shy; Clark and Ehlinger, 1987, Wilson et al., 1993, van Oers et al., 2004).Where differences in behaviour between individuals are stable across a range of situations or contexts, we refer to this variation as "personality" (Gosling, 2001). Heritability in personality traits (Dingemanse et al., 2002, Drent et al., 2003, van Oers et al., 2004) and differences in fitness or survival between personality types (Fraser et al., 2001, Dingemanse et al., 2004, Bell, 2005) suggest that personality may reflect ecologically significant variation between individuals.

Few studies measure personality in the wild (but see Coleman and Wilson, 1998, Reale et al., 2000, Reale and Festa-Bianchet, 2003, Hollander et al., 2008). To investigate the ecological significance of personality, researchers generally measure behaviour in captivity and compare the distribution or fitness of individuals in the wild thereafter (Dingemanse et al., 2004, Bell, 2005). Studying behaviour in captivity has numerous advantages, notably allowing researchers to control the conditions under which all individuals are tested (Campbell et al., 2009). However, classifying personality in captivity may be misleading for two reasons. First, behaviour changes as wild individuals adapt to the captive environment (Butler et al. 2006). Where there are systematic differences in the rate of acclimation between personality types therefore, testing in captivity may exaggerate or even generate behavioural differences between personality types. For example, risk-averse or "shy" individuals take longer to recover from handling or capture stress and also to eat in a novel environment than risk-prone or "bold" individuals (van Oers et al., 2004, van Oers et al., 2005b, Wilson et al., 1993). As food is usually withdrawn prior to personality trials and often returned within trials to stimulate behaviour, residual stress, hunger or condition may then motivate shy but not bold individuals to a greater extent in captivity than in the wild. Therefore, it is important to

test that behavioural differences between personality types extend beyond the captive environment.

Second, classifying behaviour in captivity may be misleading because behaviour is often highly context specific. Isolation from the appropriate context may suppress or subvert personality traits in captivity. For example, studies carried out in captivity, in artificially constructed dominance interactions, find no linear relationship between rank and exploratory tendency in great tits (*Parus major*), and an overall a negative correlation between these traits (Verbeek et al., 1999). However in the wild, this relationship is only negative between non-territorial juvenile males, and in contests between territorial males on neutral ground, fast-explorers dominate slow explorers (Dingemanse and de Goede, 2004). Indeed, within their own territory, males were dominant regardless of personality, so the absence of a territorial context in captivity may limit my ability to predict the ecological significance of captive personality traits. Another important contextual difference may be social isolation in captivity, as numerous studies suggest individuals modify their risk-taking behaviour in relation to the presence and identity of conspecifics (van Oers et al., 2005b, Boogert et al., 2006, StoI waset al., 2006, Apfelbeck and Raess, 2008, Pike et al., 2008). The relationship between different behavioural traits may also be context dependent. Bell and Sih (2007), for example find that aggression and risk-taking in a predator-naïve population of sticklebacks correlate only after exposure to a predator, suggesting that the absence of the predator-prey context affects captive personality trait estimates. Without comparing behaviour in captivity to behaviour in the wild therefore, it is impossible to assess whether or indeed which personality traits directly contribute to fitness differences observed between personality types.

I investigated individual variation in exploratory tendency and neophobia (risk-responsiveness toward novel objects) in a population of blue tits (*Cyanistes caeruleus*). To measure this variation, I used variants of two classic behavioural assays in captivity and developed versions of these for use in the wild: Verbeek et al.'s (1994) exploration test and Greenberg's (1983) novel object test. Verbeek et al.'s (1994) exploration test assigns exploratory tendency by movement in a novel captive environment. Whilst it is difficult to quantify movement *per se* in the wild, we may compare the movement of individuals by their presence at certain targets. Dingemanse et al. (2003), for example, have used the distance between the origin and endpoint of post-natal dispersal as a measure of differences in dispersal behaviour in the great tit. Here, I used presence or absence at new feeding sites, introduced within a network of established feeding stations, as a measure of exploratory tendency during foraging. Greenberg's (1983) novel object

test assigns "neophobia", the aversion to the unfamiliar, by the latency to return to a known resource, for example a food bowl or nest site, in the presence of a novel object (see also van Oers et al., 2004, van Oers et al., 2005b). The novel object appears to generate a motivational conflict between desires to obtain the resource and to avoid any unknown risks associated with the novel object (Richard et al., 2008). This test is often used in the wild, where novel objects are introduced to familiar feeding sites, but usually for unmarked individuals (Webster and Lefebvre, 2000, Webster and Lefebvre, 2001, Echeverria et al., 2006). Using variants of these established tests, exploratory tendency and neophobia in species from a variety of taxa are often, but not universally, correlated (but see Clark and Ehlinger, 1987, Wilson et al., 1993, Mettke-Hofmann et al., 2002, Coleman and Wilson, 1998). My aims were threefold: first, to determine whether variation between individuals in these trials was consistent and repeatable, and hence whether exploratory tendency and neophobia constitute personality traits in the blue tit. Second, as trait correlations may differ between contexts, to assess whether neophobia and exploratory tendency are themselves correlated in either captivity or the wild. And third, to compare exploratory tendency and neophobia measured in captivity with the analogous traits measured in the wild for the same, marked individuals.

2.3 METHODS

Studies were conducted between 2007 and 2009 in oak dominated woodland on the east bank of Loch Lomond, UK (56°08'N 4°37'W). In October 2007, I first established eight feeding stations at approximately 500m intervals. These feeding stations were removed at the end of Feburary 2008 and reinstalled in the same positions between October 2008 and February 2009. Each feeding station consisted of two tubular Defender™ feeders (35cm height, 7cm diameter) hung above one another from a bracket on an oak trunk, at approximately 2m and 3m above ground level respectively. The feeders were stocked with peanut granules, and covered with a tube of grey laminated paper to disguise cues about the amount of food available. There was one small feeding hole, so only one bird could feed at a time. I attached a wooden rectangular perch (8cm x 5cm) under this hole, onto which I laid flat a rectangular metal hoop antenna (8cm x 5cm; TROVAN®, United Kingdom). Between November and February, I captured birds as they approached the feeding stations, using mist-nets. I mist-netted three times at each feeding station in the 2007-8 season, and twice the 2008-9 season, generally between

dawn and noon, to ensure equal disturbance at each site. One hundred and twenty-five blue tits were trapped over this time (4-17 per site in 2007-8, 2-10 per site in 2008-9), and taken into captivity for personality trait testing. On first capture, each bird was fitted with a unique Passively Integrated Transponder ("PIT" tag; 11.5 mm x 2.1 mm, <0.1g, Trovan Unique™) attached to a plastic leg ring with Araldite™ glue (as Macleod et al., 2005). The PIT tag weighs less than 1% of the body mass of a blue tit hence is unlikely to affect individual behaviour. On entering the electromagnetic field generated within the antenna loop, the PIT tag produces an amplitude modulated code signal. Using an electronic monitoring system (Trovan™ LID665) I was able to identify individual birds as they used the feeders, from which I derived my wild measures of personality traits. In 2007-8, wild exploration trials were carried out between 1st February 2008 and 28th February 2008 and wild neophobia trials between 19th December 2007 and 28th February 2008. In 2008-9, both trials ran between the 11th January 2009 and 28th February. A total of 91 birds were detected at feeders in the wild: 61 in 2007-8 and 30 in 2008-9.

2.3.1 Personality Trials in Captivity

Birds arrived in captivity generally between 10:00 and 12:00, within 15 minutes journey time from their capture site. They were housed indoors, at a temperature of 17°C±1°C and, to conduct all tests within the captive period whilst standardising captive conditions across birds, a longer than natural 12:12 hour light:dark regime. Each bird was housed individually in a 150cm x 50cm x 50cm cage. Peanut granules, Haiths' Prosecto™ insectivorous mix and water were provided ad libitum, along with around ten *Tenebrio molitor* and two *Galleria mellonella* larvae per day. All birds were observed eating within 10 minutes of arrival in captivity. They were then left undisturbed for a minimum of 2 hours. An exploration trial was run after this period, followed by a further hour without disturbance. Neophobia trials ran between 13:00 and 17:00 on day 1 and were repeated between 08:00 and 11:00 on day 2. Following trials on day 2 in 2007-8, birds were blood sampled and then released at the site of capture at least one hour before sunset. In 2008-9, after blood sampling they were kept undisturbed in captivity for a further night, and released after a second exploration trial on the morning of day 3.

2.3.1.1 Exploratory tendency in captivity

The exploration trial was conducted within what would become the home cage of the focal bird (Fig. 2-1). Each cage contained six perches, three in each half, that were covered with plastic plant vines to increase habitat complexity. The cage bottom was lined with white paper. On arrival into captivity, the bird was introduced to one side of the cage only, selected at random, the other blocked off by an opaque metal divider. I anticipated that the two hours in the cage prior to testing would create a "familiar" and, behind the divider, a "novel" environment. To assay exploratory tendency and not neophobia, the arrangement of plastic plants and perches was the same in each cage half, so that the novel environment was novel only in that it was unexplored. Prior to the trial, the food bowl and any spilt food were removed from the cage to motivate birds toward foraging activity. After thirty minutes, the water bowl was also removed. After a further thirty minutes, the observer removed the cage divider, stepped behind a screen, and observed the focal bird through a small hole for 10 minutes. Unlike other exploration trials (e.g. Verbeek et al., 1994), individuals had the option of remaining within the familiar environment. I allowed this option to help distinguish activity due to exploration from activity due to escape behaviours in the novel environment, as the birds had only been in captivity for a short period prior to testing (Mettke-Hofmann et al., 2009). A movement was defined as a hop or flight between two perches and/or the floor, the cage wall or the front and rear of the cage. The number of movements in each side of the cage was recorded, with the endpoint of each movement defining the side of the cage: novel or familiar. After the test, food and water were returned and the bird was allowed free access to the entire cage.

In 2008-9, birds underwent a second exploration trial, on day 3. On arrival into captivity, birds were randomly allotted to a cage lined either with white paper (as in 2007-8) or brown paper. The arrangement and size of perches and artificial plant material were similar between these cage types, but different leaf shapes were used in the brown versus white-lined cages. My aim was to create two similar but distinct environments and, controlling for cage order and bird identity, there was no difference in activity (LME $t_{43} = -0.14$, $p = 0.89$) or exploration ($t_{43} = 0.49$, $p = 0.63$) between brown versus white-lined cage types. Trials were conducted as 2007-8 for days 1 and 2. After collecting a blood sample on day 2 (when birds in 2007-8 were released), I then moved each bird to one half of a new home cage, of the other cage type. They were left undisturbed until the

following morning, when exploration trials began one hour after the lights were switched on.

I accounted for differences in overall activity level between birds by deducting the number of movements in the familiar environment from the number in the novel environment. This residual activity in the novel environment from the first exploration trial was my measure of exploratory tendency. I used the number of movements in the trial rather than latency to first enter the novel environment (as used in Verbeek et al. 1994) because here 56 birds entered then exited immediately as the divider was removed, and this appeared to reflect an escape or startle response toward the removal of the divider rather than exploration (K.H. pers. obs.). To investigate whether activity in general or activity specifically in the novel environment then correlated with captive neophobia or with exploration in the wild, I conducted separate analyses using the total number of movements in the first exploration trial as a measure of activity during the captive exploration trial. Four birds were excluded from the first exploration trial due to accidental disturbance immediately prior to testing, and three (including one of the above) from the second exploration trial. Exploratory tendency (Shapiro–Wilks test: $W_{120} = 0.94$, $p < 0.0001$) and activity during the exploration trial ($W_{120} = 0.95$, $p < 0.0001$) were leptokurtic and it was not possible to normalise their distributions.

(a)

(b)

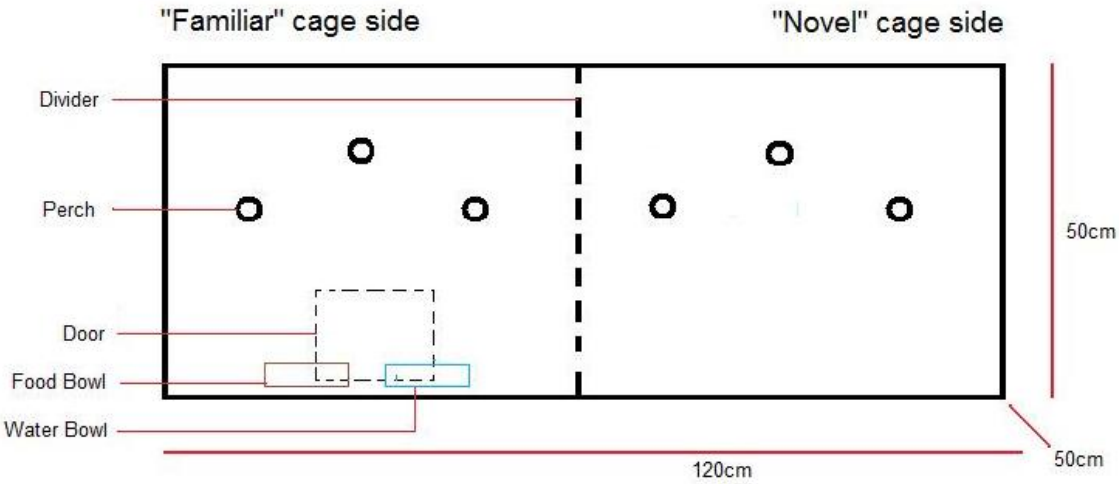


Figure 2-1 Apparatus used during the captive exploration trials. (a) Photo of cage set up. (d) Schematic of cage set up; the food was removed 1hr and water bowl 30min before the start of the trial, via the door.

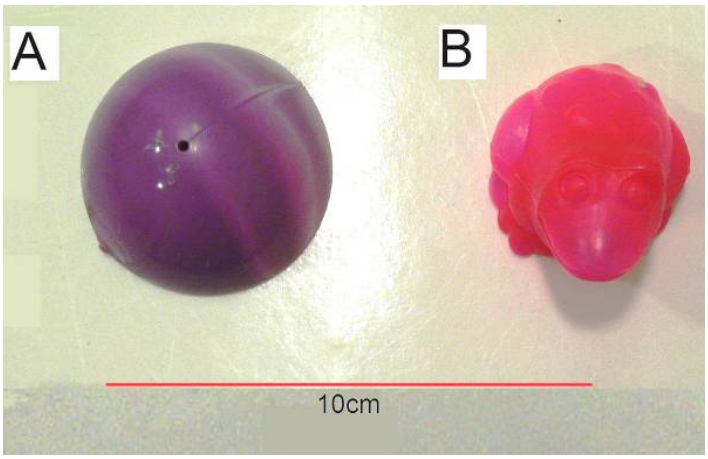
2.3.1.2 Neophobia in captivity

The neophobia trial had two phases: a novel object phase and a disturbance control phase. Each bird took part in one trial on day 1 and another (with a different novel object) on day 2. Food and water were removed for thirty minutes prior to each phase. In the novel object phase, the observer then returned the food bowl with one of two novel objects placed inside. The objects were a luminous pink plastic frog and a half of a purple rubber ball, of similar size (fig. 2-2). The latency to approach the familiar food bowl was recorded. The object was then removed and the water returned.

Independent of differences in response toward a novel object, individuals may also differ in their motivation to feed, or their response to disturbance by the observer returning the food bowl to the cage (van Oers et al., 2005b). To control for this, I also measured latency to feed by the same procedure but without a novel object, returning the familiar food bowl only. This disturbance control phase was performed either one hour before or one hour after each novel object phase. The order of novel object and disturbance control phases was randomized on each day. One bird was excluded from one trial in the disturbance control phase due to a disruption during the trial. Of 79 birds, one bird did not approach within 10 minutes in either phase, and was excluded from analyses. A further 3 birds did not approach during the novel object phase, 1 bird during the disturbance control phase, 9 birds in only one trial of the novel object phase and 3 in only one trial of the disturbance control phase. Birds which participated in both replicates performed consistently between day 1 and day 2 in disturbance control (LME with order of trials as a random effect: $F_{1, 117} = 3.27$, $p = < 0.0001$) and novel object phases ($F_{1, 106} = 2.3$, $p = < 0.0001$) so a mean was calculated per phase per individual. Birds that approached the food bowl in only one trial of a phase were given the latency of that trial rather than a mean.

Neophobia was defined as the latency to feed in the presence of a novel object. In the wild neophobia trials (see below), birds were not disturbed as the novel object was introduced – i.e. pure neophobia was measured. Therefore, to discount the affect of disturbance from neophobia in captivity, I deducted mean latency in the control disturbance phase from mean latency in the novel object phase. As such, the 4 birds that did not approach in either trial of one phase were also excluded from the analyses. Mean risk responsiveness was leptokurtic (Shapiro–Wilks test: $W_{78} = 0.89$, $p = <0.0001$) and it was not possible to normalise this distribution.

(a)



(b)

Figure 2-2 The apparatus used for captive neophobia trial. (a) A photo of the novel objects used in the captive neophobia trial: A: half a purple rubber ball, B: a plastic pink frog. (b) A photo of a blue tit approaching the novel object in a food bowl.

2.3.1.3 Between-individual sources of variation

To accurately measure repeatability of behaviour in captivity, and hence define personality traits, we must first eliminate or control for covariance between behaviour and permanent (e.g. sex) or non-permanent (e.g. condition) differences between individuals that may also generate consistent individual differences in behaviour.

Permanent variables (that would not change within a field season) were wing length, age and sex. Wing length was used as a measure of overall body size; wing length was not measured in one bird. Age (juvenile/adult) was determined from plumage traits (Jenni & Winkler 1994); there were 67 juveniles and 58 adults. Sex was determined using a molecular technique from a blood sample taken at the end of day 2 in captivity (Arnold et al. 2007); there were 32 females and 86 males, and 7 birds were not sexed. Whilst dominance in Parids is highly context specific (Dingemanse & de Goede 2004), in general smaller, juvenile and female Parids are subordinate at feeders. As such, they may be more likely to take risks during foraging, and hence be faster to explore or less neophobic than larger birds, adults or males respectively.

Non-permanent variables were a combination of morphometric and environmental variables collated at capture. Morphometric measures reflecting an individual's current state were body mass and condition. Condition was calculated as the residual of body mass at capture regressed on tarsus length (Linden et al., 1992); a condition measure was not obtained in one bird. Environmental variables that would affect opportunity for foraging immediately prior to entering captivity and hence starvation risk were day length, rainfall (mm) and minimum and maximum temperature for the day of, and day prior to, capture. Weather data were collated from Met office records for Glasgow Bishopton. Together, these variables should reflect or affect an individual's perceived starvation risk on entry at capture, and hence may have short term affects on individual behaviour in captivity.

2.3.2 Personality Trials in the Wild

2.3.2.1 Exploratory tendency in the wild

In the wild exploration trial, birds were scored for whether or not they discovered new feeders installed within the study site. In each of nine consecutive replicates in 2007-8, and 16 consecutive replicates in 2008-9, a new feeder was installed an average of 160 meters (range: 110m-260m) from one of the eight established feeding stations (Fig. 2-3). To avoid influencing concurrent neophobia trials, it was located such that the two closest feeding stations were out-with experimental manipulations. The feeder was positioned 1.5m from the nearest mature oak on a 1.5m high pole. The location was otherwise selected at random, but in 2008-9 chosen such that each permanent feeding station was closest to the new feeder on two occasions during the season, about a month apart; an arrangement used in the calculation of repeatability of wild exploratory tendency (see statistical methods). It was installed before sunrise, left undisturbed for three days, and then removed after sunset. I used PIT tag records from established feeding stations to deduce which individuals were identifiable (i.e. had not lost their PIT tags) in the wild during a replicate. As birds were added to the study as the season progressed, replication was uneven between individuals. For each replicate in which a bird participated, it was scored 0 or 1 for discovering the new feeder, using PIT tag records. Ninety-one birds were detected in the wild and included in on average 10 replicates of this trial (range 2-16). Exploratory tendency was then defined by the number of new feeders an individual did discover relative to the number it could have discovered (i.e. the number of replicates in which it participated).

Difference in site coverage by individuals may have affected the probability that they discovered new feeders, so at the end of the field season, I used PIT tag records to deduce which permanent feeders each bird had used. On average, birds used 1.8 of the eight permanent feeding stations (range 1-4). To account for differences in the distance birds would have to travel to discover each new feeder, I then calculated the distance between the nearest of these permanent feeders and the position of the new feeder in each replicate for each bird. These variables were included in the analyses of wild exploratory tendency (see 2.3.4).

Figure 2-3 Satellite image of the field site, modified from GoogleEarth™ (www.googleearth.com). White squares indicate the positions of the eight permanent feeding stations. To assay wild exploration behaviour, individual were compared for their discovery of short term (3 day) feeding stations installed 110-260m from these permanent feeding stations. The area marked in white around each permanent feeding station shows the area considered when installing these short term feeding stations, limited by natural (e.g. loch edge) and artificial boundaries (e.g. roads). The white arrow indicates the position of the SCENE, where captive personality assays were conducted.

2.3.2.2 Neophobia in the wild

In the wild neophobia trial, birds were scored for the latency to return to an established feeding station following introduction of a "novel object": a colourful feeder cover, substituted for the familiar grey cover (Fig. 2-4). Installed at least three months prior to the study the eight "familiar" feeding stations, each with two tubular feeders with grey covers, were analogous to the familiar food bowl in the captive trials. In 2007-8, for three days prior to an experimental manipulation, I used PIT tag records to establish which individuals used and hence were familiar with the grey feeders at a given site. On the fourth day, between 12:00 and 16:30 (but on one occasion at 18:30), one of the grey covers was substituted for a coloured cover (blue, green, red or yellow). This cover was left on for 3 or 4 days then the grey cover was returned. In 2008-9, the coloured cover was left on for 1 day, starting between 12:00 and 15:00, so in both years PIT tag data was censored at 24 hours after presentation of the coloured feeder cover. In each year, this process was repeated four times at each site a minimum of 10 days apart, twice modifying the upper feeder and twice the lower feeder. The four colours were presented in a different order and combination of positions (upper or lower) at each site. Using a subset of data from 2007-8, I compared the number of PIT tag records in the first hour after introduction of the novel cover to the mean of the same hour in the three previous control days, and found a significant reduction in use of the novel feeder relative to the control (Mann-Whitney *U* test: $U_{24} = -2.34$, $p = 0.03$). Therefore, at the population level, the novel feeder cover elicited a neophobic response.

After introduction of a novel cover, for each bird, I used PIT tag records to count the number of visits to the control feeder before the first visit to the novel feeder. The PIT tag readers recorded the time a bird was first detected on the feeder and then whether it was still present at 2 seconds intervals until not detected. As such, a visit was defined as a record separated from previous or subsequent records by more than 3 seconds. Birds that used the novel coloured feeder first, i.e. immediately on returning to the feeding station, were given a count of zero. Birds which encountered the same colour at more than one site were included only in their first experience of that colour.

A limitation of my method is that I do not know whether a long latency to use the novel feeder reflected aversion to the feeder or simply absence from a site. Therefore I calculated the average foraging bout length using PIT tag records from experimental periods in 2007-8 as follows: the median interval between an individual's feeding station

visits was two minutes, with an upper inter-quartile limit of 14 minutes. A feeding bout was then defined as a period of feeding station use bounded by periods of 14 or more minutes with no records of that bird. Using this definition, across birds the median feeding bout length at a feeding station was 42 minutes. Birds that took longer than this average feeding bout of 42 minutes to use a novel feeder after first returning to a feeding station were assumed to have left the site and were excluded from that replicate. Compared to birds taking under 42 minutes, these excluded birds were not particularly neophobic (or neophilic) in captivity (Mann-Whitney *U* test: $U_{97} = 330$, $p = 0.22$). Under this criterion, I obtained wild neophobia scores from seventy-eight birds, 53 from 2007-8 and 25 from 2008-9, with an average of 2 replicates per bird (range: 1 – 4). Seventy-five of these 78 birds had a captive neophobia score.

(a)

(b)

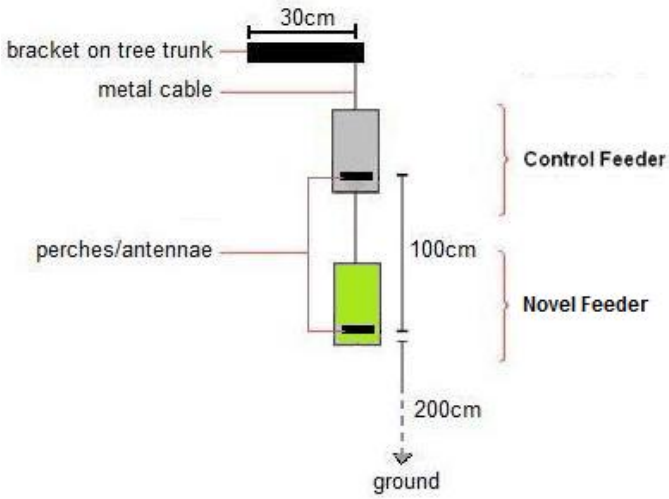


Figure 2-4 The apparatus used for the wild neophobia trials. (a) A photo of a blue tit feeding on the novel feeder (newly covered with a novel green cover). (b) Schematic of the experimental set up used to assay neophobia at the permanent feeding stations.

2.3.3 Ethical Note

All work was carried out in accordance with ASAB/ABS's guidelines for the treatment of animals in research. Work was under license of the UK Home Office and subject to ethical review by WALTHAM® Centre for Pet Nutrition and the University of Glasgow. Captive studies were completed and feeders removed 2 months before the first record of nest building in the area. Whilst I routinely weighed the birds prior to release to ensure they had not lost more than 10% body mass in captivity, there was on average a body mass gain (2.97% ± 7.3%). Following release at the site of capture, 108 out of the 125 birds were later recorded using the feeders or re-trapped in the area. Permission for holding birds in captivity and for using PIT Tags was obtained from Scottish Natural Heritage and the British Trust for Ornithology respectively.

2.3.4 Statistical Methods

Analyses were carried out using R 2.9.1 (R development core team, 2009). There were no differences in behavioural data between years so data was pooled across years.

2.3.4.1 Defining personality traits in captivity

I first determined whether permanent (sex, age and wing length) or non-permanent (body mass or condition, and weather and day length) between-individual variation at capture explained a significant proportion of variation in behaviour in each captive personality trial replicate. Captive personality traits were not normally distributed so I used nonparametric Mann-Whitney U-tests or Kendall rank sum correlations. I applied a Bonferonni correction for multiple comparisons, with a p-value of less than 0.004 for significance.

Consistency across days was analysed using a mixed model, with trial order as a random effect. I then calculated repeatability of captive personality measures using the mean squares from an analysis of variance, with the repeated measures of neophobia or exploratory tendency as the dependent variable and individual identity as the independent variable, following Lessells & Boag (1987). Repeatability is the proportion of variation in

a trait that is explained by differences between individuals, thus larger values reflect greater within individual consistency.

2.3.4.2 Defining personality traits in the wild

Personality traits were measured repeatedly in the wild (up to 16 replicates of the exploration trial and up to 4 replicates of the neophobia trial per individual). In all analyses using wild data therefore, I accounted for repeated measures by using Generalized Linear Mixed Models (GLMMs), with a wild personality trait as the dependent variable and individual identity as a random factor. Wild exploratory tendency was binary (discovered versus not discovered) and wild neophobia a count (visits to the control feeder), thus GLMMs used either a binomial or Poisson error structure respectively. In this and all subsequent analyses of wild personality traits, I also included two variables with each wild personality trait to control for experimental variation between replicates. First, in the exploration trial, feeder discovery may depend on the distance between an individual's nearest permanent feeding station and a given new feeder. Similarly, feeder discovery may be affected by the number of permanent feeding stations an individual used (i.e. their coverage of the study site). Therefore, distance and the number of sites used were included as covariates in all analyses of wild exploratory tendency. Second, in the neophobia trial, the latency to approach a novel feeder may depend on colour or height biases. Therefore feeder colour and feeder position (upper or lower) were included as fixed main effects and an interaction (colour x position) in all analyses of wild neophobia.

Analyses of repeatability used only birds that participated in more than one replicate of a trial. Repeatability of wild personality traits was calculated using the variance component estimates for individual identity from these GLMMs, following Lessels & Boag (see also 1987, Quinn and Cresswell, 2005). The significance of repeatability estimates was determined using a likelihood ratio (LRT) chi-square test between the GLMM including and a GLMM excluding individual identity.

In the exploration trial, variation in feeder discovery was low, with only 47 of 91 birds discovering any new feeders. As such, high repeatability would be misleading, resulting from all individuals scoring mostly "0"s rather than consistent individual variation (i.e. between birds with mostly "1"s and birds with mostly "0"s). Feeder discovery (and hence behavioural variation) was highest amongst individuals using the

closest permanent feeding station to the new feeder within a given replicate. In 2009, I conducted two replicates of the exploration trial within the vicinity of each permanent feeding station, around a month apart (see methods: exploratory tendency in the wild). To analyse repeatability therefore, I limited the data for each 2008-9 replicate to only birds that were using the nearest permanent feeding station and that took part in both replicates at that permanent feeding station (i.e. were PIT-tagged and not currently in captivity). Permanent feeding station identity was then included in the GLMM as a fixed effect and repeatability calculated using the variance component from individual identity nested within permanent feeding station as a random factor.

2.3.4.3 Correlations between traits

For analyses on captive traits, I performed a Kendall rank sum correlation. For analysis of wild traits, I constructed a GLMM with wild neophobia as the dependent variable. To generate a single measure of wild exploratory tendency per bird for the independent variable, which accounted for unequal replication between individuals, I created a two-vector variable with the number of feeders an individual discovered over the number of replicates in which it took part as the binomial denominator. To generate a single measure of distance between new and permanent feeding stations per individual, I took the mean distance across replicates. Along with feeder colour and position, the number of sites an individual used and this mean distance were included in the GLMM, as covariates. To test the significance of wild exploratory tendency as an explanation for variation in wild neophobia, I performed an LRT chi-square test between the GLMM including and a GLMM excluding wild exploratory tendency.

2.3.4.4 Correlations between captive- and wild personality traits

GLMMs were similar to those used when calculating repeatability of wild traits (see above). I tested whether captive personality measures explained a significant proportion of variation in wild behaviour by adding the analogous captive personality measure to these GLMMs as an independent variable, and performing a LRT chi-square test between the GLMM including and a GLMM excluding that independent variable.

2.4 RESULTS

2.4.1 Definition of the Captive Exploration Trait

I observed considerable behavioural variation among birds during the 10 minute trials. The number of movements ranged from zero to 605 (novel side: median = 132, IQR = 123; familiar side: median = 113, IQR = 118). In the second trial, birds were significantly more active (paired Mann-Whitney *U* test: U_{43} 151, $p < 0.0001$). However, exploratory tendency (activity in the novel environment minus activity in the familiar environment) did not differ between trials (paired Mann-Whitney *U* test: U_{43} 501, $p = 0.95$).

Exploration scores did not differ between sexes or ages, (all $p > 0.42$), therefore data were pooled to analyse other sources of between-individual variation. With the Bonferroni correction threshold p-value of 0.004, all other morphometric and environmental variables were non-significant. Therefore consistency and repeatability of these traits were calculated on actual scores. Controlling for trial order, exploratory tendency (LME: $F_{1, 43}$ 1.7, $p = 0.04$) and activity in the exploration trial ($F_{1, 43} = 3.39$, $p = 0.0001$) were consistent across replicates. Exploratory tendency across day 1 and day 3 ($F_{1, 43}$ 1.71, $p = 0.04$, $r = 0.27$) and activity during the exploration trials were significantly repeatable ($F_{1, 43}$ 2.56, $p = 0.001$, $r = 0.42$).

2.4.2 Definition of the Captive Neophobia Trait

I observed considerable individual variation during the 10 minute trials. Latencies to return to the food bowl in the novel object phase (median = 23s, IQR = 95.8s) or disturbance phase (median = 9s, IQR = 32s) varied between 1 and 590 seconds. Mean latency in the novel object phase was significantly greater than in disturbance phase, indicating that the presence of the novel object modified behaviour (paired Mann-Whitney *U* test: $U_{119} = 5023$, $p = 0.0006$).

Neophobia scores did not differ between sexes or ages (all $p > 0.11$), therefore data were pooled to analyse other sources of between-individual variation. As with the exploration score, all other morphometric or environmental variables were non-significant (all $p > 0.1$). Therefore consistency and repeatability of this trait was calculated on actual scores. Controlling for trial order, the neophobia score (novel object

phase latency minus disturbance phase latency) calculated for each day was consistent across days (LME: $F_{1, 103} = 1.77$, $p = 0.002$). Neophobia across day 1 and day 2 was significantly repeatable (ANOVA: $F_{1, 103}$ 1.77, $p = 0.002$, $r = 0.28$).

2.4.3 Definition of wild personality traits

In the wild exploration trial, individual discovery of feeders across two replicates conducted by a given permanent feeding station was near significantly repeatable (i.e. individuals generally found both feeders or neither feeder; GLMM: LRT χ^2 5.29, $p = 0.07$, N = 23 birds, $r = 0.16$).In the wild neophobia trial, individual latency to approach the novel feeder was significantly repeatable (GLMM: LRT $\chi^2 = 126.83$, $p < 0.0001$, N = 43 birds, $r = 0.55$).

2.4.4 Correlations between Traits within Contexts

In captivity, neophobia did not correlate with exploratory tendency (Kendall rank correlation: tau = -0.62, N = 115, $p = 0.54$; see Fig. 2-5a) or activity in the captive exploration trial (Kendall rank correlation: tau = -0.74, N = 115, $p = 0.46$). Similarly, in the wild, the proportion of feeders discovered in the exploration trial did not predict an individual's neophobia (GLMM: LRT $\chi^2 = 0.66$, N = 78 birds, $p = 0.72$; see Fig. 2-5b).

2.4.5 Correlations between Captive and Wild Measures

Wild exploratory tendency had a significant positive relationship with captive exploratory tendency (GLMM: LRT $\chi^2 = 3.889$, N = 91 birds, $p = 0.04$; see Fig. 2-6a). There was no relationship between activity in the captive exploration trial and wild exploratory tendency (GLMM: LRT $\chi^2 = 0.002$, N = 91 birds, $p = 0.97$; see Fig. 2-6b) thus the relationship between captive and wild traits relates specifically to activity in the novel environment, i.e. exploratory tendency. Wild neophobia had a significant positive relationship with captive neophobia (GLMM: LRT $\chi^2 = 48.28$, N = 75, $p < 0.0001$; see Fig. 2-6c).

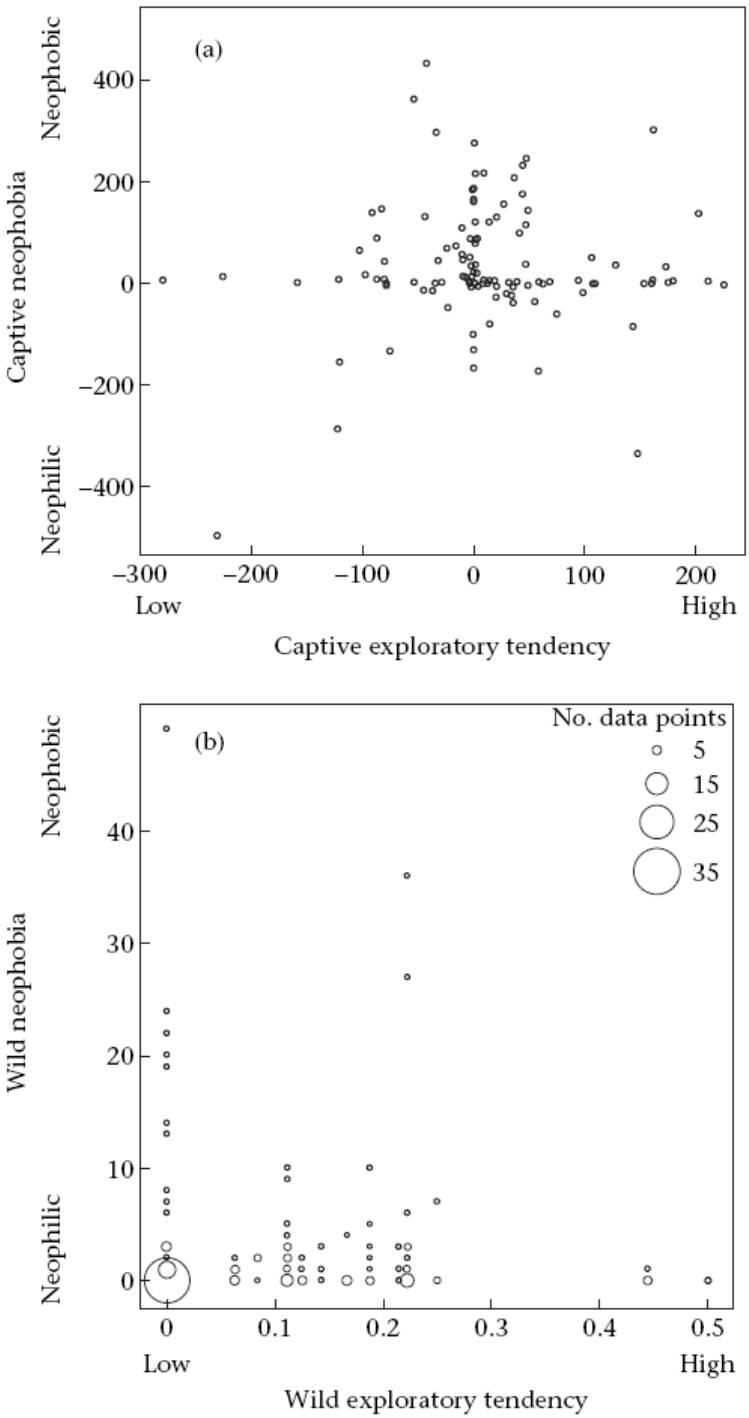


Figure 2-5 Plots of the relationship between the traits exploratory tendency and neophobia. (a) Plot of captive exploratory tendency (no. of movements in novel environment minus no. of movements in familiar environment) and captive neophobia (mean novel object phase latency minus mean disturbance control phase latency). N = 115 birds. (b) Plot of wild exploratory tendency (expressed as proportion of feeders discovered) and wild neophobic (no. of visits to familiar feeder before first visit to novel feeder); individuals are represented one to four times and where multiple data points occur on the same point this is indicated by point size. N = 78 birds.

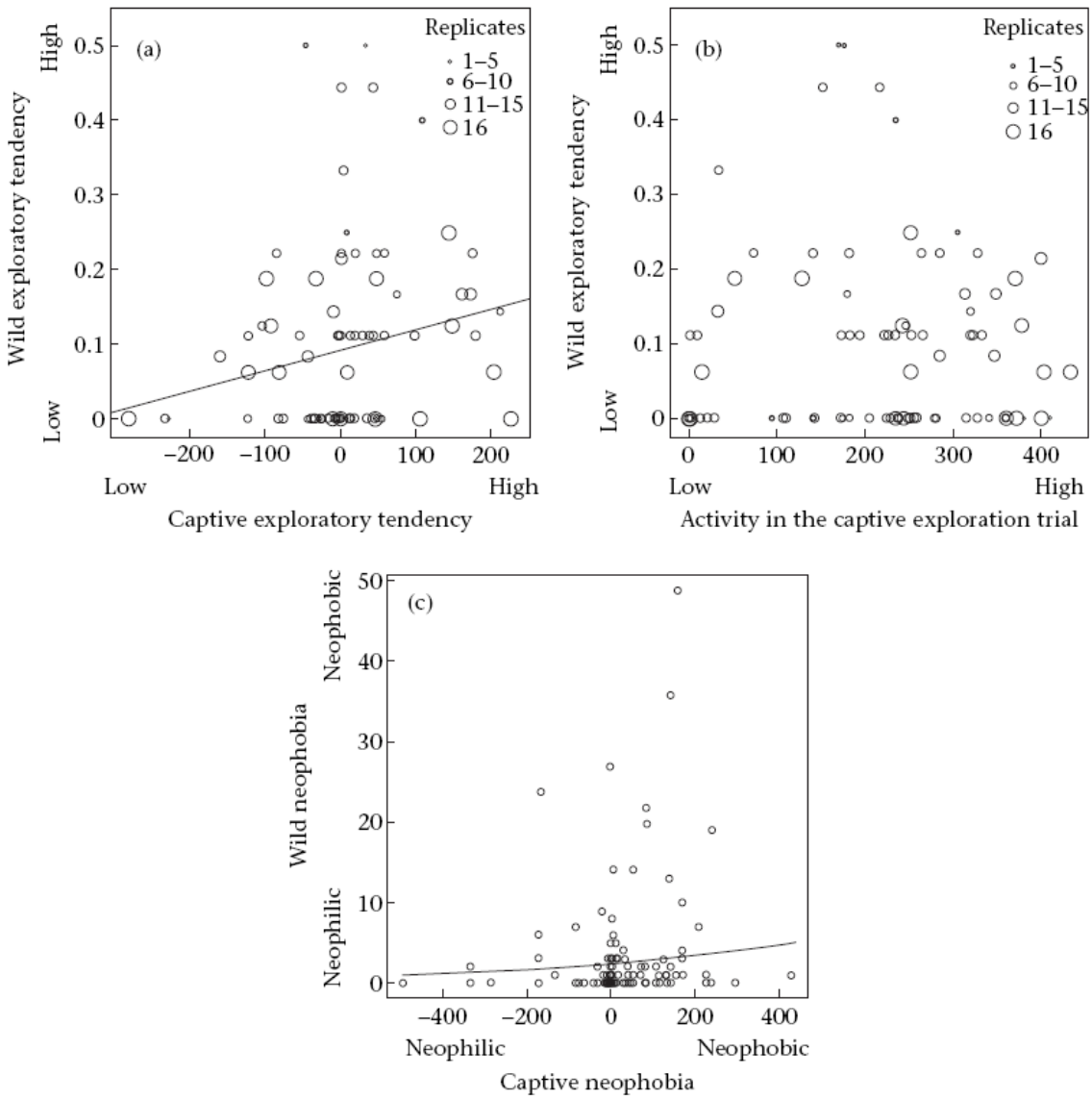


Figure 2-6 Plots of the relationships between captive and wild personality measures. **(a)** Relationship between captive exploratory tendency (no. of movements in novel environment minus no. of movements in familiar environment) and wild exploratory tendency (proportion of feeders discovered). The line is fitted from a linear regression; no. of replicates of the wild exploration trial per bird is indicated by the point size. N= 91 birds. (b) Plot of activity in the captive exploration trial (no. of movements in novel environment plus no. of movements in familiar environment) and wild exploratory tendency (expressed as proportion of feeders discovered); no. of replicates of the wild exploration trial per bird is indicated by the point size. N = 91 birds. (c) Relationship between captive neophobia (mean novel object phase latency minus mean disturbance control phase latency) and wild neophobia (no. of visits to familiar feeder before first visit to novel feeder), the line is fitted from a Poisson regression; individuals are represented one to four times. N = 75 birds.

2.5 DISCUSSION

In this study, I showed that personality traits measured in captivity were a reflection of behavioural differences between individuals foraging in the wild. First, variation between blue tits in exploratory tendency and neophobia were repeatable in captivity, and analogous traits repeatable in the wild. Second, captive measures of exploratory tendency and neophobia were not correlated within individuals, and this was also true of the analogous wild traits. Finally, captive measures of exploratory tendency and neophobia then predicted the analogous wild measures of these traits. Birds that were relatively exploratory in captivity were also more likely to find new feeders in the wild and vice versa. Similarly, an individual's neophobia measured in captivity correlated positively with its latency to approach novel colour feeders in the wild. As my wild measures of personality relate to differences in the use of feeding opportunities, the traits I have measured in captivity appear to represent ecologically relevant differences between individuals.

Whilst many studies use behaviour in captivity to explain differences in fitness observed between individuals in the wild, few directly compare behaviour between captivity and the wild, as I have done. Referring to captive studies on great tits for example, Dingemanse et al. (2004) suggest lower survival of slow than fast exploring females in food poor winters relate to differences in propensity to capitalise upon patchily distributed food. In captive studies, fast exploring great tits are quicker to form foraging routines, more aggressive, and more likely to use social cues than slow explorers: all attributes that support monopolisation of clumped resources (Verbeek et al., 1996, Verbeek et al., 1994, Marchetti and Drent, 2000). From captive studies, it appears likely that exploratory tendency also reflects differences between individuals in information-gathering: when returned to formally novel environments, search behaviour is often then directed toward locations or cues that were associated with food during the preceding novel environment trials (Mettke-Hofmann and Gwinner, 2004). My findings complement these captive observations as here, exploratory tendency in captivity appeared connected to the ability or propensity to seek out new feeding sites in the wild. In particular, the absence of correlation between activity during the exploration trial and feeder discovery in the wild suggests that it was attention to the novel environment specifically, where new information may be gathered, rather than activity *per se* that affected feeder discovery.

I also demonstrated that neophobia measured in captivity reflected differences in neophobia in the wild. Neophobia in free-living birds is associated with reactions to other novel foraging situations, for example dietary conservatism toward new food types or propensity to innovate to obtain food in a novel foraging task (Webster and Lefebvre, 2001, Thomas et al., 2003). As such, the ecological significance of my trait may be as a measure of propensity to approach and hence learn about new feeding opportunities. However, if exposure to the novel object elicits a physiological stress response, i.e. a release of the stress hormone corticosterone, it may also be a measure of response to stressors in general. Whether novel objects elicit a physiological stress response however is so far tested only in Japanese quail (*Coturnix japonica*), which do show an elevation in corticosterone (Richard et al., 2008), and starlings (*Sturnus vulgaris*), which do not (compared to a disturbance control; Apfelbeck and Raess, 2008). That great tits (Groothuis and Carere, 2005) and the blue tits in my study exhibit a behavioural aversion toward novel objects suggests the object may cause a stress response. Indeed, in great tits, individual corticosterone responses derived from a handling trial predict behavioural responses in novel object trials, suggesting similar physiological mechanisms may underlie the response to handling and novel objects (Groothuis and Carere, 2005). However, stereotypical stress behaviours are not necessarily evidence of physiological stress, for example blue tits disturbed at the nest prior to trapping exhibit aggressive behaviour and alarm call, yet show no greater corticosterone response than birds trapped unawares (Muller et al., 2006). Therefore, I should be cautious of assuming neophobia is a measure of response to stressors in general. To assess the ecological significance of the neophobia trait, future work should be addressed at investigating both whether the novel object trial elicits a physiological stress response, and also comparing neophobia with measures of risk-responsiveness toward different potential stressors.

That I did not find a correlation between exploratory tendency and neophobia in this population of blue tits, either in captivity or in the wild, was surprising. Exploratory tendency and neophobia or risk-taking are positively correlated in species from a variety of taxa, and in the closely related great tit this appears to be under genetic control (van Oers et al., 2005a). In these species, neophobia and exploratory tendency may be two measures of a single approach-avoidance trait, with risk-prone, fast exploring or "proactive" individuals at one extreme and risk-averse, slow exploring "reactive" individuals at the other. In other words, Verbeek et al.'s (1994) novel environment trial and Greenberg's (1983) novel object trial may be regarded as approach-avoidance in a novel and a familiar environment respectively (Clark and Ehlinger, 1987, Wilson et al.,

1993, Johnson and Sih, 2007). Though my captive methods differ slightly from those employed by Verbeek et al. (1994) the lack of proactive-reactive personality trait is unlikely to be an artefact of methodology, as I have tested a small sample of great tits using my protocol and found the correlation anticipated (K.A. Herborn & K.E. Arnold, unpublished data). Whilst the contrast to great tits is surprising, divergences in trait correlations between closely related species (Mettke-Hofmann and Gwinner, 2004, Mettke-Hofmann et al., 2002) and even populations of the same species (Bell, 2005, Dingemanse et al., 2007) can be explained by different selection pressures. Consequently, I suggest the traits I have assayed in the blue tit are distinct, and hence the ecological significance of each trait should be considered independently.

Differences between individuals, such as body condition or weather at capture, did not explain a significant proportion of the variation in captive behaviour. This contradicted out prediction that variables increasing starvation risk, such as short day length and poor weather (and hence reduced recent foraging opportunity) would lessen neophobia or increase propensity to explore in the short term. In the wild, Parids modify behaviour rapidly in response to environmental conditions, for example attuning foraging behaviour and hence body fat to changes in starvation and predation risk (Macleod et al., 2005). That behaviour in the captive personality trials was consistent between the first and subsequent days in captivity suggests the birds may equally adjust their perception of starvation risk rapidly to the conditions and availability of food in the captive environment. The absence of state effects is consistent with previous work on wild great tits (Hollander et al., 2008), and encouraging for studies seeking to compare personality between individuals drawn from different times or environments.

In conclusion, personality measures drawn in captivity revealed differences between individuals in their natural foraging behaviour. In directly comparing individuals between captivity and the wild, this study on blue tits joins few similar *in situ* versus *ex situ* studies of personality (birds: Hollander et al., 2008, fish: Wilson and McLaughlin, 2007, Coleman and Wilson, 1998, Brown et al., 2005, molluscs: Briffa et al., 2008). As such, it is an important validation of research based purely on captive measures of personality. Moreover, it lends weight to the growing evidence that wild animals have personality traits that are expressed consistently across contexts.

CHAPTER 3

PERSONALITY PREDICTS ENVIRONMENTAL SENSITIVITY IN THE WILD

3.1 ABSTRACT

Personality traits are behavioural differences between individuals that are consistent across time or contexts. In captive studies, personality often co-varies with tendency toward routine-formation: some personality types appear to remain behaviourally flexible and others become relatively fixed within the same context. If flexibility constitutes 'environmental sensitivity', i.e. the tendency to modify behaviour in response to environmental change, this would explain differential fitness of personality types in unpredictable environments. Here, I tested whether personality predicted variation in environmental sensitivity in wild blue tits *Cyanistes caeruleus*. In captivity, birds were scored for two personality traits: exploration and neophobia. Within individuals, these traits were consistent but not correlated. On release, birds were fitted with transponders to electronically monitor artificial feeder use in the rural woodland. First, I investigated environmental sensitivity to temperature change, measuring individual use of feeders throughout winter. Second, I investigated environmental sensitivity to changes in food supply, recording visitation to emptied feeders. At the population level, feeder use declined with increasing temperature and increasing days after food withdrawal. However, individuals differed in these responses. Environmental sensitivity toward temperature correlated with personality, but not following traditional predictions: highly exploratory bird responded flexibly to temperature, as did the most neophobic birds. Conversely, non-exploratory and neophilic birds used feeders at a fixed level regardless of temperature. Similarly, exploratory and neophobic individuals were most environmentally sensitive to changes in food supply, breaking foraging routines fastest after food withdrawal. Environmental sensitivity to temperature but not food supply also increased with age and body size, suggesting dominance interactions and learning modify expression of personality in competitive (full feeders) but not non-competitive contexts (empty feeders). Overall, personality traits may reflect significant differences in the way individuals prospect for or use information on their environments and hence cope with environmental change.

3.2 INTRODUCTION

Environmental sensitivity is the tendency to modify behaviour in response to environmental change (Boyce and Ellis 2005; Koolhaas et al., 1999). It constitutes an ability (sensory and/or cognitive) to detect variation in the environment coupled with learnt or innate preferences for higher quality or less risky options (Sih et al., 2004). Species-level environmental sensitivity reflects adaptation to the ecologically salient cues for that species (e.g. Mettke-Hofmann et al., 2002, Endler et al., 2001). If individuals of the same species differ in environmental sensitivity though, where choice exists they may differ also in their ability to make adaptive discriminations between for example food types, habitats or mates (Sih et al., 2004).

Individuals of the same species often differ in their tendency to approach novel objects or predators, explore new environments, or interact socially or aggressively with conspecifics (Gosling, 2001). Where such differences are consistent within individuals over time or contexts, this is "personality" (Gosling, 2001, Verbeek et al., 1999, Wilson et al., 1993). In some contexts, such consistency appears sub-optimal, for example individuals that are risk-prone in harsh environments where risk taking is essential are also risk-prone in benign environments where they are excessive (Bell and Sih, 2007, Johnson and Sih, 2007). However, these apparently maladaptive responses may in fact reflect an adaptive trade-off, between a generally appropriate response and the costs of responding with infinite plasticity (DeWitt et al., 1998, Ellis et al., 2006). Intriguingly, it appears the balance of this trade-off between consistency and plasticity may differ between personality types (Briffa et al., 2008; Biro et al., 2010). Terminology describing extremes of personality traits differ between studies but broadly, individuals that are "slow" to approach novel or confrontational stimuli are often also quicker to adjust behavioural routines in response to environmental change than "fast" individuals, which tend toward routine-formation (Benus et al., 1987, Benus et al., 1988, Verbeek et al., 1994, Marchetti and Drent, 2000, Koolhaas et al., 1999, Jones and Godin, 2010). Therefore it is suggested that slow (i.e. shy, neophobic, non-exploratory and/or passive) personality types may respond more quickly to environmental change than fast (i.e. bold, neophilic, exploratory and/or aggressive) types (Dall, 2004, Wolf et al., 2008). For example, passive mice (slow to attack competitors) adjusted their activity levels more rapidly in response to a change in light-dark regime than aggressive mice (Benus et al., 1988). Similarly captive fast-exploring great tits (*Parus major*) continued to visit emptied feeders whilst emptying stimulated slow-explorers to extend their search to new sites

(Marchetti and Drent, 2000; but see van Overveld and Matthysen, 2010). If plasticity does allow greater environmental sensitivity, environmental sensitivity may then explain the observed variation in coping ability within unpredictable environments between personality types in the wild (Dingemanse et al., 2004): slow individuals may respond to change more quickly than fast individuals.

Predictions on the ecological significance of personality come largely from laboratory studies, where personality traits are generally measured. To be ecologically relevant however, such predictions must be tested in the wild (Herborn et al., 2010, Minderman et al., 2009). I used two approaches to test the prediction that personality co-varies with environmental sensitivity in wild blue tits. First, I used the reaction norms approach to investigate environmental sensitivity toward temperature change. Reaction norms are linear functions describing the change in a phenotypic trait across an environmental gradient for a given individual or genotype (Via et al., 1995). Where the trait is behavioural, the intercept of the slope represents the behaviour of the individual in the average environment, and the slope the plasticity of their response to the environmental change. In behavioural studies these slopes and intercepts, also called best linear unbiased predictors or "BLUPs", can be extracted to compare individual response across discreet gradients such as predator presence (Quinn and Cresswell, 2005) social context (Frost et al., 2007) or food availability (van Overveld and Matthysen, 2010), or continuous gradients such as climate (Reed et al., 2009) or population size (Bonte et al., 2007). Here, I investigated response by blue tits (*Cyanistes caeruleus*) to variable winter temperature in terms of artificial feeding station use. Second, at the same feeding stations, I performed a manipulation to investigate individual response to changes in food supply. After 5 months of provisioning, I withdrew the food supply and measured visitation to the emptied feeders over the following days, defining environmental sensitivity by the speed of feeder abandonment. My aim in both studies was to determine whether environmental sensitivity correlated with two captive classified personality traits: exploratory tendency and neophobia. Exploratory tendency and neophobia describe responses to novelty, toward new environments and novel objects placed near familiar food respectively (Verbeek et al., 1994, Greenberg, 1995). Previously, using the same individuals, I demonstrated that an individual's exploratory tendency and neophobia in captivity predicted analogous behaviours in the wild (Herborn et al., 2010). In many species, these traits are positively correlated and considered aspects of a single proactive-reactive trait, with exploratory and neophilic individuals at the fast extreme (Brelin et al., 2008, Benus et al., 1991, Carere et al., 2005, Reale and Festa-Bianchet, 2003, Bourne and Sammons,

2008). However, this is not the case for blue tits (Herborn et al., 2010), so I considered relationships between personality and wild behaviour independently. First, I investigated whether the following predicted measures of average feeder use: personality, sex, age and body size. Sex, age and body size are correlates of competitive ability (Dingemanse and de Goede, 2004, Braillet et al., 2002) and hence starvation risk (Krams et al., 2010) in Parids. I investigated correlates of feeder use because artificial feeding stations are often used to conduct personality studies in the wild (Echeverria et al., 2006, Humber et al., 2009, van Overveld and Matthysen, 2010, Dingemanse and de Goede, 2004, Herborn et al., 2010). As such, systematic variation in feeder use between personality types could bias our interpretation of the ecological significance of captive-classified personality traits. Second, I investigated whether individual feeder use, age, sex, body size or personality predicted environmental sensitivity to temperature or food supply in the wild. Based on studies of rodents and great tits in captivity, I predicted that neophobic and non-exploratory individuals would be most environmentally sensitive.

3.3 METHODS

I monitored feeder use in wild blue tits over two winters, between 2007 and 2009. I collected data at eight artificial feeding stations, which were spaced at approximately 500m intervals through deciduous woodland on the east bank of Loch Lomond, UK (56°08'N 4°37'W, see Fig. 2-3). These feeding stations were installed in the same positions in October of each year and continually baited with peanut granules until the end of February. Each consisted of two opaque tubular Defender™ feeders (35cm height, 7cm diameter) hung from the same bracket on an oak trunk at approximately 2 and 3m above ground level respectively. There was one small feeding hole on each feeder, onto which I attached an 8cm x 5cm wooden rectangular perch to hold a metal hoop antenna of the same dimensions (TROVAN®, United Kingdom). I mist-netted three times at each feeding station in the 2007-8 season, and twice the 2008-9 season, capturing 125 blue tits (4-17 per site in 2007-8, 2-10 per site in 2008-9). On first capture, I fitted each bird with a leg-ring mounted Passively Integrated Transponder ("PIT" tag; 11.5 mm x 2.1 mm, <0.1g, Trovan Unique™; as Herborn et al., 2010). Within the electromagnetic field of the antenna, the PIT tag produces an amplitude modulated code signal, allowing identification of birds on the feeders by an electronic monitoring system (Trovan™ LID665). I also measured wing length and determined age (juvenile/adult) from plumage

traits (Jenni & Winkler 1994). Up to 12 birds per mist-netting day were then taken into captivity for personality testing (see below). They were returned to their site of capture after 2 or, in 2009, 3 days, and feeder use recorded intermittently by moving the electronic monitoring system between feeding stations thereafter. The temperature response data was collected between 22/12/07 and 25/02/08 and 12/01/09 and 26/02/09. The feeder abandonment study was conducted at the end of the 2008-9 season between 02/03/09 and 07/03/09.

3.3.1 Personality Trials

Personality tests were conducted over two days in captivity. For husbandry and detailed methods, see Herborn et al. (2010). Briefly, trials were conducted within the home cage of the focal bird (150cm x 50cm x 50cm). On arrival in captivity, they were enclosed within one half of the cage, and left undisturbed to feed for at least 2 hours. The first trial was the exploration trial, in which I measured behavioural response to the "novel" half of the cage (see below). After this trial, birds had access to the entire cage. Neophobia trials ran between 13:00 and 17:00 on day 1, following at least one hour without disturbance after the exploration trial, and were repeated between 08:00 and 11:00 on day 2. In 2007-8, following trials on day 2, birds were blood sampled as part of a separate study and for genetic sexing and then released at the site of capture at least one hour before sunset. In 2008-9, birds were released after a second exploration trial on the morning of a third day in captivity, to test repeatability of that trait.

3.3.1.1 Exploratory tendency

On arrival into captivity, birds were enclosed within one half of the home cage, and left undisturbed for at least two hours. I anticipated this time would create a "familiar" and, behind the cage divider, a "novel" environment. Each half of the cage contained three perches, which were covered with plastic plant vines to increase habitat complexity. To assay exploration independently of neophobia, the dimensions and arrangement of perches in each cage half was the same, so that the environment was novel only in that it was unexplored. To motivate birds toward foraging activity, the food bowl was removed one hour and water bowl 30 minutes prior to the trial. To start the trial, the observer

removed the cage divider, stepped behind a screen, and observed the focal bird through a small hole for 10 minutes. Unlike other exploration trials (e.g. Verbeek et al., 1994), I therefore allowed birds the option of remaining within the familiar environment. This helps distinguish exploration from activity due to escape behaviours in the novel environment (Mettke-Hofmann et al., 2009). In the trial, a movement was defined as a hop or flight between two perches and/or the floor, the cage wall or the front and rear of the cage. I recorded the number of movements, and the endpoint of each movement: novel or familiar. After the test, food and water were returned and the bird was allowed free access to the entire cage.

I defined exploration as the number of movements in the novel environment minus the number in the familiar environment. I have previously demonstrated that this trait is repeatable within individuals in captivity (Herborn et al., 2010). To determine whether activity *per se*, or specifically activity within the novel environment, i.e. exploratory tendency, predicted behaviour in the wild, I also calculated the total activity in the trial (novel and familiar environments summed). Derived from the same data, exploration and total activity were not independent thus were analysed separately (see statistical methods).

3.3.1.2 Neophobia

To motivate birds toward foraging behaviour, the food and water bowls were removed from the cage for 30 minutes prior to the trial. To start the trial, the observer then returned the food bowl with one of two similarly sized novel objects placed inside: a luminous pink plastic frog and a half of a purple rubber ball (see Fig. 2-2). The latency to approach the familiar food bowl was recorded. After 10 minutes, the object was removed and the water returned. Birds underwent one trial on day one and a second on day two in captivity, with the order of objects randomised per bird.

Independent of differences in neophobia, individuals may also differ in their motivation to feed, or their response to disturbance by the observer (van Oers et al., 2005a). To isolate neophobia, I recorded latency to feed by the same procedure but without a novel object, returning the familiar food bowl only. This disturbance control phase was performed either one hour before or one hour after each novel object phase. The order of novel object and disturbance control phases was randomized on each day. Neophobia was then calculated as mean latency in the novel object phases minus mean

latency in the disturbance control phases. Previously, I demonstrated that latency within these phases and neophobia calculated for each day separately were both repeatable across days (Herborn et al., 2010).

3.3.2 Environmental sensitivity to temperature in the wild

For each bird, I extracted two values from the PIT tag data: average feeder use and change in feeder use in response to air temperature. Starvation risk decreases with increasing air temperature and there are physical costs to carrying fat (Hake, 1996). As such, I predicted that feeder use should be high when it is cold and low when it is warm. Here I first identified whether average feeder use varied with personality, age, sex and body size. I then used change in feeder use with maximum daily temperature as my measure of environmental sensitivity and assessed whether variation in this response could be explained by personality, average feeder use, age, sex or body size.

3.3.2.1 Data collection

Feeder use was measured in the first four hours following sunrise. I collected between 8 and 14 mornings of feeder use data at each of eight feeding stations per year. PIT tag readers were set to record the time at which an individual first landed on a feeder and whether they were still present at two second intervals thereafter. From this data, I extracted the number of detections of each bird to a given feeding station within each recording day, reflecting the total time that bird spent on the feeders. Birds were scored if they were detected using the focal feeding station at least once on a given day, indicating that they were foraging in the vicinity. Feeder use was measured in tandem with a wild neophobia study in which, on four occasions, the appearance of one of the two feeders at a station was slightly modified for up to three days (see Herborn et al., 2010; Chapter 2). The effects of this modification appear short lived, with birds generally returning to the modified feeder within 42 minutes. However, to minimise carry-over effects there was at minimum a 6 day interval between those experimental manipulations and records collected here. Thirty-three percent of birds used more than one of the 8 permanent feeding stations within the same field season (average 1.8, range 1 – 4), either regularly or by switching within the season. In those birds, low feeder use at the focal feeding station could reflect time divided between the focal feeding station and another currently

not connected to the electronic monitoring system. Therefore records were excluded when a bird was detected feeding at another feeding station two days either side of a given record. Birds were only included in the study if they were recorded at least four times at feeders within the same winter. Of these birds, there were on average 5.7 records per bird within each season (range: 4 – 10). To study population level responses to temperature (see statistical methods) birds PIT-tagged in 2007-8 that returned to the field site in 2008-9 were included as a new individual, but excluded in their second year from analyses of individual level responses to temperature.

To identify correlations between feeder use and air temperature, maximum daily temperature was collated from Met office records for Glasgow Bishopton (23km south; www.metoffic.gov). Maximum daily temperature ranged between -5.7°C and 12.4°C during the study period, with an average within day variation of 6°C. Rainfall (mm) and maximum temperature on the day prior to capture may affect an individual's condition and hence motivation to forage during the feeder records, and day length the opportunity for foraging later within that day. These variables were also collated and controlled for in analyses (see statistical methods).

From the feeder use data, I derived two measures per individual (see statistical methods): average feeder use and environmental sensitivity to temperature, which was the change in feeder use with temperature.

3.3.2.2 Statistical methods

Analyses were carried out using R 2.9.1 (R development core team, 2009). I calculated population level response to temperature, and then identified correlates of individual variation in first average feeder use and then environmental sensitivity to temperature. There were no differences in captive behavioural data between years so data was pooled across years.

To identify the population level response to temperature, I used a linear mixed model (LMM) with feeder use records from individuals as the dependent variable. Each bird contributed between 4 and 10 records to the feeder use data, so individual identity was specified as a random effect. However these records were collected under different maximum daily temperatures, ranging between 1.5°C and 12.2°C. To account for the variation in the temperature range under which each individual was measured, I

employed within-subject centring to partition the effect of temperature into within and between-subject components. The mean temperature under which an individual was measured was calculated and entered as the main fixed effect (between-subject component). The maximum daily temperature associated with each record of that individual was then deducted from this mean and specified with individual identity as a random effect, thus each individual's change in behaviour was defined by a random slope within the LMM (within-subject component). Finally, I added day length and maximum temperature and rainfall in the day proceeding records as covariates, as these may also affect individual condition and hence propensity to use feeders within records (Dall et al., 2004). Feeder use was log transformed to normalise the residuals of the LMM.

To assess whether individuals differed in average feeder use or in environmental sensitivity to temperature, I used a reaction norms approach (see Dingemanse et al., 2010, van de Pol and Wright, 2009). The random intercept per individual from the population level LMM represents the behaviour of the individual in the average environment, and the random slope their change in behaviour in response to temperature. To determine whether individuals differed in average feeder use or environmental sensitivity to temperature, I used likelihood ratio tests (LRT) to compare the maximal LMM to one excluding random slopes or random intercepts respectively.

To identify sources of variation in individual level response to temperature, I constructed general linear models (GLMs) with the random intercepts (i.e. average feeder use) or random slopes (i.e. environmental sensitivity to temperature) extracted from the population level LMM as dependent variables. To identify first correlates of average feeder use, I constructed a GLM with average feeder use as the dependent variable and age, sex, wing length, exploratory tendency and neophobia as the independent variables. Age, sex and wing length were included to account for affects of dominance interactions at feeders on behaviour, as small birds, females and juveniles are generally subordinate in Parids (Dingemanse and de Goede, 2004, Braillet et al., 2002), and age also as a measure of foraging experience. Wing length was dependent on an additive relationship between sex and age (linear regression, LM: $F_{2, 52} = 4.4$, $P = 0.017$) so "corrected wing length" refers to wing length as the residual of a LM of wing length against sex and age. As the slope generally increases with the intercept (Crawley, 2007), to identify correlates of environmental sensitivity I constructed a similar model but with random intercept, average feeder use, as a sixth dependent variable. To test whether behaviour related to activity in the novel environment specifically or to total activity within the exploration

trial, I constructed a separate model with the same variables but substituting exploratory tendency for total activity. The initial models included all two-way interactions, and were simplified by stepwise backwards regression, using a threshold p-value of 0.05, until only significant interactions or main effects (significant or non) remained.

3.3.3 Environmental sensitivity to food supply in the wild

Natural food is ephemeral and the ability to respond quickly to sudden changes in food availability may equip individuals to cope better within unpredictable environments (Wolf et al., 2008). As a second measure of environmental sensitivity therefore, I compared individuals for their response to the withdrawal of food from the eight permanent feeding stations. Variation in the propensity to abandon sites after three days was my measure of environmental sensitivity to food supply, with birds that had stopped visiting the feeding stations within this time regarded most environmentally sensitive to food supply. I also compared individuals after one and five days to investigate this response in more detail. As with the test of environmental sensitivity to temperature, I also investigated the effect of average feeder use (measured on a day prior to food withdrawal), sex, age and wing length on response to food withdrawal.

3.3.3.1 Data collection

Two days prior to manipulating food supply, I used PIT tag records to determine which birds were present at each of the 8 feeding stations. Birds were included in the study if they were recorded at a given feeding station on the day of the manipulation at least once prior to and once after emptying the feeders, hence experienced the change in food supply. Twenty-six blue tits fitted these criteria. The manipulation was carried out in two blocks, at 4 feeding stations on 02/03/09 and the remaining 4 on 03/03/09. PIT-tag readers were installed within 30 mins of sunrise on those days and removed after sunset. I emptied the feeders between 09:20 and 10:40. The PIT-tag readers were rotated between the two sets of feeding stations for 6 days after the first manipulation, reinstalled within 30 mins of sunrise and removed at sunset on each occasion. From these records, I extracted the number of visits by each bird to each feeding station on the day of the manipulation and after three and five days. PIT-tag readers were set to record the time at

which an individual first landed on a feeder and whether they were still present at two second intervals thereafter. Therefore a visit was defined as a record separated from other records by more than three seconds. However in birds that visited more than once on day one, the median interval between visits was 3.44 mins and mean 17.30 mins, indicating that in general, birds that visited on multiple occasions returned to the site intermittently between long absences from the site (range of intervals: 4 seconds to 3.5 hours).

3.3.3.2 Statistical methods

The manipulation was staggered over two days, but starting date did not explain variation in feeder use prior to the trial (GLM with quasi-Poisson errors: $t_{1, 24} = 1.26$, $P = 0.22$) on day 1 ($t_{1, 24} = 0.88$, $P = 0.39$), day 3 ($t_{1, 24} = -0.49$, $P = 0.63$) or day 5 (GLM with binomial errors: $z_{1, 24} = -0.72$, $P = 0.47$) so the data was pooled. I used GLMs to first investigate relationships between personality traits and feeder use prior to the manipulation or on day 5. For feeder use prior to the manipulation, visitation was treated as a count, and I used a quasi-Poisson error structure to account for over-dispersion. On day 5, most birds did not visit or visited only once (see results), so visitation was treated as a binary variable (visited, did not), and I used a binomial error structure. To investigate relationships between exploratory tendency and visitation on days 1 and 3, I used generalized linear mixed models (GLMMs). The dependent variable, feeder use, was measured as a count so models had a Poisson error structure. Personality traits and day (day 1 or day 3) were my main independent variables. I included wing length as a covariate and sex and age as fixed factors to control for variation in feeder access and foraging experience, as in the temperature response analyses. Within this subset of birds, wing length was independent of sex and age so actual wing lengths were used in the analyses. I included feeder visitation two days prior to the manipulation as a covariate to control for individual differences in "prior feeder use", analogous to the random intercepts in the temperature response analyses. Finally, to avoid over-parameterising the models I include only the interactions between personality traits and day, as I was interested in changes in visitation behaviour between personality types over days. I simplified the models by stepwise backwards regression, using a threshold p-value of 0.05, until only significant interactions or main effects (significant or non) remained. As in the previous analysis, I ran a separate model with exploration substituted for activity in the exploration trial. Nine individuals had been taken into overnight captivity for personality testing once previous,

in winter 2007-8. In case personality scores were affected by over-year familiarity with the aviary set up, I re-ran GLMMs excluding these individuals.

3.3.4 Ethical Note

All work was licensed by the UK Home Office, with permission for taking birds in captivity and for using PIT Tags obtained from Scottish Natural Heritage and the British Trust for Ornithology respectively. Studies were carried out in accordance with ASAB/ABS's guidelines for the treatment of animals in research, and subject to ethical review by WALTHAM® Centre for Pet Nutrition and the University of Glasgow. Whilst in captivity, no bird lost (or gained) more than 10% body mass, and there was on average a body mass gain (2.97% ± 7.3%). Following release, 108 out of the 125 birds were later identified in the wild via PIT tag records or re-trapping.

3.4 RESULTS

3.4.1 Environmental sensitivity to temperature

At the population level, feeder use declined with increasing maximum daily temperature (see Table 3-1, Fig. 3-1a). However, removal of the random slope term significantly reduced the fit of the model (LRT χ^2 = 13.42, N birds = 82, P = 0.001), indicating that individual birds differed in their temperature response. Removal of the random intercept term also significantly reduced the fit of the model (LRT χ^2 = 209.9, N birds = 82, P < 0.0001), so the average feeder use also differed between birds. There was a correlation of 0.17 between random intercept and random slope, such that birds with higher random intercepts (i.e. greater feeder use) responded less to changes in temperature (i.e. had shallower slopes; Fig. 3-1b). Therefore, as well as a variable of interest, average feeder use was included in individual level models to control for variation in the slope.

Average feeder use did not correlate with age, sex, corrected wing length, neophobia, exploratory tendency or, in equivalent models substituting exploratory tendency for activity in the exploration trial, activity (see Fig. 3-2). Individual level environmental sensitivity was predicted by a significant additive relationship between age, wing length, neophobia and exploratory tendency: large, adult, neophobic and highly

exploratory birds were most responsive to temperature (i.e. had more negative random slopes; see Table 3-2 and Fig. 3-3). Sex did not predict environmental sensitivity (Fig. 3-3e). In equivalent models substituting exploratory tendency for activity in the exploration trial, activity did not explain a significant proportion of variation in environmental sensitivity (from a GLM with wing length, neophobia and age as significant main effects: activity: $t_{1, 53} = -0.76$, P = 0.45; see Fig. 3-3f).

3.4.2 Environmental sensitivity to a manipulation of food supply

Prior feeder use (measured 2 days prior to the manipulation) was not related to exploratory tendency (GLM with quasi-Poisson errors: $t_{1, 24} = 0.92$, P = 0.37) or neophobia ($t_{1, 24} = -1.68$, P = 0.11). After the manipulation of the food supply, at the population level, feeder visitation declined from a median of 3 visits per individual per feeding station per day (range 1 – 27) on day 1 to zero (range 0 – 8) on day 3. Thirteen individuals did not visit on day 3. A significant interaction between exploratory tendency and days indicates that exploratory individuals visited the emptied feeding stations more frequently than less exploratory individuals on day 1, but that more exploratory individuals visited less than less exploratory individuals on day 3 (Table 3-3, Fig. 3-4a). As my measure of environmental sensitivity was propensity to stop visiting feeders by day 3, exploratory individuals were regarded most environmentally sensitive. There was no interaction between day and neophobia, but neophilic individuals visited more than neophobic individuals overall across days 1 and 3, so neophobic individuals were regarded most environmentally sensitive (Table 3-3, Fig. 3-4b). Sex, age and wing length did not predict environmental sensitivity to food supply (Table 3-3). The results were unchanged by re-analysing the data excluding birds that had been taken into captivity once previously, in the winter of 2007-8 (from a GLMM including age, sex, wing length and prior feeder use as fixed effects, exploration x day: $z_{1,15} = -3.29$, P = 0.005, effect ± s.e. = -0.04 ±0.012; neophobia: $z_{1,12} = -2.38$, P = 0.035, effect ± s.e. = -0.02 ±0.007). In analyses substituting exploration for activity in the exploration trial, there was no interaction between day and activity ($z_{1, 21} = -0.84$; P = 0.4) and the main effect was non-significant following removal of the interaction term ($z_{1, 19} = 0.52$, P = 0.61; see Fig. 3-4c). On day 5, only 4 of the 26 birds visited the emptied feeding stations. Visitation on day 5 was independent of exploratory tendency (GLM with binomial errors: $z_{1, 24} = -0.3$, P = 0.76) and neophobia ($z_{1, 24} = -0.12$, P = 0.9).

Table 3-1 Results from LMM of population level feeder use in response to environmental variables

Predictors	t (d.f.)	P-value	R (S.E.)
Mean-centred temperature	-2.33 (80)	0.024	-0.0839 (0.036)
Maximum temperature in previous day	-2.47 (527)	0.014	-0.0199 (0.008)
Rain fall (mm) in previous day	-3.35 (527)	0.0009	-0.0105 (0.003)
Day length	-5.13 (527)	< 0.0001	-0.00003 (0.000005)

The results are of a LMM with log feeder visitation as the dependent variable and random intercepts and slopes with mean-centred temperature for each bird; n feeder use records = 614 and n birds = 82

Table 3-2 Results of a GLM of individual level environmental sensitivity to temperature (i.e. individual changes in feeder use with temperature)

Predictors	t (d.f.)	P-value	R (S.E.)
Average feeder use	0.82 (49)	0.42	0.0079 (0.0097)
Age	3.2 (49)	0.002	0.0228 (0.007)
Sex	1.12 (49)	0.27	0.0094 (0.0084)
Corrected wing length	-2.07 (49)	0.044	-0.0045 (0.0022)
Neophobia	-2.09 (49)	0.042	-0.0006 (0.0003)
Exploratory tendency	-2.54 (49)	0.014	-0.0001 (0.0004)

The dependent variable environmental sensitivity and independent variable feeder use are BLUPs extracted from the LMM on Table 3-1; n = 56 birds.

Table 3-3 Results from GLMM on individual variation in visitation to feeding stations in 1 and 3 days after they were emptied. Environmental sensitivity was defined as the propensity to cease visitation by day 3

Predictors	z (d.f.)	P-value	R (S.E.)
Prior feeder use	3.881 (18)	0.0001	0.013 (0.003)
Age	0.705 (18)	0.48	0.278 (0.394)
Sex	-0.927 (18)	0.35	-0.4 (0.432)
Wing length	-0.932 (18)	0.35	-0.127 (0.137)
Neophobia	-2.6 (18)	0.009	-0.004 (0.005)
Exploratory tendency x day	-4.82 (22)	<0.0001	-0.01 (0.002)

The results are of a model with feeder visitation as the dependent variable, individual identity specified as a random effect, and a Poisson error structure; n visitation records = 52, n birds = 26

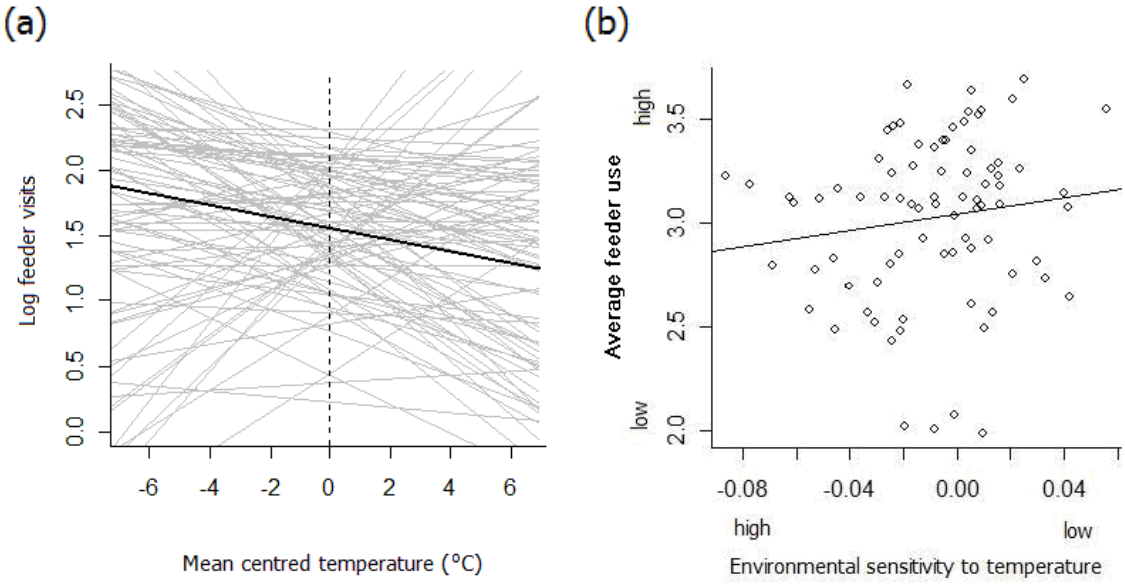


Figure 3-1 Plots of population level and individual level reponse to temperature in terms of feeder visitation and average feeder use. (a) Individual and population level response to increasing maximum daily temperature. The mean temperature an individual experienced was centred on zero (dashed line). The temperature on each day that an individual was measured for feeder use is expressed in degrees above or below this mean per individual, on the x axis. The grey lines are the slope of feeder use against mean centred temperature for each individual and a black line the population slope to mean centred temperature across individuals. (b) Relationship between individual environmental sensitivity to temperature (i.e. random slopes extracted from LMM of feeder use and temperature) and average feeder use (i.e. random intercept), illustrated with a regression line.

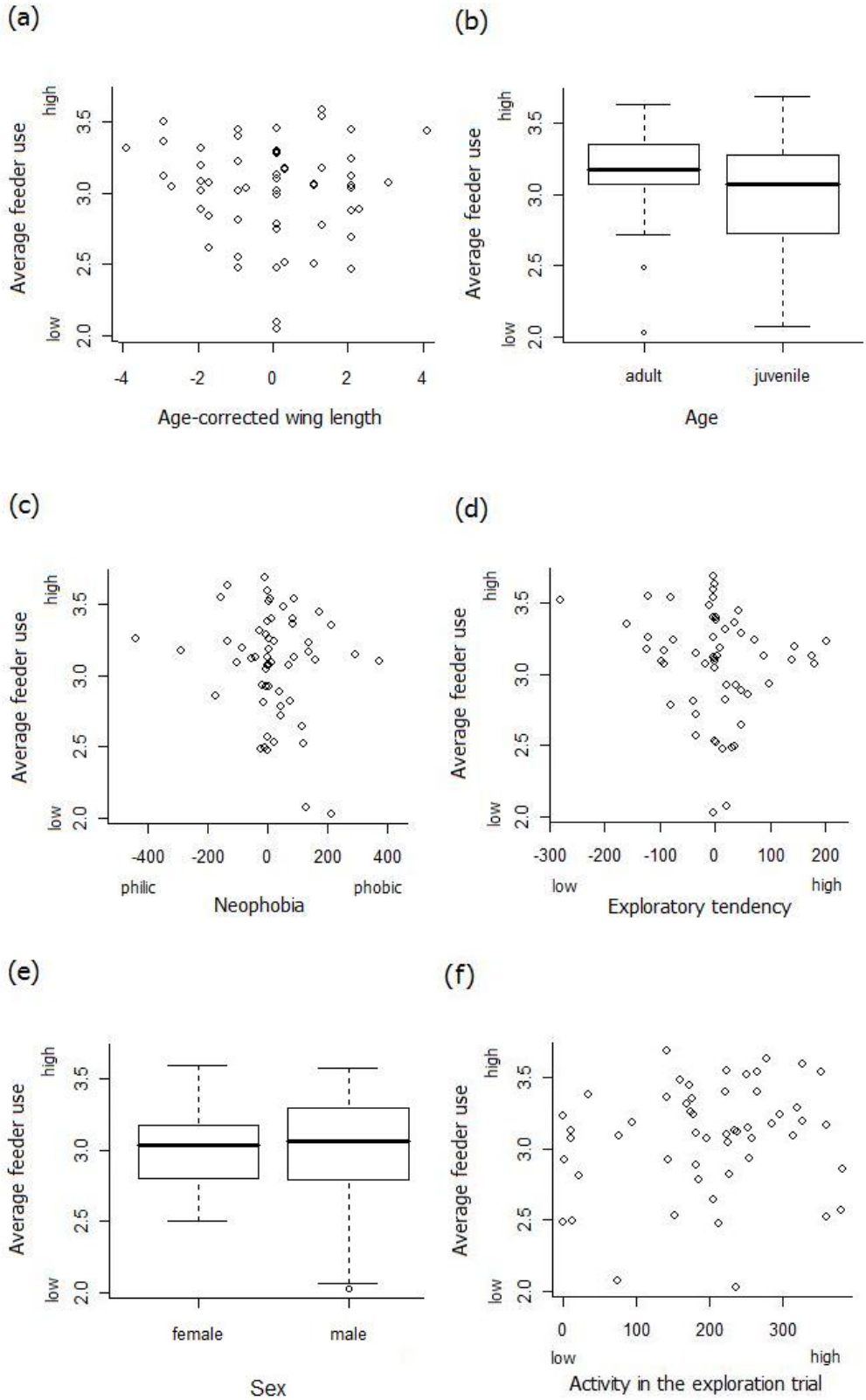


Figure 3-2 Individual level plots of non-significant relationships between average feeder use (i.e. random intercept extracted from LMM of feeder use and temperature) and (a) corrected wing length, (b) age, (c) neophobia, (d) exploratory tendency, (e) sex and (f) activity in the exploration trial.

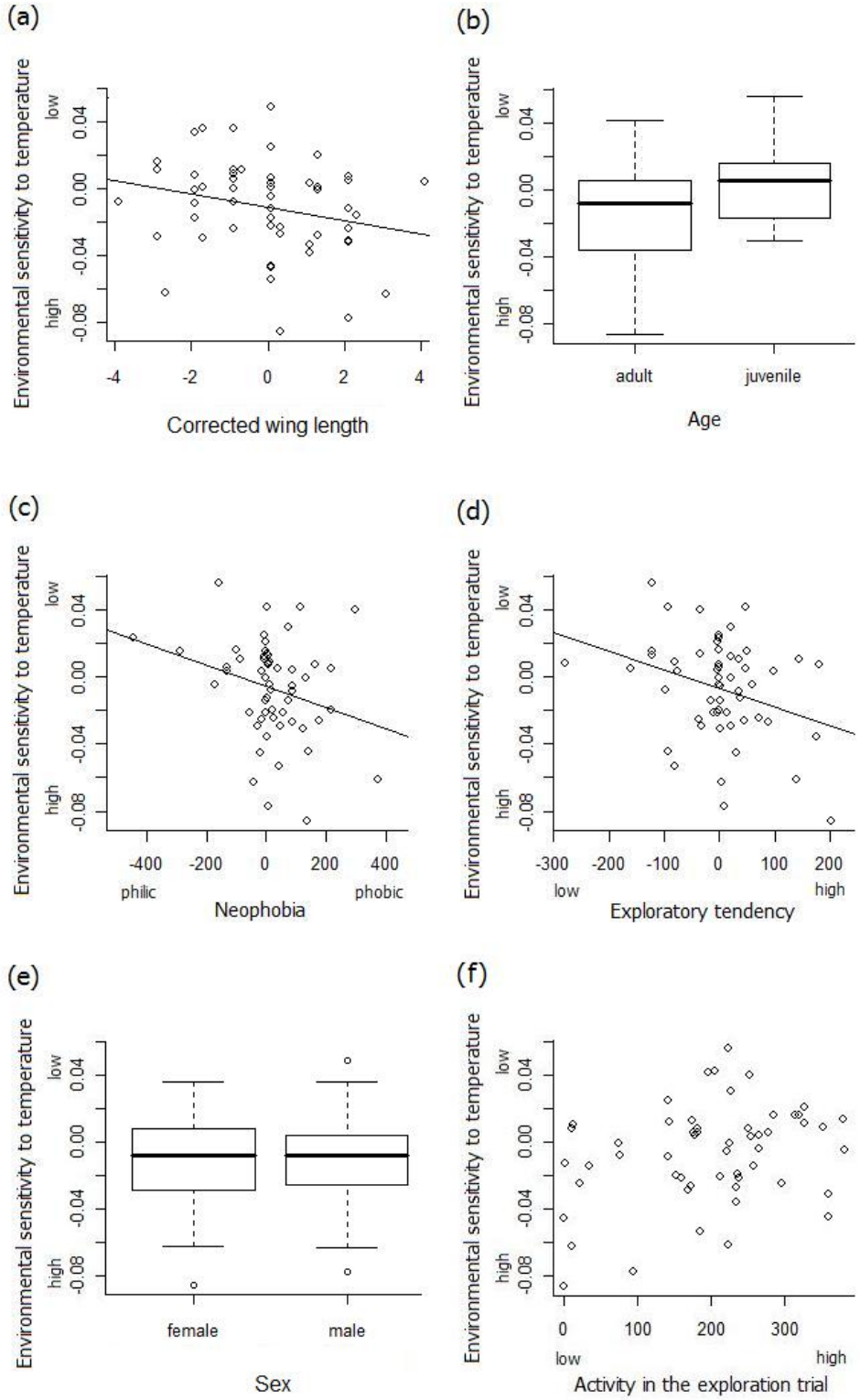


Figure 3-3 Individual level relationships between environmental sensitivity to temperature (i.e. random slope extracted from LMM of feeder use and temperature) and (a) corrected wing length, (b) age, (c) neophobia, (d) exploratory tendency, (e) sex and (f) activity in the exploration trial.

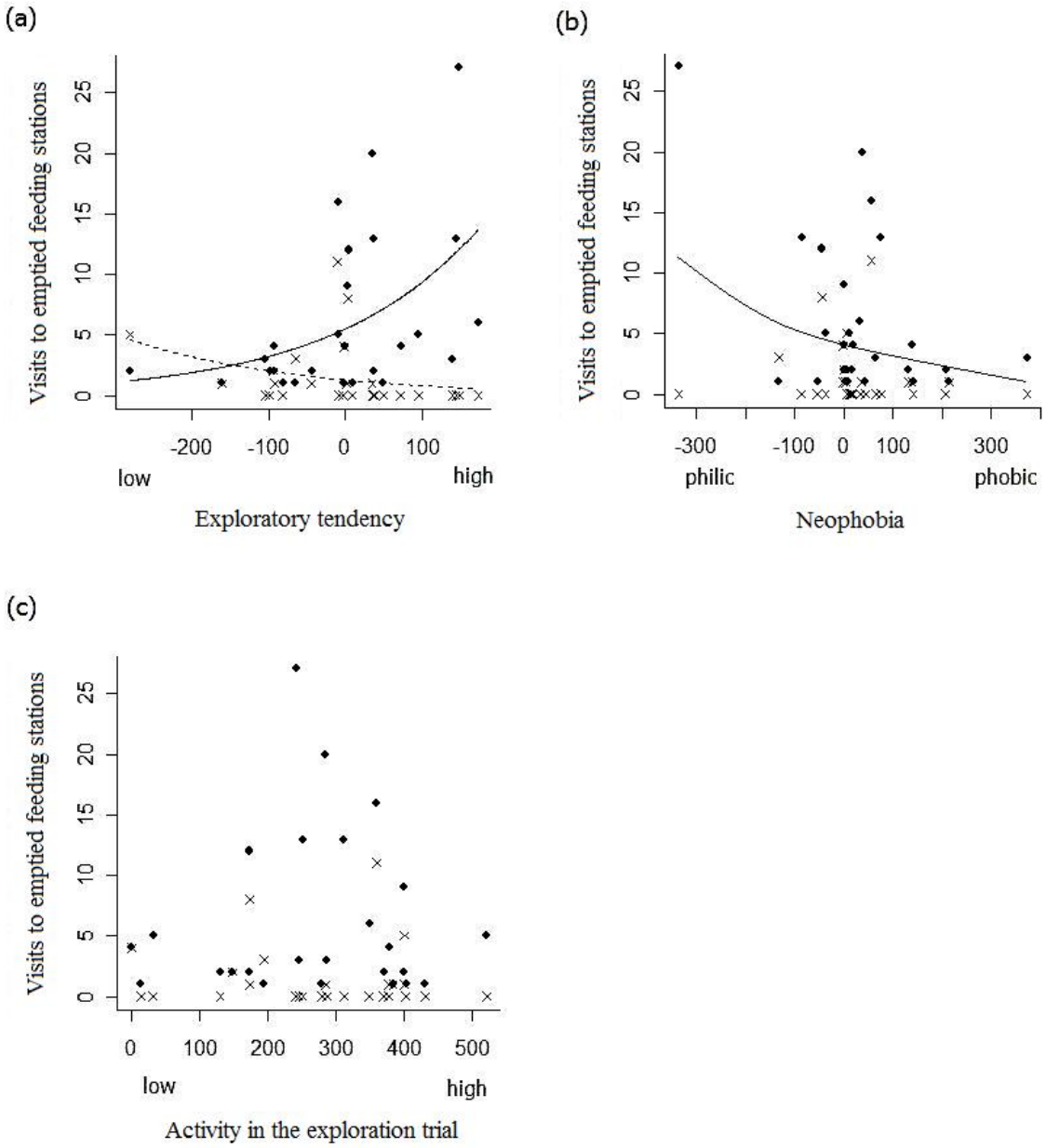


Figure 3-4 Relationships between visits to emptied feeding stations and (a) exploratory tendency, (b) neophobia and (c) activity in the exploration trial on the day of the food supply manipulation (day 1: filled circles) and after 3 days (day 3: crosses). An interaction between day and exploratory tendency is illustrated by the use of lines and dashed lines for days 1 and 3 respectively. I interpret high environmental sensitivity to food supply as a greater tendency to reduce feeder use by day 3, whether overall (i.e. neophobic birds were more environmentally sensitive than neophilic birds) or in contrast to day 1 (i.e. more exploratory birds were more environmentally sensitive than less exploratory birds).

3.5 DISCUSSION

This is one of only two studies (the other: Overveld and Matthyssen, 2009) so far to examine variation in behaviour between personality types, rather than survival or fitness, in response to environmental change in the wild. Such studies of environmental sensitivity are important to understanding the ecological significance of personality traits (Sih et al., 2004). At the population level, feeder visitation dropped off with increasing temperature and with days following food withdrawal. However, these responses differed between birds: some showed a steep decline whilst other used feeders at a fixed level independent of temperature and then continued to visit feeders for several days after they were emptied. Neophobic and, contrary to expectation from captive studies (e.g. Benus et al., 1988, Benus et al., 1990, Marchetti and Drent, 2000), exploratory individuals were most environmentally sensitive to temperature and food supply. In contrast, neophilic and non-exploratory birds were relatively fixed in their level of feeder use despite changing temperature, and continued to visit the emptied feeders for a longer period. Therefore personality traits relating to novelty did represent systematic variation in environmental sensitivity. Also, birds with longer wings and adults were more sensitive to temperature, but not food supply, than small and juvenile birds. Relating to the use of a real feeding opportunity, personality traits may represent major differences in the way individuals prospect for and use information on their environments and hence respond to environmental change.

Plasticity in foraging behaviour could stem either from feed-forward mechanisms, whereby individuals respond to internal cues such as hunger or body condition, or feed-back mechanisms, using environmental cues to respond pre-emptively (Krebs and Davis, 1997). In the environmental sensitivity to temperature analysis, I controlled for environmental variables (rain fall and temperature the day before measurement) that may affect body condition on entering feeder use trials, thus have assayed specifically the latter: sensitivity to feed-back from the environment. I identified no correlates of the rate of average feeder use, and average feeder use did not affect environmental sensitivity to temperature, so my results are not simply an artefact of differences in feeder use between personality types, ages or body sizes (Crawley, 2007). Indeed, contrary to expectation (but consistent with Dingemanse and de Goede, 2004), neither average feeder use from the temperature analysis nor the records of prior feeder use from the food withdrawal

experiment: two measures of feeder dependency, correlated to personality. In captive studies, neophilic individuals generally form foraging routines at reliable feeding sites more quickly than neophobic individuals, often at the cost of discovering (and dividing time between) new feeding opportunities elsewhere (e.g. Benus et al., 1988, Benus et al., 1990, Verbeek et al., 1994, Marchetti and Drent, 2000). I predicted personality may similarly co-vary with feeder use in the wild. That such systematic differences were not identified is encouraging for studies using artificial feeding sites to assay behaviour in the wild, where variation in tendency toward feeder use *per se* could then bias results. I also found no interaction between neophobia and exploration in my study, which is consistent with the independence of these traits in blue tits and further proof of consistency between captive and wild behaviour in this species (Herborn et al., 2010).

Neophobic birds were more environmentally sensitive to both temperature and food supply than neophilic birds. This is in keeping with the expectation from other species that slow individuals are more plastic in their behaviour than fast individuals (Verbeek et al., 1994, Marchetti and Drent, 2000, Koolhaas et al., 1999). In both the captive and wild contexts, neophobic blue tits adjusted their use of known feeding sites (the food bowl or feeding station) in relation to environmental change (a novel object, temperature or food availability), so reactions toward novel objects may be a general measure of responsiveness to environmental stimuli. Often, neophobic individuals are fast to learn negative changes in familiar situations (Exnerova et al., 2010; Marchetti and Drent, 2000) but slow to learn positive associations in novel situations (Webster and Lefebvre, 2001, Thomas et al., 2003). They may therefore be more sensitive to negative than positive experiences, here for example perhaps prior experience of condition loss with low temperature. In a study on blue tits from the same population, Arnold et al. (2007) showed that nestling diets associated with poor foraging environment (i.e. rich in spiders relative to caterpillars) were associated with development of neophilic personality. The authors suggest this may equip birds to “take risks” on fledging into an impoverished environment. Taking these studies on blue tits together, variation in neophobia in this species may constitute different strategies (plastic versus fixed) for coping with detrimental environmental change.

Birds with high exploratory tendency were more environmentally sensitive to both temperature and food supply than birds with lower exploratory tendency. This appears contradictory to Marchetti and Drent’s (2000) study on captive great tits, in which slow explorers were flexible and fast explorers routine-formers (see also Benus et al., 1988). However in that study, exploration was measured as the latency to approach all

parts of a novel environment, whereby fast birds may be considered "superficial" and slow birds "thorough". Using the same experimental set up and species, van Overveld and Matthysen (2010) measured instead activity (number of hops or flights) in a two minute period. Corroborating my study, Overveld and Matthysen then showed that fast explorers were quickest to break unrewarding foraging routines in the wild. Similarly, Dingemanse et al. (2003) found that fast-exploring juvenile great tits, classified by activity scores, travelled further from the familiar environment (hence foraging routines) during post-natal dispersal. What comparison of these studies shows is that interpretation of "exploratory tendency" is dependent on the method by which exploration is scored. It may also be important to incorporate space use with activity. In one of few studies on environmental sensitivity, on wild starlings (*Sturnus vulgaris*) Minderman et al. (2009) used a trial analogous to the great tit studies but classified the behaviour with a principle components analysis (PCA), thus separated out axes describing separately activity (in their terms "speed of exploration") and space use (time spent on perches versus the ground). On reintroduction to that formally novel environment, only space use predicted individual response to an environmental change (an escape hatch), which they suggest is another measure of environmental sensitivity. In my trial, I allowed birds the option to avoid exploration altogether, by providing access to a familiar environment throughout. I then weighted activity in the novel environment by activity in a familiar environment, creating a score that combined space use with activity. Previously, using the same individuals, I showed that exploration in captivity positively predicted the birds' propensity to find new, short-lived feeding sites in the wild, whilst total activity (movement in novel and familiar environments combined) did not (Herborn et al., 2010). Similarly, here total activity did not predict environmental sensitivity toward either temperature or food supply. Therefore blue tits that were relatively active specifically within novel environments are more flexible foragers than less exploratory birds, both in their propensity to seek out or use new feeding sites and, here, in their use of known feeding sites.

When I experimentally manipulated food supply, I found a positive relationship between exploratory tendency and feeder visitation immediately after the loss of the food supply (day 1). This represents a change in behaviour as, consistent with other studies (Dingemanse and de Goede, 2004; van Overveld and Matthysen, 2010) personality types did not differ in feeder use prior to the manipulation. However, by day 3, only the less exploratory individuals continued to visit the feeders. This is in contrast to the results with neophobia, whereby neophobic individuals visited at a higher rate on both days, in

line with the expectation of routine-formation (Marchetti and Drent, 2000). This suggests that exploration predicts the rate at which unprofitable feeding opportunities are abandoned. In Overveld & Matthysen's (2009) study on great tits, the abrupt removal of an artificial feeding site stimulated fast explorers to move to new areas whilst slow explorers remained within the vicinity of the old feeding site. As the great tits in that study did not differ in feeding range size, the authors suggest that response may reflect differences in the way individuals combine past and current information: fast explorers returned to former, distant profitable feeding sites, whilst slow explorers continued to update their information on the currently unprofitable site. My finding is consistent with that study, and suggests some generality of the exploration trait between closely related species.

An alternative explanation for environmental sensitivity in exploratory blue tits though may be variation in response toward social rather than other environmental cues. Whilst slow individuals in many species appear more sensitive to changes in environmental stimuli than fast individuals, social stimuli may be an exception. Individuals that are fast in one personality trait often react more quickly to competitive (Verbeek et al., 1996) and social signals (Marchetti and Drent, 2000, but see Frost et al., 2007) than slow individuals and take longer to recover from social defeat (von Holst, 1998, Carere et al., 2001). Therefore exploratory blue tits may be more sensitive to competition than less exploratory individuals, hence avoid feeders where possible (i.e. except when temperatures are very low), but also more sensitive to social signals, hence avoid feeders when conspecifics are absent (i.e. when feeders were empty). I have not investigated sociability or aggression in the blue tit, thus cannot distinguish these alternative explanations.

Finally, I discovered that small and juvenile birds were less environmentally sensitive to temperature than large and adult birds. This observation is consistent with intraspecific variation in starvation risk: small and juvenile birds have low resource holding potential (Dingemanse and de Goede, 2004, Braillet et al., 2002), thus may feed with opportunity (i.e. low feeder use by competitors) as much as necessity (i.e. low temperatures), obscuring trends with temperature. In support of this interpretation, I found no affect of age or wing length in the food withdrawal experiment when feeder use hence competition was lower. However, the relationship between age and wing length in the temperature study was additive, suggesting age made an independent contribution to the observed variation in environmental sensitivity. Environmental change is only a useful cue if it predicts the future, by covariance between phenotype and fitness (Van

Noordwijk and Muller, 1993). By survival through one or more previous winters, adults may learn this covariance, through experience of the association between mass gain (feeder use) and condition under a wide range of temperatures. Inter-year environmental sensitivity toward temperature is evident also in studies comparing plasticity across breeding attempts: the extent to which blue tits and great tits respond to spring temperature as a cue to breed is dependent on their prior experience of breeding under higher or lower spring temperatures (Nussey et al., 2005) or food supply (Grieco et al., 2002). Nussey et al. (2005) also observed that plasticity in breeding behaviour was heritable in a population of great tits, and that it had increased within the population by selection in the previous 32 years. Current global temperature change is associated with shifts in the breeding phenology of many species, in some cases threatening to mismatch the timing of interdependent species, such as Parids and their moth larvae prey (Both et al., 2009, Visser et al., 2004). Therefore studies such as ours, which investigate individual level plasticity, are important to assessing the capacity of populations not only to survive short term environmental change but also to evolve in response to long term environmental change (Visser, 2008).

CHAPTER 4

PERSONALITY TYPES DIFFER IN OXIDATIVE PROFILE

4.1 ABSTRACT

Oxidative stress occurs when pro-oxidants, which damage body tissue, exceed the antioxidants that counteract them. I predicted that individuals differing consistently in behaviour, i.e. "personality", would differ also in their oxidative profile (pro-oxidants, antioxidants, oxidative stress and oxidative damage), for example due to differences in physical activity or responsiveness to stress. The personality traits measured, neophobia (latency to approach food near novel objects) and object exploration (latency to approach novel objects), were consistent within individuals and uncorrelated. Measures of oxidative profile: antioxidant capacity ("OXY"), pro-oxidant level (reactive oxygen metabolites, "ROMs"), oxidative stress ("OS", ratio of ROMs to OXY) and an end-product of oxidative damage: malondialdehyde (MDA), were uncorrelated. Object exploration and neophobia related positively and additively to OXY: neophilic or fast-exploring birds had higher OXY than neophobic or slow-exploring birds. ROMs and OS related only to neophobia: neophilic birds had lower ROMs and lower OS than neophobic birds. Variation in MDA was described by an additive quadratic and linear relationship with neophobia and object exploration respectively: fast-exploring and extremely neophilic or neophobic birds had lower MDA than birds with other combinations of traits. From these results, I draw three conclusions. First, personality types differ in oxidative profile. Second, though physiological differences between personality types (e.g. metabolic rate, stress responsiveness) generally range along a linear continuum, the physiological costs may not. For example, MDA differed between extreme neophilic or neophobic birds and intermediates. 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 trait correlations, "behavioural syndromes", arise between populations.

4.2 INTRODUCTION

Oxidative stress occurs when pro-oxidants, which are produced during normal metabolism and damage the body tissue, exceed antioxidant capacity which counteracts pro-oxidants (Finkel and Holbrook, 2000). Costs, in tissue damage and also investment into cellular repair and replacement, accrue under oxidative stress. As such, intraspecific variation in "oxidative profile" (pro-oxidants, antioxidants, oxidative stress and oxidative damage) often then predicts variation in health and longevity (Harman, 1956, Hulbert et al., 2007, Costantini, 2008). An individual's metabolic rate, and thus rate of pro-oxidant production, is context dependent (Ferguson et al., 2008, Alonso-Alvarez et al., 2004). However within contexts, individuals of the same mass are expected to have the same metabolic rate yet often differ still (Careau et al., 2008, Krol and Speakman, 2003, Careau et al., 2009). 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 (Gosling, 2001). For example, some individuals are consistently fast to approach novel stimuli, competitors or environments (i.e. neophilic, aggressive and fast-exploring) whilst others are consistently neophobic, passive and slow-exploring (Wilson et al., 1994). To respond quickly and actively, "fast" personality types may have a generally higher metabolic rate than "slow" types ("performance" or "sustained maximal limit" model; e.g. Drent and Daan, 1980), or channel more energy toward these activities from a limited energy budget (the "allocation" model; e.g.Wiersma et al., 2005, Wiersma et al., 2004, Wiersma and Verhulst, 2005, Cutts et al., 2002). Differing systematically in metabolic rate therefore, I predicted that personality types would differ also in oxidative profile.

Few studies investigate the relationship between oxidative profile and personality explicitly. An exception is recent work by Costantini and colleagues (2008a), which showed that mice from strains characterised by 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 et al., 2003; Ewalds-Kwist & Selander, 1996; Dingemanse et al., 2004;

Cavigelli et al., 2009), suggesting a cumulative cost to personality. In LAL-SAL mice for example, young LAL mice have higher antioxidant capacity than SAL mice yet no lower oxidative stress, and ultimately shorter life spans (Costantini et al., 2008; Ewald-Kwist & 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 most active (hence most metabolically active) individuals have shortest lifespan, a finding consistent across a broad taxonomic range (Biro and Stamps, 2008). Specifically, we could therefore predict that fast personality types suffer higher oxidative damage than slow personality types.

Second, in species with well defined personality types, individuals that are fast to engage with novel or threatening stimuli often have lower gluccocorticoid (stress hormone) levels than their slower counterparts, including for example SAL versus LAL mice (*Mus musculus*; Veenema et al., 2003), "proactive" (neophilic/aggressive/fast-exploring) versus "reactive" 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). Gluccocorticoids stimulate the metabolism to facilitate rapid behavioural responses, such as the fight or flight response (Cockrem, 2007). Thus slow personality types, that have consistently higher or more reactive stress responses, may then be expected to suffer higher oxidative stress than fast types, diverting more energy away from other activities into stress responses. Interestingly, dietary supplementation of poultry over days to achieve chronic levels of the gluccocorticoid corticosterone ("CORT", the avian stress hormone) raises lipid peroxidation (a measure of oxidative damage; Lin et al., 2004b), but acute exposure to CORT, via injection, does not (Lin et al., 2004a). With chronic exposure perhaps akin to persistent differences in stress reactivity as predicted by personality, and a single acute exposure analogous to short term fluctuations in stress that may be experienced by any individual (for review: Cockrem, 2007), this provides experimental support for stress responsiveness as a mechanism for personality differences in oxidative stress.

In this study, I investigated personality and oxidative profile in captive-bred European greenfinches (*Carduelis chloris*). First, I measured differences between hungry individuals in their latency 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, or "neophobia" (Mettke-Hofmann et al., 2002). Latency to approach in those trials may also be motivated by the novel object itself, however, for information gathering. To distinguish the affects of neophobia and information gathering on oxidative profile I also measured latency to approach novel objects in the absence of food, or "object exploration" (Mettke-Hofmann et al., 2002). Specifically I 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. I also tested whether neophobia and exploration were correlated within individuals. 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). Such correlations imply proximate links between traits, via genetic linkage or shared physiology (Verbeek et al., 1994), hence perhaps oxidative profile. However, correlations can also occur when two traits are not mechanistically connected but rather 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. My measure of pro-oxidant status and oxidative damage were an intermediate step and end-product of the lipid peroxidation cascade respectively: reactive oxygen metabolites (ROMs) and malondialdehyde (MDA). I measured anti-oxidant capacity (OXY) as the capacity of the plasma to resist oxidation by a pro-oxidant, hypochlorous acid. Oxidative stress (OS) was then defined as the ratio of ROMs to OXY x 1000 (Costantini and Dell'Omo, 2006). 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 (Wiersma et al., 2004, Costantini et al., 2007, Larcombe et al., 2010). Therefore I also investigated affects of body mass on oxidative profile. Finally: to determine whether neophobia or exploration co-varied with oxidative profile, and consequently whether personality types may differ in their oxidative costs.

4.3 METHODS

The study utilised 22 birds from a colony of captive bred greenfinches, 13 males and 9 females. Birds were sourced from several private breeders, but all were aged between 15 and 17 months and had been in the colony for at least seven months. Birds were kept

singly, in 120 x 50 x 50 cages, but in auditory and visual contact. Out-with trials, birds had *ad libitim* 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 in accordance with ASAB/ABS's guidelines for the treatment of animals in research, and subject to ethical review by WALTHAM® Centre for Pet Nutrition and the University of Glasgow. No birds became ill or died during this experiment. Neophobia trials were conducted between 26/08/08 and 04/09/08 and exploration trials between 05/09/08 and 08/09/08.

4.3.1 PERSONALITY TRIALS

4.3.1.1 Neophobia

Each bird took part in four neophobia trials across an eight 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 toward foraging activity. After 30 mins, the water bowl was also removed. After a further 30 mins (1h in total without food), the food bowl was returned to the cage and the latency to approach 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 cookie-cutter of approximately 3cm x 2cm x 1cm. Birds that did not approach within 30 mins were given a maximum latency of 1800 seconds. Phases were alternated each day; the first phase a bird received was randomized. Bird identity explained a marginally significant proportion of the variation in approach latency during disturbance phases (Linear mixed 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. Independent of response toward 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 I conducted the disturbance phases: for each object I 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 for each bird, converted to z scores, provided 4 measures of neophobia for each bird, one per object.

4.3.1.2 Object exploration

Each bird took part in two object exploration trials, conducted on consecutive days. The home cage contained six perches, three in each half. Prior to a test, the food and water bowls were removed as per the neophobia trial. 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 mins. 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 objects was randomised per bird. The latency to first land on the object perch was recorded. After 30 mins, the object was removed and the food and water bowls returned. Therefore each individual had two object exploration latencies. 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 affects between trials or the order in which objects were encountered.

4.3.2 Oxidative profile

Oxidative profile measures were derived from a blood sample of up to 300µl collected on 30/10/2008, taken within 3 minutes 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 minutes at 14,000g, and was then frozen at -80°C until analysis. ROMs were measured by the d-ROMs test and OXY by the Oxy-Adsorbent test (Diacron, Grosseto, Italy, as Costantini et al., 2007). MDA was measured using H.P.L.C. (as Young and Trimble, 1991). Body mass (g) was recorded immediately after blood sampling.

4.3.2.1 OXY analysis

OXY was measured as the capacity of the plasma to withstand oxidation by hypochlorous acid (HOCl). The plasma sample was defrosted at room temperature and then a 2µl of the sample or 2µl of a HOCl calibrator were each diluted 1:99 with distilled water (dH_20). A 200µl chromagen solution of 0.01 mol1-1acetic acid/sodium acetate buffer (pH 4.8) and N,N-diethyl-phenylenediamine was combined with 5µl of the diluted plasma, calibrator or dH_2O (control), and then incubated at 37°C for 10 minutes. Finally 2µl of the calibrator was added to each sample. Alkyl-substituted aromatic amine in the chromogen solution are oxidized by HOCl remaining in the sample (i.e. not quenched by plasma OXY), and produce a pink derivative, the intensity of which is measured at 490mm using a microplate spectrometer (Multiskan Spectrum, Thermo Scientific). OXY concentration is inversely proportional to the intensity of the pink, and was expressed as the µmol of HOCl/ml of sample, calculated as follows:

$$\text{OXY} = \frac{\text{Absorbance } dH_2O - \text{Absorbance sample}}{\text{Absorbance } dH_2O - \text{Absorbance calibrator}} \times \text{calibrator concentration}$$

4.3.2.2 ROM analysis

ROMs were measured as the pro-oxidant capacity of the plasma equivalent to mM hydrogen peroxide (H_2O_2). ROMs are expressed as Carratelli Units (CARR U), with 1 CARR U equivalent to the pro-oxidant capacity of 0.08mg H_2O_2. The HOCl calibrator and the above chromagen solution were combined in a 1:100 ratio. 4µl of the diluted plasma, calibrator or dH_2O were then added to the mixture and incubated at 37°C for 30 minutes. Colour intensity was measured at 490nm using the microplate spectrophotometer (Multiskan Spectrum, Thermo Scientific). The process cleaves hydrogenperoxides in the sample into two free radicals. These free radicals then react with alkly-substituted aromatic amine in the chromagen solution and produce a pink colour with intensity directly proportional to the hydroperoxide (pro-oxidant) content of the sample. The ROM concentration is then expressed as CARR U, according to the following equation:

$$\text{CARR U} = \frac{\text{Absorbance sample}}{\text{Absorbance calibrator}} \times \text{calibrator concentration}$$

4.3.2.4 MDA analysis

MDA, an indicator of lipid peroxidation and oxidative stress, was measured by reaction with thiobarbituric acid, following Young and Trimble (1991). A solution of thiobarbituric acid (0.44 M, 100µl) and phosphoric acid (1.22M, 100µl) was added to a test tube containing 50µl plasma from a bird, 50µl of a malonaldehyde bis(dimethyl acetyl) standard (Sigma Aldrich) or 50µl of dH_2O (control). For one bird where only 45µl of plasma were available 5µl of distilled water were added for consistent volume and MDA concentration later proportionally scaled to the sample size. A nitrogen blanket was added to create an inert atmosphere around the solution, and test tubes sealed, vortexed and then incubated for one hour at 70°C. After cooling in a water bath at room temperature, I pipetted 200µl of the mixture into a centrifuge tube that contained sodium hydroxide (1 M, 100µl). Methanol (500µl) was added and samples were vortexed. Finally, samples were centrifuged (10 minutes, 4010rpm). I used a Summit HPLC system (Dionex, Idstein, Germany) with Chromeleon software (Dionex) and an acclaim 120 C18 5µl column (Dionex) and guard to measure fluorescence (excitation 532nm and emission 553nm) of this supernatant. The mobile phase (40:60 methanol:phosphate buffer; 40mM, pH 6.5) had a flow rate of 1ml min-1.

4.3.3 Statistical Methods

Analyses were carried out using R version 2.9.1 (R Core Development Team, 2009). There were no significant sex differences in oxidative profile measures (ANOVA: ROMs $F_{1, 20} = 0.32$, $P = 0.58$; 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 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 $F_{6, 15} = 0.39$, $P = 0.88$; exploration $F_{1, 20} = 1.84$, P = 0.16). Therefore data were pooled across sexes and breeders. Relationships within and between oxidative profile measures and personality traits were analysed using general linear models (GLMs). To identify relationships between oxidative profile and personality, I first constructed GLMs with each measure of oxidative profile as the dependent variable and either neophobia or exploration as the

dependent variable. I specified both linear and quadratic expressions of the personality traits, to examine whether oxidative profile differed between the linear ends of the trait continua (neophobic versus neophilic or fast versus slow explorers) or instead between intermediate and extreme (neophobic and neophilic or fast and slow-exploring) personality types. To identify higher order interactions, I then constructed a GLM for each oxidative profile measure with all two way interactions between linear and quadratic expressions of both personality traits as the dependent variables. These models were simplified by backwards stepwise regression, removing non-significant ($P > 0.05$) interactions and then main effects in turn until only significant ($P < 0.05$) or no independent variables remained. All oxidative profile measures and body mass were log-transformed to normalise the residuals of these models.

4.4 RESULTS

4.4.1 INDIVIDUAL VARIATION IN PERSONALITY

In the neophobia trial, mean latency to approach the food bowl was significantly greater when a novel object was present than absent (paired Wilcoxon rank sum test: $V = 351$, $N_1 = N_2 = 22$, $P = 0.0001$), thus the presence of the object modified behaviour, i.e. induced neophobia. Individual neophobia was significantly repeatable (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 I used a mean z value per individual as the neophobia score for remaining analyses.

In the object exploration trial, I measured latency to approach novel objects in the absence of food. Individual latency was significantly repeatable over two trials (ANOVA: $r = 0.47$, $F_{21, 22} = 2.69$, $P = 0.013$). Therefore I used a mean latency per individual as the object exploration score for remaining analyses. There were no linear (GLM: $F_{1, 19} = 0.57$, $P = 0.46$) or quadratic relationships (GLM: $F_{2, 18} = 0.29$, $P = 0.76$) between individual neophobia and object exploration.

4.4.2 INDIVIDUAL VARIATION IN OXIDATIVE PROFILE

Variation in the time of blood sampling (10:00 – 16:00) 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 (up to 3 minutes; 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) or 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.

4.4.3 RELATIONSHIPS BETWEEN PERSONALITY AND OXIDATIVE PROFILE

I identified linear relationships between neophobia and three aspects of oxidative profile: neophilic birds had significantly lower ROMs ($t_{1, 20}$ = 2.57, P = 0.018; Fig. 4-1a), higher OXY ($t_{1, 20}$ = -2.25, P = 0.036; see Fig. 4-1b) and lower OS ($t_{1, 20}$ = 3.17, P = 0.005; Fig. 4-1c) than neophobic birds. There was no linear or quadratic relationship between neophobia and MDA in analyses including only neophobia as an independent variable (Fig. 4-1d).

I identified no relationship between object exploration and ROMs ($t_{1, 20}$ = 0.88; P = 0.39; Fig. 4-2a). There was a marginal relationship between object exploration and OXY, indicating that fast-explorers had higher OXY than slow-explorers ($t_{1, 20}$ = -2.09, P = 0.05; Fig 4-2b). There were no relationships between object exploration and OS (Fig. 4-2c). There was a significant linear relationship ($t_{1, 20}$ = 2.17, P = 0.04) but also quadratic relationship between exploration and MDA ($t_{2, 19}$ = -2.28, P = 0.034; Fig. 4-2d): fast-explorers had lower MDA than slow-explorers, but both fast- and slow-explorers had lower MDA than intermediate individuals.

In GLMs starting with all two-way interactions between both neophobia and object exploration as the independent variables, results pertaining to ROMs and OS were unchanged. In another GLM, I identified additive relationships between object exploration and neophobia together and OXY. This suggests that, though both traits had significant relationships with OXY in single trait analysis, the relationship of each trait to OXY was independent ($F_{2, 19}$ = 4.5, P = 0.025). Variation in MDA was best explained by

an additive relationship between the linear expression of object exploration and the quadratic expression of neophobia ($F_{2,\ 19}$ = 4.06, P = 0.034): birds that were fast-exploring and extremely neophilic or neophobic had lower MDA than birds that were slow-exploring or were intermediate in their neophobia scores.

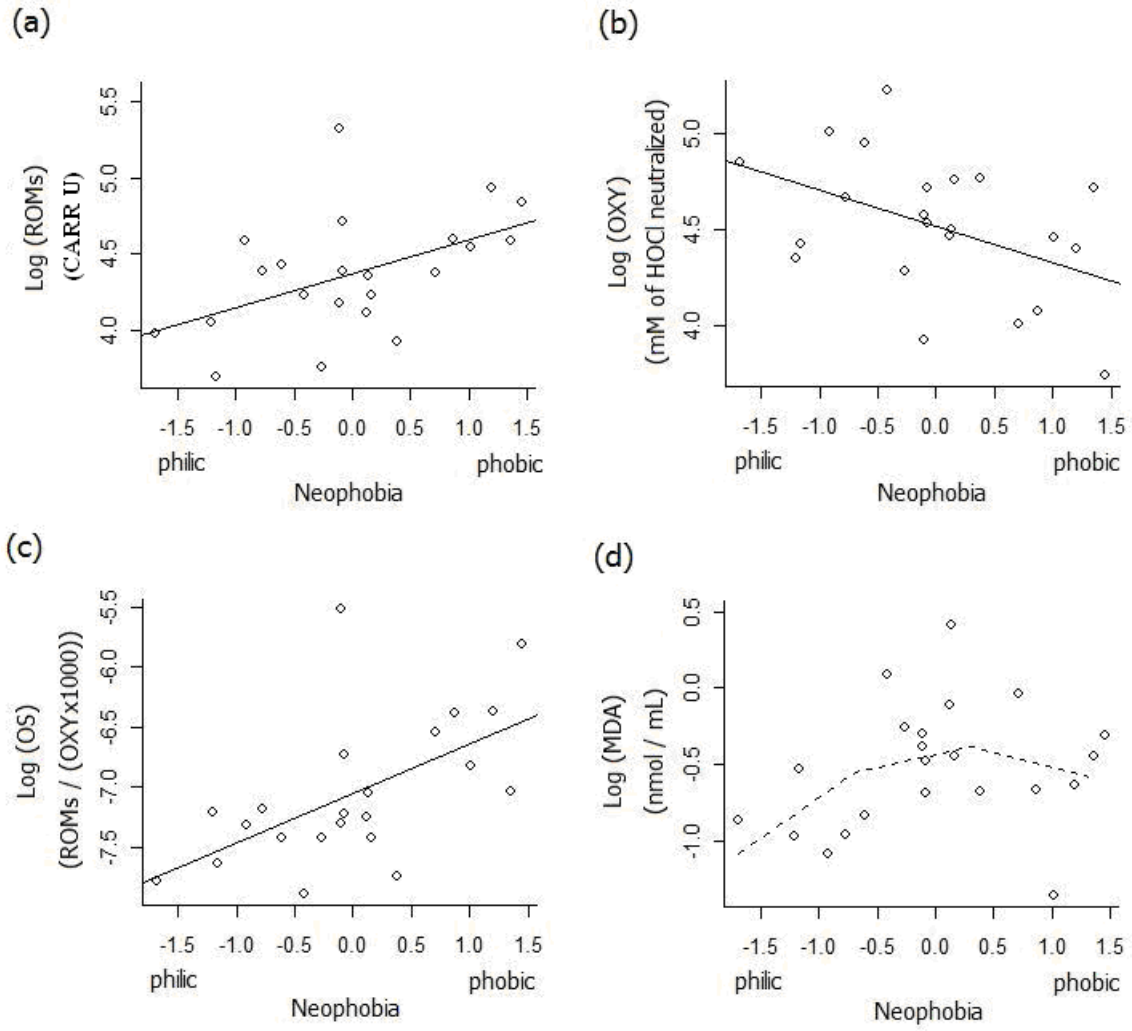


Figure 4-1 Relationships between oxidative profile and neophobia. Significant relationships from analyses with only neophobia as a dependent variable are represented with lines, and from analyses including object exploration as a covariate with dashed lines. (a) Neophilic birds had higher ROMs, (b) lower OXY and consequently (c) higher OS than neophobic birds. (d) Neophobia only significantly related to MDA in analyses including object exploration as a covariate, where the relationship with MDA was quadratic.

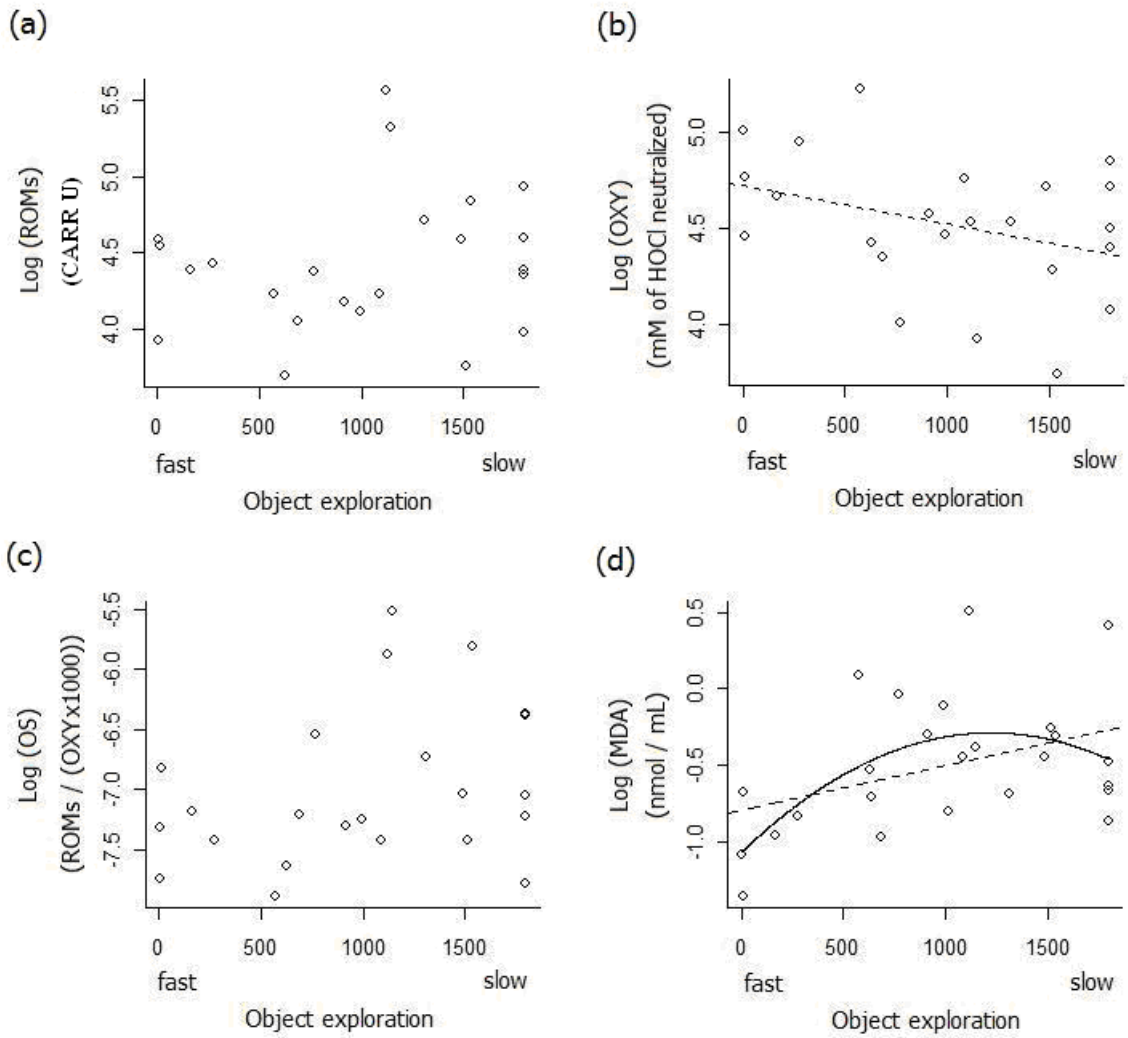


Figure 4-2 Relationships between oxidative profile and object exploration. Significant relationships from analyses with only object exploration as a dependent variable are represented with lines, and from analyses including neophobia as a covariate with dashed lines. (a) Exploration did not correlate with ROMs. (b) There was a negative relationship between object exploration and OXY (marginal in analyses excluding neophobia and significant in analyses including neophobia as a covariate), such that fast explorers had higher OXY than slow explorers. (c) There was no relationship between exploration and OS. (d) Fast and slow explorers had lower MDA than intermediates (i.e. a quadratic relationship) and, in analyses including neophobia as a covariate, fast-explorers had lower MDA than slow-explorers.

4.5 DISCUSSION

Neophobia and object exploration were consistent within individuals across days, thus constitute personality traits in the greenfinch. Both traits related to oxidative profile. Most relationships were linear, suggesting higher oxidative costs at the "slow" (neophobic/slow-exploring) than the "fast" extreme: neophilic birds had higher OXY, lower ROMs and consequently lower OS than neophobic birds; fast-explorers had higher OXY and lower MDA than slow-explorers. However there were also quadratic relationships between MDA and personality: the extremes (neophilic and neophobic birds and, in single trait analyses, very fast and slow explorers) had lower MDA than intermediate responders. Whilst the relationships between neophobia or object exploration and OXY or MDA appear similar, 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. I found no relationship between body mass and oxidative profile. Interestingly, I found no direct relationship between ROMs and OXY nor, though ROMs are a step in the lipid peroxidation chain that produces MDA, between OS and MDA.

This study confirmed my prediction that personality types would differ in oxidative profile. Measuring multiple aspects of oxidative profile, as I have done, is critical to drawing inference on variation in oxidative profile (Costantini and Verhulst 2009, Monaghan et al. 2009). For example, the fast ends of both trait axes (neophilic birds and fast-explorers) 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 neophilic birds do have lower OS than neophobic birds, fast-explorers achieve only the same plasma oxidative balance as slow-explorers. Costantini and colleagues (2008) found that passive strains of mice, like fast-exploring greenfinches, had higher OXY than aggressive strains yet equivalent OS. Passive mice also have shorter life spans (Ewalds-Kwist and Selander 1996) and are more physiologically stress-responsive than aggressive mice (Veenema et al. 2003). Unifying these studies, Costantini and colleagues (2008) suggest the apparent antioxidant surplus may in fact be a buffer against short term, stress-induced increases in free radical production. Accordingly, short life span is suggested as the cumulative cost of this up-regulation. From my results on OXY and ROMs, I may similarly predict short life span in fast-exploring greenfinches. 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 2000) and ability to control sparse resources (Dingemanse and de Goede 2004), have recently received considerable attention. Less common studies on potential cumulative, physiological costs, through variation in oxidative profile (e.g. Costantini et al., 2008) 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, I found that fast-explorers had lower MDA than slow-explorers. Similarly, I found a positive relationship between neophobia and OS but a quadratic relationship with MDA, such that neophobic birds with highest OS 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 1) on plasma samples, and 2) in aqueous solution (Bartosz, 2010). As such, important lipid-soluble antioxidants such as α – Tocopherol (vitamin E) and Ubiquinol (coenzyme Q) that occur mostly in the cell-membranes are underestimated by these methods. Contrasting my results on OS with MDA therefore, I suggest there may be further personality variation in cell-membrane antioxidant capacity. Specifically: a greater cell-membrane capacity in fast than slow-explorers and in neophilic and neophobic extremes than intermediate responders.

The relationship between neophobia and MDA raises a further interesting point: physiological differences between personality types generally range along a linear continuum, for example in several species, from low to high stress responsiveness (baseline and/or elevated gluccocorticoid level) with increasing neophobia (for review: Cockrem 2007). However my results demonstrate that the physiological costs of personality may not be linear: intermediately neophobic birds higher MDA than neophilic and neophobic extremes. This quadratic relationship may be explained by differential budgeting into antioxidant defences: OXY was higher in neophilic birds, therefore neophobic birds may either benefit from investing less energy into plasma antioxidant systems or by investing instead into e.g. plasma-membrane antioxidant systems, not measured in the OXY assay. Indeed, these traits may constitute two-dimensional continuum in greenfinches, with general "responsiveness" to novel objects (whether neophilic or neophobic) a shared mechanism differentiating oxidative profile (i.e. plasma or plasma-membrane antioxidant systems) between extremes and (less responsive)

intermediates. It is a common assumption of personality research that responsiveness to stimuli falls along a single-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 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 extremes (Dingemanse et al. 2004). Whilst the physiology of fast and slow personality types are often well characterized by selection line studies (e.g. Carere et al. 2003, Cavigelli and McClintock 2003, Martins et al. 2007, Veenema et al. 2003), these results suggest that the physiology of intermediate personality types warrant further investigation.

That neophobia and object exploration, 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 (2002) found no general correlation between neophobia and object exploration. Moreover, the expression of each trait correlated to different ecological variables: exploration was fastest in species that appeared to benefit most from information gathering, that inhabited relatively changeable (e.g. forest edge) versus homogeneous habitats and cryptic versus conspicuous prey (e.g. buds versus fruit/flowers). Conversely, neophobia appeared related to dietary risk: novel insects are potentially noxious, and insectivorous species were more neophobic than leaf-eating species. In physiological studies too, neophobia appears 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, I predict that whilst both trials presented an opportunity for information gathering, only the neophobia trial elicited a stress response, hence assayed individuals for stress responsiveness. Overall though, I 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 slow-exploring but extremely neophobic or neophilic birds. 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 costs vary within and between personality traits may therefore provide new insight into the selection mechanisms differentiating behavioural syndromes between populations.

Finally, as metabolic rate increases with body size across species (Lovegrove, 2000), I expected OS, ROMs and MDA to increase with body size within greenfinches. In line with a number of within-species studies on metabolic rate though (for review: Careau et al., 2008), I found no relationship. 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 that found in wild-caught greenfinches (0.66 ± 0.33 nmol MDA/mL plasma compared to 1.23 ± 0.68 nmol MDA/mL plasma; Horak et al., 2006). Wild birds may differ substantially from caged birds in the demands on their antioxidant systems. For example, one prolonged (> 1h) flight in homing pigeons can cause an immediate depletion of 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., 2010). 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 an exercise studies (Dotan et al., 2004). However, variation in oxidative profile between personality types suggests that, even within benign, homogenous 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), migration (Costantini et al., 2007) 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.

CHAPTER 5

CONTEXT-DEPENDENT PREFERENCES FOR LOCAL OR SPATIAL CUES IN THE EUROPEAN GREENFINCH

5.1 ABSTRACT

Using local cues such as colour or shape to identify ephemeral food can increase foraging efficiency. The visual appearance of food may change over time, however, therefore animals should use spatial cues to re-find food that occurs in a temporally stable position. tested this hypothesis by measuring the cue preferences of captive greenfinches *Carduelis chloris* when relocating food hidden in a foraging tray. In these standardised associative learning trials, greenfinches favoured local cues when returning to a foraging context that they had encountered before only once ("one-trial test") but switched to spatial cues when they had encountered that scenario on ten previous occasions ("repeated-trial test"). suggest that repeated encounters generated a context in which individuals had a prior expectation of temporal stability, and hence context-dependent cue selection. Next, trained birds to find food in the absence of local cues but tested them in the presence of visual distracters. Birds were able to learn spatial cues after one encounter, but only when visual distracters were identical in colouration to the spatial cue. When a colourful distracter was present in the test phase, cue selection was random. Unlike the first one-trial test though, birds were not biased *toward* this colourful visual distracter. Together, these results suggest that greenfinches are able to learn both cues, local cue biases represent learning, not simply distraction, and spatial cues are favoured over local cues only in temporally stable contexts.

5.2 INTRODUCTION

The distribution of food in the natural environment is generally ephemeral. As birds are predominantly visual foragers, learning about the appearance of food types (generating a 'search image': Tinbergen, 1960; Dawkins 1971; Lawrence 1986) can increase their efficiency at locating such food before it decays, moves or is depleted. Also, by generalising amongst experiences of similarly coloured (Gamberale and Tullberg 1996; Baddeley et al. 2007) or patterned (Swaddle, Che and Clelland, 2004) food types object-specific or "local cue" learning can aid birds in recognising potential in unfamiliar (e.g. seasonal or patchily distributed) food types. Where food occurs reliably in a particular position though, it may also be located by learning spatial relationships with landmarks in the broader environment (e.g. trees, rivers, houses; Cheng 1988; Collett et al., 1986; O'Keefe and Nadel, 1978). When the position of food is temporally stable, local cues (aspects of food or feeding site appearance) are generally less permanent than spatial cues. In spatiotemporally stable circumstances therefore, a more reliable strategy may be to relocate food by spatial rather than local cues (Bennett, 1993).

The question of whether expectation of temporal stability in food location influences cue selection has often been tested using food storing or "caching" species. Food storing species hide food about their environment and then retrieve it later when seasonal food is scarce (Sherry, 1984): tendencies which make them a useful model for testing hypotheses on spatial memory. In a one-trial memory task where either a spatial or a local cue may be used to relocate food, these storers tend to favour the spatial cue whilst closely related non-storing species use both cue types equally (Clayton and Krebs 1994; Brodbeck 1994; Brodbeck and Shettleworth 1995; but see Hodgson and Healy 2005; LaDage et al. 2009). To relocate caches over long periods, during which local cues to their location may change, it is suggested that storers may be under particular selection pressure (compared to non-storers) to remember spatial information. However, tests of whether storers have superior spatial memory retention to non-storers, the central tenet of the "adaptively specialised memory hypothesis" (Krebs, 1990), are inconclusive (e.g. Shettleworth et al., 1990; Hilton and Krebs, 1990; Healy and Krebs, 1992; Healy, 1995) and some authors suggest that it is not memory but rather cue prioritisation that differs between species (Shettleworth and Westwood, 2002; Shettleworth, 2003). For example, when spatial cues prove unreliable, storers often attend to local cues as a second choice

(Brodbeck 1994; Brodbeck and Shettleworth 1995; Clayton and Krebs 1994). This raises an interesting, and untested point: if non-storing species have equivalent memory capacity for spatial cues to storers (at least within days: Krebs et al., 1990; Hilton and Krebs, 1990; Krebs and Healy, 1995) then do they prioritise spatial cues when the location of food is perceived to be temporally stable? Foraging on ephemeral food, the tendency to use both cue types equally in one-trial memory tasks may reflect selective pressure toward information gathering (Humber et al., 2009), as local cues may be useful when ephemeral food is scattered but spatial cues when it is clumped.

A prior expectation of temporal stability in a feeding site may materialise if individuals repeatedly return to a site to resample the distribution of food, and find that it is unchanged (McNamara and Houston 1980). For example, the great tit (*Parus major*), a non-storer, shows equivalent retention times for local and spatial memories (Healy 1995) thus may draw on either at long intervals, but if allowed to encounter a stable distribution of food repeatedly over a long interval, then prefer spatial over local cues (Hodgson and Healy 2005). Similarly, pine siskins (*Carduelis pinus*) generally use spatial position rather than colour to distinguish between repeatedly encountered well, medium or poorly stocked feeders in the wild (Humber et al. 2009). However, no study has yet compared cue selection between scenarios encountered once and repeatedly to assess whether spatial biases in these species represents a *change* in tactic with a prior expectation of temporal stability in food location.

The aim of this study was to test whether repeated encounters with an invariant foraging scenario would cause a non-storing species, the European greenfinch (*Carduelis chloris*), to favour spatial cues over local cues. A limitation of previous studies on cue selection, raised by LaDage and colleagues (2009), is that stimulus design may generate biases toward particular cue types by rendering some easier to learn than others (Shettleworth, 2005) or by "overshadowing" other available cues (Cheng, 2008; Cheng et al., 2007; Gray et al., 2005). Noting that previous studies often used a local cue that may be regarded more complex than the spatial cue (e.g. trial-specific complex patterns for local cues versus few spatial locations used across trials: Brodbeck et al., 1994; Clayton and Krebs, 1994), LaDage and colleagues (2009) attempted to provide local and spatial cues of equivalent complexity, using single blocks of colour for local cues. These simple local cues were favoured over spatial cues by the mountain chickadee (*Poecile gambeli*), a storer. Here I attempt to eliminate cue perceptual salience as an explanation for cue selection by using an invariant situation for both a one-trial and repeated-trial associative learning test. Examining the change in behaviour within an invariant foraging scenario,

rather than initial response to the stimuli, I examine specifically the affect of perception of temporal stability. I also conducted two trials to examine the mechanisms underlying cue selection within the one-trial tests. First, in the "one-trial spatial test", I tested whether greenfinches were able to learn spatial cues in the absence of local cues. And second, in the "one-trial distraction test", I tested whether their propensity to use spatial cues was affected by the presence of conspicuous visual distracters, as the local cue in the one-trial test may then similarly distract birds from spatial cues in the one-trial test.

5.3 METHODS

The study utilised a colony of 27 captive bred greenfinches aged between 15 and 17 months. Birds were sourced from several private breeders but were housed in one room for at least 7 months prior to trials. Each bird was housed individually, in a 120cm x 50cm x 50cm cage. All but the front panel of the cage were opaque, and screens were erected for an hour prior to and during trials to remove opportunity for the focal bird to copy the cue selections of other birds. As greenfinches are social outside of the breeding season however, they were kept in auditory contact and cages were positioned to allow visual contact outside of trials. Except during trials, birds had *ad libitim* access to Haith's™ greenfinch mix and water, and were provided with 10 defrosted frozen garden peas per day (but up to 14 during training, see below). The study was conducted from the 26th August to the 19th October 2008, throughout which the room was maintained at a temperature range of 16°C to18°C with a 14:10 hour light:dark regime. All work was carried out in accordance with ASAB/ABS's guidelines for the treatment of animals in research, and subject to ethical review by WALTHAM® Centre for Pet Nutrition and the University of Glasgow. No bird became ill or died during this experiment.

5.3.1 TRAINING FOR CUE SELECTION TRIALS

My aim was to investigate whether individuals favoured local or spatial cues when relocating food. I used methods for studying cognition in small passerines established by Hodgson and Healy (2005). Briefly, in their home cage, each bird was trained to forage for food in a 24cm x 20cm white plastic ice cube tray. The tray had two rows of seven square wells that were approximately 2cm in depth and of 2.5cm diameter (see Fig. 5-1).

During the trials the birds were required to remove a cotton wool ball of approximately 1 gram that plugged a well to find a food reward (3 garden peas) hidden beneath (as Donaldson, 2009). This relatively simple learning scenario was adopted after pilot trials indicated that finches have a more limited cognitive and/or motivational capacity than Parids (Hodgson and Healy, 2005, Arnold et al., 2007).

Birds were trained to use this apparatus over 12 days, during which they underwent one training session in the morning, between 8:00 and 12:00 and a second between 13:00 and 17:00. On days 1-3, birds were familiarised with feeding from the tray: the tray was presented with a garden pea in each of seven wells and birds were left undisturbed to eat for one hour. For the remaining training, the food bowl was removed for one hour prior to each training session to motivate the birds toward foraging. On training days 4-6, the tray was then returned with seven peas randomly distributed amongst the wells and three cotton wool balls placed loosely on top. This habituated the birds to eating in the presence of the cotton wool balls; all birds had done so by day 6. On days 7-9, a cotton wool ball was placed over seven wells. There was an empty well or no well above, below and to the side of each covered well (i.e. cotton wool balls covered positions 1, 3, 5, 7, 9, 11 and 13, or 2, 4, 6, 8, 10, 12 and 14, randomised across replicates; see Fig. 5-1a). There was a reward in every covered well, and this familiarised the birds with manipulating the cotton wool balls to access food; all birds had opened at least one well by day 9. On days 10-12, again seven wells were covered but three were rewarded with two garden peas and four empty (see Fig. 5-1a). I covered empty along with rewarded wells to encourage the birds to search for food in preparation for trials; three birds failed my learning criterion of opening at least one rewarding and one empty well in the same training session and were excluded from the cue selection trial. To maintain training throughout the study, this final phase was run daily for birds that were not involved in trials on a particular day. Eight (out of ten) females and 16 (out of 17) males completed the training, and were entered into the cue-selection trials.

During training, I made two observations on well-opening behaviour that informed my experimental design for the cue selection trials. First, during the final phase of training (days 10 – 12) when, as in the trials, some rewarding and some unrewarding wells would be encountered (Fig 5-1a), the number of wells opened per bird per replicate ranged between 0 and all 7 (mean = 4.3). Birds did not significantly increase (or decrease) their propensity to open wells as the training phase progressed (Linear Mixed Model with bird identity as a random effect: $t_{119} = 1.78$, $P = 0.08$), so this variation did not relate to improvement across trials. Calculating repeatability (r) of the wells opened

per individual per replicate, using the mean squares from an analysis of variance (ANOVA) as Lessells and Boag (1987), I found that the 24 birds which passed training exhibited consistent and repeatable differences in well-opening tendency, i.e. some always opened more and some fewer (ANOVA: $F_{1, 120} = 4.87$; $P = < 0.0001$, $r = 0.66$). Second, during days 10 – 12 of training, the mean proportion of birds opening first a well on the left or right did not differ from the random expectation of 50:50 (G-test: $G_1 = 0.24$, $P = 0.62$) and the mean proportion opening first wells on the ends (wells 1, 7, 8 or 14) versus middle of the tray (wells 2, 3, 4, 5, 6, 9, 10, 11, 12, 13; Fig 5-1a) did not differ from the random expectation of 29:71 ($G_1 = 2.12$, $P = 0.15$). Therefore the position of the first well opened was random. However the subsequent wells opened were generally those closest to the first well (K.H., pers. obs.). These observations necessitated simplification of the foraging task in the trials down from seven to at most three covered wells, and restriction of analyses to the first selected well only (see below).

5.3.2 Study 1: Cue selection in the One-Trial versus Repeated-Trial Test

The primary aim of this study was to identify preferences for local or spatial cues in a foraging context that had been encountered only once and in a similar context that had been encountered repeatedly.

Each test had two phases: a learning phase and a test phase. In the learning phase, after 1 hour without food the focal bird was presented with a tray with one well covered by a coloured cotton wool ball (dyed red, green, blue or yellow; Fig 5-1b). This well contained a reward, and all other wells were empty and uncovered. Once opened, the bird was allowed to eat from the well for 15 seconds, generally eating less than one pea, before the tray was removed. In the one-trial test, birds proceeded directly to the test phase. In the repeated-trial test, birds received 10 consecutive presentations of the learning phase on one day, with the same configuration of colour and position, prior to the test phase. I used only one well during training because birds differed in their propensity to open wells (see above); otherwise, birds consistently opening more or fewer of any additional (empty) wells across these 10 presentations would enter trials with different levels of information on the distribution of food elsewhere in the tray. In the test phase, each bird was presented with a tray in which three wells were covered by cotton wool balls: a white cotton wool ball covered the correct well from the learning phase (i.e. correct position: the “spatial cue”), a cotton wool ball of the same colour as in

the learning phase but placed over a novel well (i.e. correct colour: the "local cue") and a third novel well was also covered with a white cotton wool ball (i.e. wrong colour, wrong position: a "distracter"; Fig. 5-1c). The conformation of the three wells was the same for all individuals within each replicate and was randomised across replicates. Only three wells were covered to allow at least one well space between covered wells whilst permitting some flexibility in the conformation of rewarding wells, as during training, birds tended to open adjacent wells (see above) so may be attracted to wells that were closer together. All wells were unrewarded in the test phase so that odour cues could not be used. The first well opened were noted. Each bird took part in four one-trial tests and four repeated-trial tests. Within trials each replicate used a different colour but the same four colours were used in both trials so that cue colour could not explain differences between trials. The order of trials and colours were both randomised for each bird. Each bird took part in only one test (one-trial or repeated-trial) per day. I took two measures to limit the possibility of carry-overs in cue learning (and hence biases) between trials. First at the end of each trial, to re-train the birds that the white and coloured cotton wool balls which covered empty wells in the test phase could be rewarding in future trials, when returning the food bowl I also presented the birds with a tray with two covered rewarded wells, one white and one of the test colour. This tray was left in the cage for an hour, during which time birds unanimously opened both wells. Second, the colours of local cues and wells used for spatial cue position were systematically randomised to differ between trials on consecutive days. Therefore specific colours or positions would not be informative in consecutive trials.

Differences in cue selection between the one-trial and repeated-trial tests may relate to repeated encounters prior to testing or equally to the longer time interval between the first learning phase and the test phase in the repeated-trial test. To determine which was most important, I calculated the mean time to complete the repeated-trial across birds, which was 31 minutes. On the two days following the one-trial and repeated trial tests, on two occasions (one per day), each bird then underwent a training phase and test phase separated by this interval, hence forth the "one-trial duration test". Colours used were blue and yellow, the order of which was randomised per bird.

5.3.3 Study 2: Spatial Cue Use in the Absence of Local Cues

In the one-trial test, the presence of a local cue may affect a bird's ability to learning spatial cues by creating a distraction. Therefore I carried out two tests to investigate spatial cue use in the absence of local cues. These trials used the same general format as the one-trial test. In the first trial, during the training phase only one well was covered by a white cotton wool ball, under which was the pea reward (Fig. 5-1d). In the test phase, three wells were covered by white cotton wool balls, one in the correct position and two in novel, randomly selected positions (Fig. 5-1e). Thus, if birds learned the spatial location, they would open the correct well more often than the chance expectation of one in three occasions. This was the "one-trial spatial test". For the second trial, again only one white cotton wool ball was used to cover a well in the training phase. In the test phase, I again placed a white cotton wool ball in the correct position, but one white and one coloured distracter in the test phase (Fig. 5-1f). This was the "one-trial distraction test". The colours chosen for the short term distraction trial were green and red. The colour of the distracter did not affect cue selection (G-test: $G_2 = 0.009$, $p = 1$). Each bird underwent two one-trial spatial tests and two one-trial distraction tests, with trials and (for the distraction trial) colours presented in a random order across four consecutive days. A bias toward the spatial cue was again tested using deviations from a two to one chance expectation.

5.3.4 Statistical methods

In study one, for each trial I compared the distribution of selections using the G-test (Sokal and Rohlf, 1994; Hodgson and Healy 2005) or, where expected values were below 5, a fisher exact test with values rounded to the nearest integer. Seven birds failed one of the four replicates of the one-trial test, i.e. did not open a well within the 30 minute test phase. Similarly five birds, including four of those which failed one one-trial replicate, failed one of the four replicates of the repeated trial test, i.e. did not open a well within 30 minutes of either a learning phase or test phase. To avoid pseudoreplication (up to four replicates per bird per test) and also account for this variation in trial numbers, rather than the sum of selections for each cue type across birds I used the sum of the proportion of selections per cue type per bird. The resulting distribution, which then summed to the number of birds, was compared to the expected random distribution of 1:1:1. I repeated analyses including only those birds which completed all replicates within a test, i.e. for 17 birds in the one-trial test and 19 birds in the repeated trial test. To

avoid psuedoreplication in that restricted analysis, I calculated the average distribution of cue selections across replicates for each test. Finally, using only those 17/19 birds that completed all replicates within a test, I compared the two average distributions directly, to identify whether cue selection differed between the one-trial and repeated-trial tests.

In study two, in both the one-trial spatial test and one-trial distraction test there were only two outcomes in the test phase: distracter or spatial cue, which at random would be selected in a 2:1 ratio (i.e. two distracters and one correct position). To avoid pseudoreplication, I calculated the average distribution of cue selections across replicates of the two tests. I used a binomial test to compare these observed distributions to the expected 2:1 ratio of distracters to spatial cues.

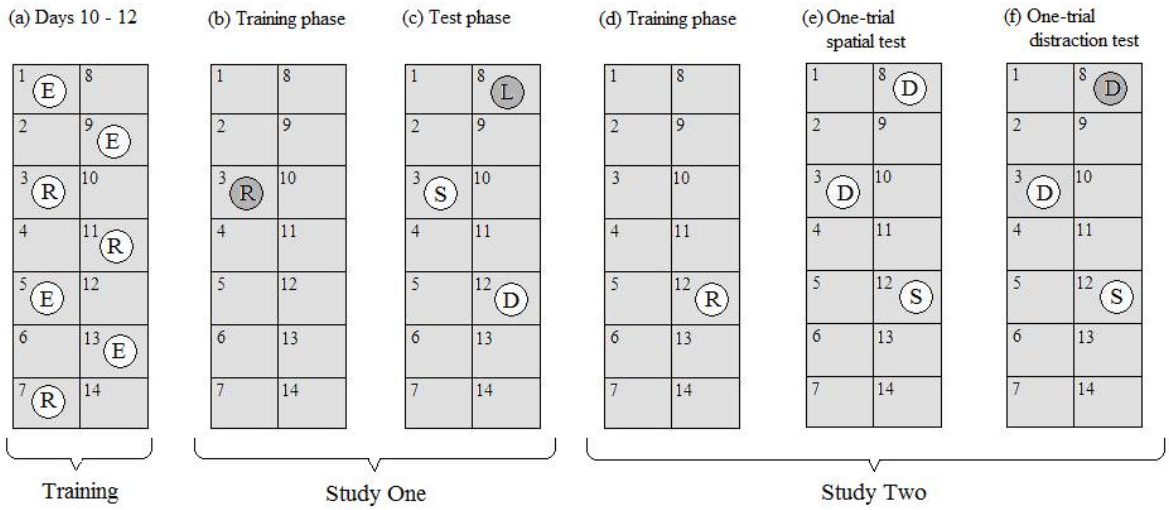


Figure 5-1 Diagrams of example layouts for the tray used during training and cue selection trials. (a) Days 10 – 12 of training to use the apparatus. (b) The training phase of study one and (c) the test phase of study one. (d) The training phase of study two. (e) The test phase study one's one-trial spatial test. (f) The test phase of study one's one-trial distraction test. Each numbered box represents a well on the ice cube tray, with wells 1 and 8 nearest the back of the cage and wells 7 and 14 nearest the front. Circles represent cotton wool balls plugging wells, with coloured cotton wool balls (blue, green, red or yellow) shown in dark grey. Cotton wool balls labelled R concealed a well containing a food reward and E an empty well. Cotton wool balls labelled S are spatial cues, L are local cues and D distracters; S, L and D wells were all unrewarded.

5.4 RESULTS

5.4.1 STUDY 1: CUE SELECTION IN THE ONE-TRIAL VERSUS REPEATED-TRIAL TEST

There was no difference in the distribution of selections between sexes in either the one-trial test or repeated-trial test (for both, Fisher's Exact test P = 1), so data was pooled across sexes. There was no difference in the cue selection distribution across the four replicates of the one-trial test (Fisher's exact test: P = 0.15) or repeated-trial test (Fisher's exact test: P = 0.76; see Fig. 5-2). In both the one-trial (G test: one-trial: $G_2 = 11.13$; p = 0.004) and repeated-trial tests ($G_2 = 6.06$, p = 0.048), the distribution of choices differed significantly from random (see Fig. 5-3). When birds that had failed one replicate of the one-trial test were excluded, results were similar (G test: one-trial: $G_2 = 8.21$; P = 0.016). When birds that failed one replicate of the repeated trial test, the distributions of selections was no-longer significantly different to random ($G_2 = 4.74$, P = 0.09). Overall however, comparing the distribution of selections between the one-trial and repeated-trial test, there was a significantly change from a predominantly colour cue biased search in the one-trial test to a predominantly spatial cue biased search in the repeated trial test (Fisher's exact test: P = 0.0002). In the one-trial duration test, however, the distribution of cue selections was not significantly different to random (G_2 0.51, P = 0.77), thus the spatial cue bias in the repeated-trial test related to the repeated encounters with the tray prior to testing rather than the longer duration of the repeated-trial than one-trial test.

5.4.2 STUDY 2: SPATIAL CUE USE IN THE ABSENCE OF LOCAL CUES

In the one-trial spatial test, there were three white cotton wool balls with one in the correct spatial location and two distracters (i.e. no coloured local cue). In this test birds used the spatial cue more often than expected by chance (binomial test: P = 0.048; see Fig. 5-4). Therefore, greenfinches appear capable of learning spatial cues after one encounter with a simple foraging context.

In the one-trial distraction test, there were two white cotton wool balls, one in the correct spatial location and one in a novel location (a distracter) and one coloured cotton wool ball in a novel location (a coloured distracter). In contrast to the one-trial spatial test with only white distracters, the distribution of selections in the presence of a coloured

visual distracter was not significantly different to random (binomial test: P = 0.67; see Fig. 5-4). As the one-trial spatial and distraction tests differed only in the test phase, the presence of uninformative visual stimulus appears to affect the propensity to use spatial cues that have been learnt. However, unlike the first one-trial test, birds were not biased *toward* the visual distracter over the spatial cue thus were visually-biased only when an informative local cue was available.

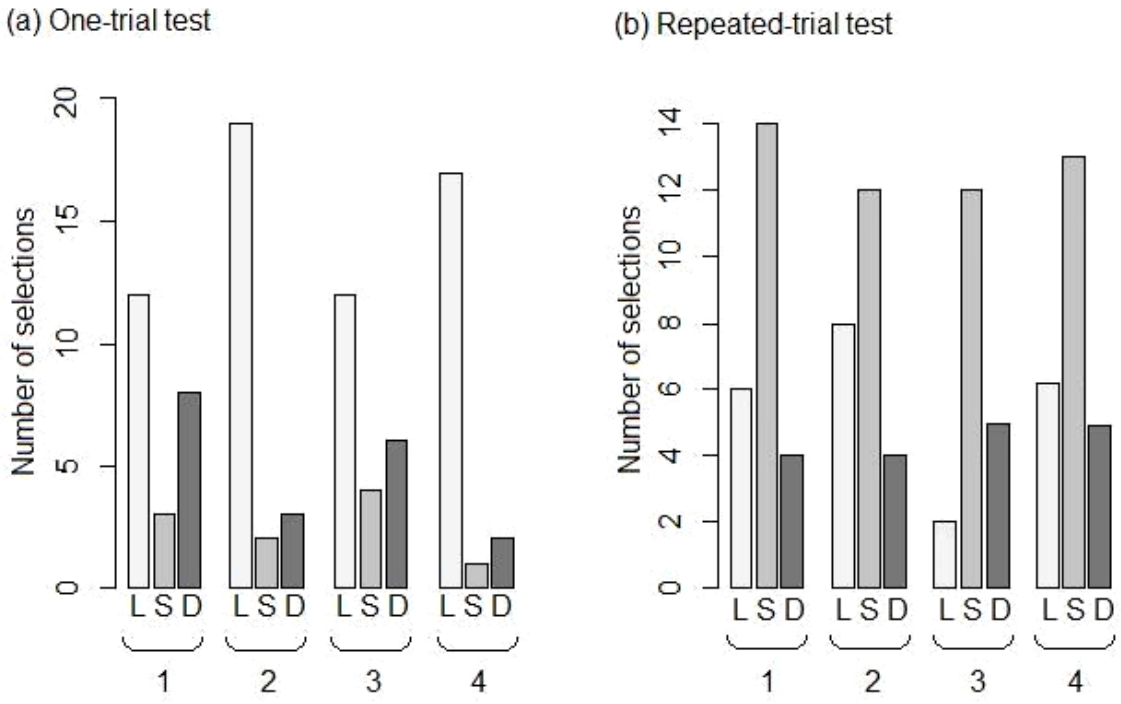


Figure 5-2 Distribution of cues selected in study one for all birds across the four replicates of the (a) one-trial test and (b) repeated trial test. Local cues are represented with pale grey bars and denoted L, spatial cues medium grey and denoted S and distracters dark grey and denoted D

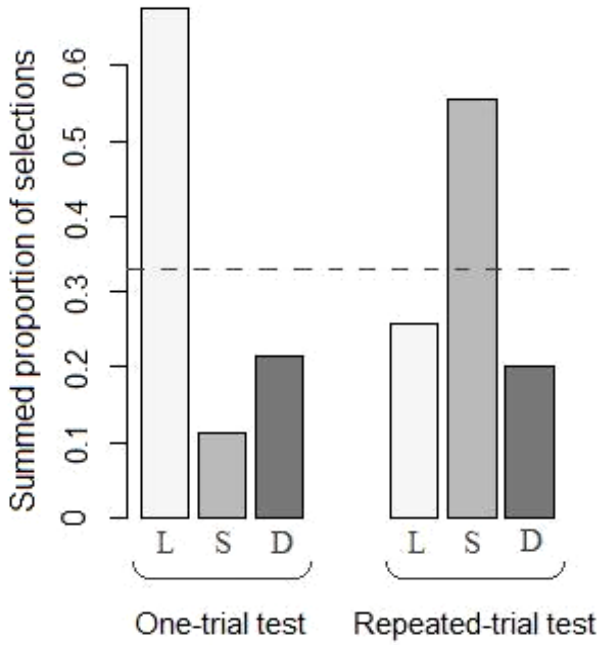


Figure 5-3 Summed proportion of cue selections across birds in study one for the (a) one-trial test and (b) repeated-trial test. Local cues are represented with pale grey bars and denoted L, spatial cues medium grey bars and denoted S and distracters dark grey bars and denoted D. The dashed line at 0.33 represents random choice.

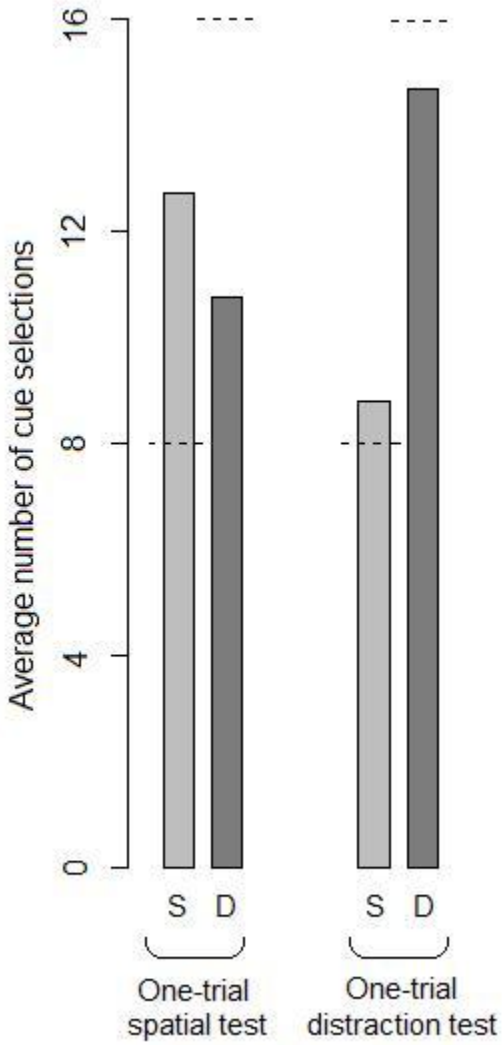


Figure 5-4 Average distribution of cue selections across birds in study two for the (a) one-trial spatial test and (b) one-trial distraction test. Spatial cues are represented with pale grey bars and denoted S and distracters dark grey bars and denoted D. Dashed lines at 0.33 and 0.667 represent random choice of spatial cues and distracters respectively (i.e. 1 : 2 ratio of spatial cues to distracters).

5.5 Discussion

Here, I demonstrated that greenfinches favoured local over spatial cues in a one-trial test, but spatial cues in a repeated-trial version of the same test. This cue-switch was dependent on repeated encounters with a foraging scenario prior to testing, as cue selection after a single encounter and an absence equivalent to the duration of the repeated-trial test was random. Therefore, I have evidence that cue selection altered when individuals had experience of a temporally stable foraging context. I then established that greenfinches were able to learn spatial cues in the absence of local cues (one-trial spatial test), but only in a visually simple foraging environment. Birds did not use this spatial learning when confronted with a visual distracter (one-trial distraction test). However, they were not biased *toward* the visual distracter, thus the bias toward the local cue in the first one-trial test reflects associative learning rather than simply distraction.

In the one-trial test, greenfinches differed from other non-storers tested by similar methods (Clayton and Krebs 1994; Brodbeck 1994; Brodbeck and Shettleworth 1995) in expressing a bias toward local cues, rather than equal use of local and spatial cues. There are two explanations for this result. First, as discussed in the introduction, this may be an artefact of stimulus design, as cues which are simpler to distinguish from alternatives are easier to learn (the "cue complexity hypothesis": LaDage et al. 2009). For example the propensity to use spatial cues in both black-capped chickadees (*Poecile atricapilla*) and dark-eyed juncos (*Junco hyemalis*) declines with increasing proximity of distracters to cues (Shettleworth and Westwood, 2002). In my experiment, the local cue was a uniform, conspicuous colour (a coloured cotton wool ball on white background): attributes which enhance the rate or accuracy of learning in birds (Ruxton et al. 2004). This may therefore have rendered the local cue significantly simpler to learn than the spatial cue, giving the local cue greater *perceptual* salience than the spatial cue (Shettleworth, 2005). Alternatively, however, the local cue may have greater *functional* salience than the spatial cue. As an aid to detecting new rather than relocating old (possibly exhausted) feeding sites, being "visually-oriented" may be an adaptation in the greenfinch toward a generally unpredictable distribution of food. Indeed, their sensitivity to uninformative visual distracters could equally be interpreted as responsiveness toward local information *per se*. Across species, reactions toward uninformative visual stimuli appear also to relate to the temporal stability of prey. The presence of visual distracters for example is more detrimental to spatial learning in non-storers than storers (McGregor and Healy 1999). This susceptibility to distraction may therefore be an adaptive response bias, as an aid to

prospecting for alternative food types. For example frugivory in parrots is associated with increased exploration of novel visual stimuli, which may in turn aid the discovery of previously un-encountered (e.g. seasonal) fruit (Mettke-Hofmann et al 2005). Therefore, when not equipped with information about the temporal stability of the feeding situation (i.e. in the one-trial tests), I suggest that responses to uninformative visual stimuli as well as informative local cues may both be evidence of a local cue biased foraging strategy in the greenfinch.

Using spatial cues may incur a search cost, if the signalled food decays or is depleted prior to using that spatial cue. These costs are mitigated when individuals have a prior expectation that food will 1) be temporally stable and/or that 2) searching without spatial information will be less efficient than searching with spatial information. In storing species, these prior expectations may be innate, evolving along with the propensity to store (Krebs 1990). In species with generally ephemeral food such as the greenfinch, however, this information must be acquired through experience. There are a number of examples where increasing the costs of alternative search strategies causes individuals to favour spatial cues. For example, increasing the energetic costs of a random search strategy, by weighting down flaps that conceal food, causes an increase in spatial cue use by zebra finches (*Taeniopygia guttata*; Sanford and Clayton 2008). Spatial cues are also particularly useful when *local* search strategies are unreliable. For example, in contrast with sticklebacks (*Gasterosteus aculeatus*) from ponds, fish from streams favour spatial cues (e.g. current direction, body orientation) over local landmarks when navigating a maze to find food, presumably because the turbid stream disturbs and thus renders local cues unreliable during learning (Odling-Smee and Braithwaite 2003). Here, greenfinches favoured spatial over local cues only in the repeated-trial test, where they were able first to generate a prior expectation that the foraging situation was temporally stable. I suggest that the preference for spatial cues reflects an innate prior expectation that local cues become less reliable over time.

There are a number of limitations that must be noted when considering the results of this and similar studies. First, due to variation between individuals in tendency to open wells, and also a tendency for birds to open wells in a non-random manner (selecting neighbouring wells as a second choice), I was limited to a very simple experimental design. Though a cue-shift was still evident, selection of local or spatial cues from a larger array of alternatives would provide more convincing evidence of cue-shifting behaviour. Second, during training birds were introduced to only white cotton wool balls, which (on days 10 to 12) were occasionally rewarding and occasionally not. This training

phase was necessary to equip birds with an expectation of unpredictability in reward in trials, with the aim of encouraging accurate (cue-based) foraging. However, it is possible that the birds developed an expectation that "white" was an unreliable cue, which may also explain (or contribute to) the lower use of spatial than local cues summed over study one. Similarly, positive experiences with coloured (in training phases) but not white cotton wool balls in study one may have carry-over effects in study two. These possibilities cannot be excluded. However, there was no evidence of temporal variation in the distribution of cue selections across days: as coloured cues would prove as "unreliable" as white cues as trials progressed, I would expect these in particular to be used less in latter trials. Moreover, I used different colours and positions to signal reward during training on subsequent days, which can reduce carry-over effects. For example, in starlings (*Sturnus vulgaris*) the sudden disappearance of high quality food types causes a shift in behaviour toward reduced foraging on lower quality food and increased prospecting ("successive negative contrast", Freidin et al., 2009). However, when equipped with a cue (a differentially coloured feeder) to signal higher quality food, the disappearance of the food (and cue) elicits a lower SNC response. Here, I anticipated that the change in cue colour and spatial position between trials (i.e. disappearance of previously used cues) would reduce carry-over between trials. Finally, I re-trained birds to expect food under both coloured and white cotton wool balls at the end of each trial, though whether this was successful cannot be tested.

In summary, this is the first study to explicitly compare cue selection between a one-trial and repeated-trial test in birds. As predicted, propensity to use spatial cues was dependent on first gaining experience of the temporal stability of the foraging context, through repeated encounters. Greenfinches were also unusual amongst non-storers (Clayton and Krebs 1994; Brodbeck 1994; Brodbeck and Shettleworth 1995) in expressing a bias toward local cues in one-trial tests, rather than equal use of spatial and local cues. Through a series of trials, I was able also to demonstrate that this bias was an associatively learnt, rather than a result of inability to learn spatial cues and/or mere attraction toward distracting visual stimuli. In the wild, the natural food of the greenfinches is short-lived and highly variable in distribution (Newton, 1972). The ability to learn both local and spatial cues on first encounter with foraging situations, and to opt for spatial cues within temporally stable contexts, may reflect adaptation toward information gathering and flexibility for species foraging on ephemeral food (Humber et al., 2009).

CHAPTER 6

WILD BIRDS EXHIBIT COLOUR BIASES IN TEMPORALLY STABLE FORAGING LOCATIONS

6.1 ABSTRACT

Birds are predominantly visual foragers and colour cues may play an important role when locating food. However, the colour of a natural object changes over time so colour cues may be perceived unreliable, hence disregarded, when re-locating food in temporally stable feeding locations such as bird feeders. Here, I tested this at eight urban sites located in parks in the city of Glagow, UK. When simultaneously presented with feeders coloured red, blue, green or yellow, the great tit (*Parus major*), long tailed tit (*Aegithalos caudatus*) and robin (*Erithacus rubecula*) were all biased toward red feeders. In great tits and long tailed tits, this appeared to be a conspicuousness bias: red feeders were favoured specifically over green on a background of predominantly green foliage. Robins exhibited a bias against blue feeders and coal tits (*Periparus ater*) against yellow feeders. The blue tit (*Cyanistes caeruleus*) showed no colour bias: blue tit feeder selection was instead influenced by the position of feeders and presence of con- and heterospecifics. Feeder use in those species that showed colour biases was generally lower than in blue tits and varied over time and with the weather. Rather than becoming dependent on artificial feeding sites therefore, I suggest these birds utilise feeders as part of a larger foraging range. As such, a perception of temporal instability in food position (i.e. experience with natural ephemeral food) in the broader environment may generate a context-independent bias toward local rather than spatial cues in colour-biased birds. As three species were biased toward red feeders, particularly over green and blue, and aversions toward yellow were evident, red bird feeders may attract the greatest number and diversity of garden birds.

6.2 INTRODUCTION

"Bird-gardening", the design of gardens and parks to attract or accommodate birds, is a popular hobby in Britain: up to 75% of households actively encourage birds by provisioning food (Cowie and Hinsley, 1988). As domestic gardens in England and Wales cover more than four times the area of English nature reserves, this is a considerable resource for wintering birds (Cannon et al., 2005). Gardens and other urban green spaces also improve quality of life and foster environmental awareness in urban populations, engaging people with nature (Cannon, 1999). Therefore increasing the attractiveness of gardens and parks to birds may be of benefit to both birds and gardeners (Brittingham and Temple, 1992; Cannon, 1999). Bird feeders are designed to be aesthetically pleasing, whether blending in subtly or as decorative ornaments in themselves. However, wintering birds are often nomadic or short-term immigrants (Cramp and Perrins, 1994): feeder design could be further employed to actively attract these passers-by. One visual feature, colour, has dramatically influenced nectar-feeder design in the US, where red feeders that mimic the colours of hummingbird-pollinated flowers dominate the market. Surprisingly, no research is yet published on the affect of feeder colour on feeder use in European passerines. This was the objective of this study.

Food (hence feeder) colour may influence foraging behaviour in several ways. First, colours which contrast strongly with the background are simply more conspicuous to birds than less contrasting colours (Wyszechi and Stiles, 2000). In nature therefore, red feeders may elicit stronger reactions (whether attractions: Osorio et al., 1999, Schmidt et al., 2004; or aversions: Gamberale-Stille and Guilford, 2003) than green feeders by contrasting more against predominantly green foliage (Burns and Dalen, 2002). Second, biases toward detecting particular colours can be acquired when food is ephemeral: over repeated encounters birds learn to hone in on distinctive features of food such as colour to increase foraging efficiency (the 'search image': Tinbergen, 1960; Dawkins 1971; Lawrence 1986). Third, naive (hand-raised) birds often exhibit spontaneous biases toward or against particular colours (see Roper and Cook, 1989). Together these acquired or innate colour biases can then influence decisions on encountering unfamiliar food types (Miklosí et al., 2002): novel food coloured red, the colour of many bird-dispersed fruits but also noxious insects, can stimulate spontaneous attractions in frugivores but aversions in insectivores (Gamberale-Stille and Tullberg, 2001; Schmidt and Schaefer, 2004; Honkavaara et al., 2004; Moreby et al., 2006; Exnerová et al., 2006). Similarly, colours

that are rare in natural foods, such as blue, elicit "neophobic" aversions in many bird species (Gionfriddo and Best, 1996; Hartley et al., 1999, 2000; Miklosí et al., 2002).

Where food occurs reliably in a particular position though, such as at feeders, it may also be located by learning spatial relationships with landmarks in the broader environment (e.g. trees, rivers, houses; Cheng 1988; Collett et al., 1986; O'Keefe and Nadel, 1978). Local (object-specific) cues such as colour are generally less permanent than spatial cues, for example a fruit's colour changes as it ripens but the position of the fruit tree does not. As such, spatial cues may be perceived as more reliable hence superior to local cues in temporally stable foraging contexts (Bennett, 1993). From captive studies, there is considerable evidence that birds favour spatial over local cues when they have a learnt or innate expectation that the spatiotemporal distribution of food will be predictable (e.g. Clayton and Krebs 1994, Hodgson and Healy 2005, see Chapter 5). Noisy miners (*Manorina melanocephala*;) for example deplete nectar from flowers as they forage so use spatial cues to keep track of which visually similar flowers have already been visited (i.e. a "win-shift" strategy; Sulikowski and Burke, 2007, 2010). When searching for insects though, the distribution of which changes over time, they attend instead to local cues (i.e. forage spatially at random: Sulikowski and Burke, 2007, 2010). Similarly across species, food-storing behaviour: the act of hiding food for later retrieval hence generation of temporally stable food distributions is associated with spatial cue biases: in one-trial associative learning tasks, with a choice of local cues (e.g. colour) or spatial cues to relocate food, food-storers generally favour spatial cues (e.g. black-capped chickadees, *Poecile atricapilla*; marsh tit, *Parus palustris*; European Jay, *Garrulus glandarius*; Brodbeck, 1994; Clayton and Krebs, 194; Brodbeck and Shettleworth, 1995; Humber et al., 2009; but see LaDage et al., 2009). In contrast, closely related species with ephemeral food use both cue types equally (e.g. blue tits, *Cyanistes caeruleus*; dark-eyed Junco, *Junco hyemalis*; jackdaw, *Corvus monedula*) or favour local cues (*Carduelis chloris*; chapter 5). As with the noisy miner, food-storers also use local cues when appropriate: when spatial cues prove unreliable, food-storers often attend to visual cues as a second choice (Brodbeck, 1994, Brodbeck and Shettleworth, 1995, Clayton and Krebs, 1994).

This raises an interesting point: whilst the focus of these studies is often the special spatial abilities of nectar-eating or food-storing birds, a lack of cue bias in non-storers may equally reflect adaptation toward a flexible foraging strategy. In winter, the diets of temperate bird species often change as natural insect food becomes less abundant and seed food more sporadic than in summer (see Cramp and Perrins, 1994). Temperate

bird species often expand foraging territories or become nomadic in winter: adaptations which increase the chance of finding this ephemeral food (Newton, 1972, see Cramp and Perrins, 1994). Similarly, under such unpredictability, selection may favour learning both spatial and local information, as local cues may be useful when ephemeral food is scattered but spatial cues when it is clumped (Humber et al., 2009).

Bird feeders are an artificial, temporally stable foraging opportunity for wild birds (Humber et al., 2009). This poses an interesting question: do wild birds perceive feeders, filled with natural foods such as seeds, as ephemeral? Or do they learn that they are temporally stable? In the few studies that have investigated the subject, there is little evidence that birds become dependent on single artificial food sources. Brittingham and Temple (1992b) found that black-capped chickadees provisioned by feeders in a rural area continued to forage in a variety of locations elsewhere. Similarly blue tits (*Cyanistes caeruleus*) in rural areas have been shown to locate new feeding opportunities even when provisioned in fixed locations throughout the winter (Chapter 2) and in urban areas to provision their chicks with around 70% natural food (Cowie and Hinsley, 1988). On a broader scale, the numbers of birds using garden feeders generally mirrors national population trends but, in times of low natural food availability, exceeds them (Cannon et al., 2005; Chamberlain et al., 2005; 2007). Together, these studies suggest flexibility in the use of artificial food sources despite their constant availability. Brittingham and Temple (1992b) suggest this reflects adaptation toward naturally ephemeral food, hence continued prospecting of the environment even within temporally stable contexts. However, the use of alternate feeders could also be explained by costs to feeder use: feeders generate a clumped distribution of birds in urban environments, which enhances competition, predation risk and interactions with diseased birds (Dunkley and Cattet, 2003). In this study, I will provide insight into whether urban birds perceive bird feeders as temporally unstable, as response to local cues (colour) suggests an ephemeral foraging strategy.

Specifically, the aims of the study were to establish whether five common European garden passerines: the robin (*Erithacus rubecula*), great tit (*Parus major*), blue tit (*Cyanistes caeruleus*), coal tit (*Periparus ater*) and long tailed tit (*Aegithalos caudatus*) exhibit colour biases when locating feeders in a temporally stable foraging situation. The colours used were a green and blue chosen to match the colours most commonly available in bird feeders, and a red and yellow selected for similar brightness. Other potential sources of variation in feeder use (daily temperature, time, date) and

feeder selection (inter or intraspecific interactions, feeder position, feeder content) were identified and statistically or experimentally eliminated. By rotating colour feeders amongst positions within each of eight sites on a weekly basis, I was able also to assess whether colour biases had a permanent affect on feeder selection, or only when feeders are newly installed in a given location.

6.3 METHODS

6.3.1 STUDY SITES

The study was conducted at eight sites through urban parks in central Glasgow, UK (see Fig. 6-1). Urban parks share a plant and bird community with local residential areas, thus species using feeders in parks are representative of those in urban gardens (Chamberlain et al., 2005). To capture wintering behaviour, when food is most commonly provisioned in gardens (Chamberlain et al., 2005), feeders were installed in 01/02/07 and observed until 20/03/07.

In each site, I installed four hanging fat-block feeders (13cm x 13cm x 5cm). Such feeders are commonly used by Parids (Hinsley and Bellamy, 2005). A wooden dowel perch (length 16cm, diameter 9mm) was adhered to the base with solvent (Bostik™ "All Purpose") to accommodate other passerines. The four feeders were painted: bottle green, navy blue, mustard yellow or deep red (B&Q™ "Colours" Non-drip Gloss paint). Green and blue colours were selected to match those commonly available for bird feeders; red and yellow colours were selected for a similar brightness, as perceived by humans, to reduce preferences based on achromatic variation. All feeders were then coated with transparent varnish (B&Q™ Quick Dry Gloss) to eliminate chemical or tactile differences between the paints that may affect feeder selection.

To maximise species diversity at each site and also minimise ecological variation between feeders within sites, the four feeders were positioned in a row along a boundary between woodland or herbaceous plants and open ground (Cowie and Simons, 1991). The first four sites were established in location 1, 2, 3 and 4 on the 31/01/07; the second 4 sets were installed in locations 5, 6, 7a and 8 on the 01/02/07 (see Fig. 6-1). Site 7 was moved to site 7b on the 25/02/07 due to low attendance at site 7a. Each feeder was suspended from the branch of a tree using a metal cord at least 1.20m above the ground. The feeders

within each set were placed 4m to 8m apart: close enough to minimise ecological variation between feeders but far enough apart to reduce the chances of non-selective movement between feeders (i.e. landing on the nearest perch following inter- or intra-specific displacement from a preferred feeder). Feeders at each site were positioned so that all could be observed simultaneously.

Throughout the study period, I provisioned feeders with lard, de-husked sunflower seeds (Bill Oddie's Bird Food Recipes™) and chopped peanuts (Pets at Home™ own brand), mixed in a 1:1:1 volume ratio. Feeders were emptied and re-filled with a fresh 8cm x 8cm x 2cm block of this mix every fourth day so that the lard could not become rancid, and were topped up on the afternoon prior to observation days (see below) to ensure equal food availability during data collection.

The five study species were selected *a posteri*, having used the feeders regularly and at the majority of study sites. Feeders were also used by chaffinches (*Fringilla coelebs*; n = 20 observations), starlings (*Sturnus vulgaris*; n = 14 observations); blackbirds (*Turdus merula*; n = 9 observations) and greenfinches (*Carduelis chloris*; n = 2 observations). However, observations of these species were sporadic and exclusive to one or a few sites thus these "non-focal" species were excluded from analyses. All birds were not ringed, so individual behaviour could not be scored. However, I selected sites at least 200m apart and in two clusters over 500m apart (see Fig. 6-1), so all sites were at minimum one robin territory apart (150m^2; Tobias, 1997) and half the sites at least an average Parid foraging range apart (500m^2; Sirwardena, 2006). By covering a large area, individual biases are less likely to be overrepresented (Crawley, 2002).

6.3.2 Data Collection

Colour biases were identified using timed observations of birds at feeders. Every minute, for 30 mins, I recorded the number of individuals of each species on each of the four feeders within a site. As individuals could not be recognised this was a measure of relative feeder use per species. If for example N = 30 for blue tits within a 30min observation this could mean the same individual fed for 30 mins or 30 individuals each fed for less than one minute. To separate position from colour biases, the positions of the four colours within each site were rotated every 7th day. To compare biases expressed when colour cues are novel in a given position with those where both colour and location

were stable each set of feeders was observed for 30 minutes on the mornings of both the 1st and the 6th day following rotation, hence forth the "new" and "stable" feeder arrangements. As feeders were installed in two groups of four sites, four thirty minute observations were carried out per day, starting at sunrise, sunrise + 35 minutes, sunrise + 70 minutes and sunrise + 105 minutes. The number and species of birds observed on each feeder were recorded each minute. In case feeder use varied with the time of day, the order of observation of sites 1-4 and 5-8 were systematically rotated each day.

To identify temporal trends in feeder use, I used the cumulative total number of birds of a species per 30 min observation (first to fourth) for "within day" variation and per date for "across season" variation. Weather may also affect feeder use, for example feeder use in blue tits declines with increasing maximum daily temperature (Chapter 3) and in a range of species with increasing minimum weekly temperature (Chamberlain et al., 2005). Therefore the maximum temperature (°C) per observation day was collated from MET office reports (www.metoffice.gov.uk). Maximum temperature was independent of date (linear regression: $F_{1, 30} = 2.47$, $P = 0.13$).

Finally, feeder selection may be influenced by the presence of con- and heterospecifics at feeders. For example, an individual may signal the location of the feeders to other birds or competitively exclude others from particular feeders (Cresswell et al., 2001; Krams, 2001). To investigate intra- and interspecific dynamics, I identified all occasions where two individuals of any species were observed within the same minute. If observed on the same feeder, these were scored 1, if not 0. Instances where more than two birds were present or where feeders were in use by non-focal species were excluded from this data.

6.3.3 Ethical note

Care was taken to ensure that lard could not become rancid whilst in the feeders: it was mixed cold (rather than melted, which denatures preservatives), kept in a refrigerator until required or for a maximum of one week and replaced every fourth day in the field. On that day, I also wiped feeders down with a bird-safe disinfectant, as the congregation of birds at feeders has been implicated in disease transmission (Bradley and Altizer 2006). There is little evidence that wild birds become dependent on single food sources (Brittingham and Temple, 1992). However after completion of the experiment, the

feeders were allowed to empty naturally for 5 days, with uneaten food replaced with equal volumes of fresh food on the 3rd day, so that individuals could make a gradual transition to alternate food sources.

6.3.4 Statistical Methods

I first identified intra- or interspecifc effects on feeder selection. For each species or species pair, influences on feeder selection were identified using a Wilcoxon signed rank test with a null mean expectation of 0.25 (i.e. 1 in 4 chance of occurring on the same feeder).

I then investigated variation in feeder use with maximum temperature, within days (represented by observation order, i.e. 30 minute blocks since sunrise) and across the season (i.e. days since feeder installation). For each species, I constructed a generalized linear mixed model (GLMM) with the count of birds per observation as the dependent variable, hence a Poisson error structure, and a three way interaction between maximum temperature, observation order and date. To control for variation in feeder attendance between sites, site was specified as a random effect. I used stepwise backwards regression to simplify these models using a threshold p value of 0.05, until only main effects (significant or non-significant) and significant interactions remained. In 28 of the 128 observations, no birds were observed (for a summary of observations per species see Table 6-1). As the aim of the study was to investigate feeder selection, for the remaining analyses all occasions where a particular species was not present within a given observation were excluded. These zero scores would be informative to feeder use patterns. Therefore, the main effects: maximum temperature, observation order and date were included as covariates in subsequent models to control for weather or temporal variation in feeder use, but their significance as main effects not tested.

Finally, for each species I constructed a generalized linear mixed model (GLMM) to investigate feeder selection. The dependent variable was a count of the number of birds per colour per observation so I used a Poisson error structure. Feeder position nested within site was specified as a random effect. Feeder colour and feeder arrangement (new or stable) were my variables of interest: I specified an interaction between these variables. Maximum temperature, observation order (1 to 4, with observation 1 starting at sunrise and each subsequent observation 35min after), and date were covariates. To first

identify whether birds expressed biases toward particular positions within sites, I performed a likelihood ratio test (LRT) between this maximal model and a model with only site as a random effect. To assess whether colour biases differed between occasions when feeder were in new or stable locations, I then test the significance of removing the interaction between colour and observation with a likelihood ratio test (LRT) between the maximal model and a model including colour and observation type as main effects (i.e. an additive model). Finally, to test colour as a main effect, independent of observation type, I performed an LRT between this additive model and a model with all independent variables except colour. As intra and inter-specific dynamics affected feeder selection in the majority of species (see 6.4.1), I repeated these analyses but counting all records of the same species on a feeder simultaneously as one record and excluding all occasions where more than one bird (of the same or different species) was observed at a site simultaneously.

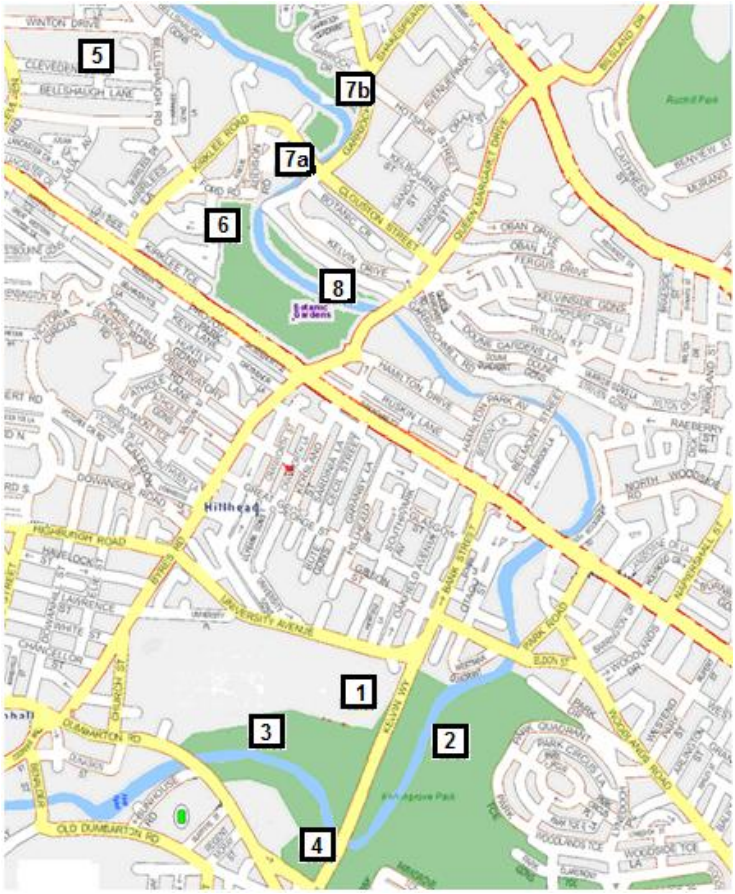


Figure 6-1 Map of the Glasgow urban parks study site. Study sites are marked with black open squares, numbers correspond to locations as follows: 1) Meadow bordered by road and public footpath; 2) Riverside beech and coniferous stand along public footpath; 3) Oak and birch woodland bordering grass along public footpath; 4) Oak stand bordering grass by car park; 5) Unenclosed apple orchard within residential area; 6) Herbaceous and deciduous woodland bordering grass along public footpath; 7a) Deciduous woodland along public footpath; 7b)Deciduous woodland bordering grass along public footpath (replacing 7a on 25/02/07 due to low attendance at site 7a); 8) Beech woodland along public footpath. Scale 1cm = 165m; modified from www.multimap.com

Table 6-1 Summary of observations per species over the study period.

Species	N	N single	observations	single observations
Blue tit	392	240	87	86
Great tit	137	91	48	43
Coal tit	74	51	31	24
Long tailed tit	99	60	30	30
Robin	136	88	48	42

N is the total number of records of a species summed across observations; N single is the number of records taken when no other bird (same or different species) was present on the feeders within the same minute. Observations is the number of the 128 30min feeder observations in which a member of a given species was recorded; single observations is the number of observations in which a member of a given species was recorded at least once in a minute with no other birds

6.4 RESULTS

6.4.1 INTRA AND INTERSPECIFIC DYNAMICS

To assess the affect of intra and interspecific interactions on feeder selection, on all occasions where two birds were observed on feeders simultaneously I first tested whether they were observed significantly more often than expected on the same or different feeders (i.e. chance expectation of 0.25, with 4 feeders in each site). Three species: the blue tit, great tit and long tailed tit showed intraspecific interactions. The blue tit avoided conspecifics (V = 2754, p = <0.0001, μ 0.65, n = 78); the great tit (V = 221, p = 0.0096, μ 0.57) and long tailed tit (V = 273, p = < 0.0001, μ 0.91, n = 23) occurred with conspecifics. Blue tits and robins were also more likely to feed apart than together (V = 47, p=0.001, μ = 0.08, n = 24), but blue tits were observed more frequently than expected by chance on the same feeders as long tailed tits (V = 108, p = 0.035, μ 0.56, n = 16). No other interspecific interactions were identified.

6.4.2 THE BLUE TIT

Blue tit feeder use increased within days and also across the season, but was independent of daily maximum temperature (Table 6-2a). Feeder selection was not affected by colour, either in interaction with feeder arrangement (new or stable) or as a main effect (Table 6-3a, Fig. 6-2a). Results were same when only single observations were analysed (Table 6-3a), so colour biases were not masked by competitive exclusion forcing blue tits onto non-preferred feeders. Blue tits were biased toward particular positions within sites (Table 6-2a).

6.4.3 THE GREAT TIT

Like the blue tit, great tit feeder use increased within days and was independent of daily maximum temperature (Table 6-2b). However, in contrast to blue tits feeder use declined across the season. Great tits exhibited an overall bias toward red, and a significant interaction with feeder arrangement indicates that green feeders in particular were used

less when in new than stable locations (Table 6-3b; Figure 6-2b). Results were unchanged by analysing single observations, thus colour biases were not an artefact of individuals responding to social signals hence congregating on feeders with other great tits or long tailed tits. There was no position bias (Table 6-3b).

6.4.4 THE COAL TIT

There were no correlates of feeder use by coal tits (Table 6-2c). Overall, coal tits used yellow feeders less than all other colours, with no affect of feeder arrangement (new versus stable; Table 6-3c, Fig. 6-2c). However, this bias was lost when analyses were limited to single observations of birds. There was no position bias (Table 6-3c).

6.4.5 THE LONG TAILED TIT

Long tailed tit feeder use increased both across the season and with maximum temperature, and there was an interaction between these (Table 6-2d). Feeder use did not differ within days. Long tailed tits exhibited an overall bias toward red feeders and, like the great tit, a significant interaction with feeder arrangement indicates that green feeders were used less during new than stable location observations (Table 6-3d, Fig. 6-2d). Results were unchanged by analysing single observations. There was no position bias (Table 6-3d).

6.4.6 THE ROBIN

In contrast to the long tailed tit, a significant interaction between date and temperature indicates that robin feeder use decreased with across the season and with increasing maximum temperature, but was also independent of time of day (Table 6-2e). Robins exhibited an overall bias toward red, and a significant interaction with feeder arrangement indicates that blue feeders were used less during new than stable location observations (Table 6-3e, Fig. 6-2e). There was no position bias (Table 6-3e).

Table 6-2 Results from GLMMs on feeder use by each species during 30 minute observations in relation to observation order (i.e. starting sunrise, sunrise +35min, sunrise +70min and sunrise + 105min), maximum temperature (°C) and date

Species	z (d.f.)	P-value	R (S.E.)
Predictors			
(a) Blue tit			
Time of day	3.82 (116)	0.0001	0.175 (0.046)
Maximum temperature (°C)	-1.24 (116)	0.26	-0.029 (0.024)
Date	5.7 (116)	< 0.0001	0.021 (0.004)
(b) Great tit			
Time of day	2.94 (116)	0.003	0.231 (0.079)
Maximum temperature (°C)	1.11 (116)	0.27	0.047 (0.042)
Date	-4.12 (116)	< 0.0001	-0.029 (0.007)
(c) Coal tit			
Time of day	0.1 (116)	0.33	0.104 (0.106)
Maximum temperature (°C)	1.55 (116)	0.12	0.014 (0.009)
Date	-0.28 (116)	0.77	-0.016 (0.056)
(d) Long tailed tit			
Time of day	1.59 (115)	0.11	0.149 (0.094)
Date x maximum temperature (°C)	-2.7 (115)	0.007	-0.011 (0.004)
(e) Robin			
Time of day	-1.15 (115)	0.25	-0.094 (0.081)
Date x maximum temperature (°C)	2.57 (115)	0.01	0.007 (0.003)

The results are of models with feeder visitation as the dependent variable, site specified as a random effect, and a Poisson error structure

Table 6-3 Results on feeder selection by each species, from LRT between models including and excluding the listed predictor for single (maximum one bird recorded per minute) and all records

Species	**Single records**			**All records**		
Predictors	LRT χ^2	P-value	N	LRT χ^2	P-value	N
(a) Blue tit						
Colour x feeder arrangement	1.202	0.64	344	1.711	0.75	348
Feeder position	4.446	0.14	344	2.16	0.035	348
Colour	5.824	0.06	344	7.395	0.12	348
(b) Great tit						
Colour x feeder arrangement	32.59	< 0.0001	176	15.23	0.002	192
Feeder position	1.257	0.26	176	< 0.0001	1	192
Colour	36.72	< 0.0001	176	29.18	< 0.0001	192
(c) Coal tit						
Colour x feeder arrangement	1.809	0.61	96	4.035	0.26	124
Feeder position	0.001	0.98	96	0.153	0.7	124
Colour	4.077	0.25	96	12.49	0.006	124
(d) Long tailed tit						
Colour x feeder arrangement	8.225	0.042	80	10.08	0.018	80
Feeder position	0.672	0.41	80	0.236	0.63	80
Colour	22.63	< 0.0001	80	24.21	< 0.0001	80
(e) Robin						
Colour x feeder arrangement	6.247	0.1	168	8.124	0.044	192
Feeder position	0.096	0.79	168	0.636	0.43	192
Colour	11.9	0.008	168	16.12	0.001	192

The maximal model specified records per colour per observation as the dependent variable, colour x feeder arrangement, maximum temperature, observation order and date as covariates, position nested within site as a random effect. Results for colour x feeder arrangement or feeder position are obtained by comparing this model to models excluding these predictors. Results for colour are obtained by testing the significance of removing colour from a similar maximal model but with colour and feeder arrangement as main effects only.

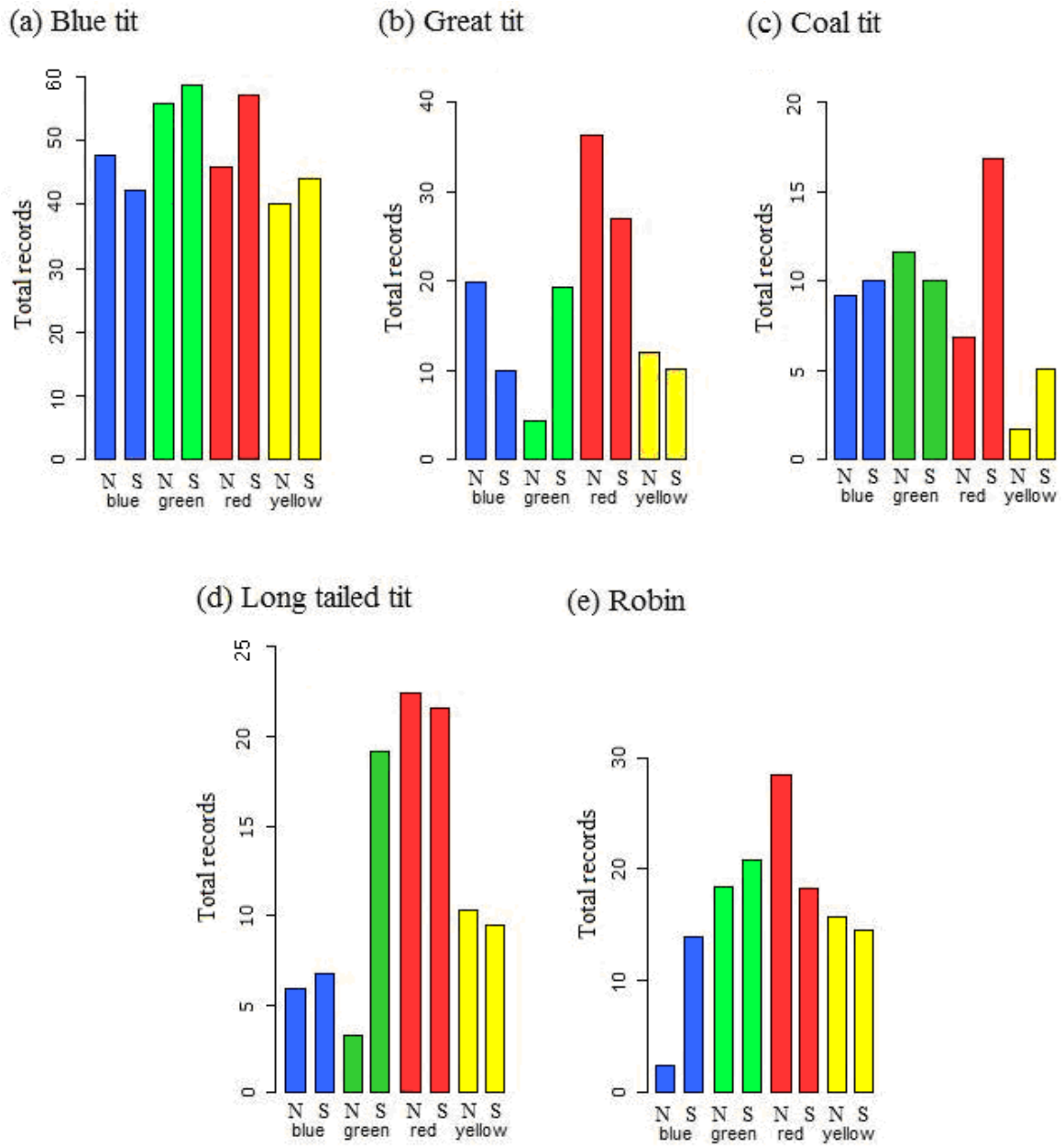


Figure 6-2 Bar charts of the total records per species per colour summed across the study period. Colours are labelled beneath the bars with "N" denoting new feeder arrangement observations, conducted in the morning following feeder rotation, and "S" stable feeder arrangement observations, conducted six days after feeder rotation

6.5 DISCUSSION

In this study, I sought to investigate whether colour affected feeder selection in five common garden passerines. I was particularly interested to see whether green, a colour which typifies European bird feeders, was attractive. Whilst four out of five species did show colour biases, which were independent of other influences on feeder selection such as position biases or (except for coal tit) the presence of con-and heterospecifics, none favoured green. Three species: the great tit, long tail tit and robin, were biased toward red. The great tit and long tailed tit were biased against green, the robin against blue and the coal tit (in total records) against yellow. As local cues such as colour become increasingly unreliable over time, I had predicted that birds would not respond to colour when foraging in temporally stable situations such as bird feeders. This prediction was only supported in the blue tit, whose feeder selection was influenced instead by feeder position and inter and intraspecific interactions. Therefore the results for four of five species contradict captive expectations that spatial cue use replaces local cue use in temporally stable locations (Chapter 5). Moreover, the lack of preference for green suggests that, in comparison with the US nectar-feeder market, European bird feeder design has responded more to aesthetic than functional demands.

In keeping with previous avian colour preference studies (e.g. Burns and Dalen, 2002; Schmidt et al., 2004; Exnerová et al., 2006), red appears to be a potent signal to foraging birds. Red has the greatest contrast against the predominantly green foliage of the backdrop, and green the least. Thus the bias toward red over green expressed by the great tit and long tail tit in novel observations may suggest reliance on local cues when locating feeders, with feeders relocated in order of conspicuousness (red > green) following feeder rotation. The alternative, an aversion to green itself, is unlikely. First, green feeders were used as often (great tits) or more often (long tailed tits) than blue or yellow feeders in stable observations. Second, a short-term neophobic aversion during novel observations is counterintuitive: studies on poultry suggest that birds are both slow to learn aversions toward green and quick to disregard this colour when other aspects of food such as palatability are informative (Rowe and Guilford, 1999; Rowe and Skelhorn, 2004). As green is common in nature, this inability to form negative associations with green may be a safeguard against forming generally maladaptive aversions (Rowe and Skelhorn, 2006).

In robins, the bias toward red in novel observations came at the expense of blue. Blue is a rare colour in nature (Miklosí et al., 2002). Previous studies on robins suggest they are averse toward novel colours (Marples et al., 1998), so I attribute this bias to neophobia. If true, the robin would join a taxonomically disparate group of birds which show neophobic reactions toward blue (Gionfriddo and Best, 1996; Hartley et al., 1999, 2000; Miklosí et al., 2002). The coal tit's aversion toward yellow (when using total records) on the other hand was not consistent with neophobia: coal tits avoided yellow both in new and stable location observations. The possibility of an aversion toward yellow is quite intriguing: in omnivorous species such as Parids, base colour aversions are often latent unless coupled with other insect cues, such as shape or pyrozine odours, which may help distinguish palatable plant foods from aposematic insects (Gamberale-Stille and Tullberg, 2001; Kelly and Marples, 2004; Lindström et al., 1999; Rowe and Skelhorn, 2004). Whilst I can suggest several negative associations between yellow and plants, synonymous for example with decaying leaves, or unripe fruits, Paridae also forage for yellow pollen and flower and leaf buds (see Hinsley and Bellamy, 2005). And whilst negative associations with yellow aposematic insects such as *Vespidae* wasps may be important in this predominantly insectivorous genus, the aversion toward red insects that is quite universal to the Paridae (Exnerová et al., 2006) did not cause aversions toward red feeders.

It is notable that no species exhibiting a colour bias also exhibited position biases: position biases were evident only in blue tits. Position biases can reflect ecologically significant variation between foraging opportunities, for example both accessibility and exposure to predators can influence the foraging decisions of free-living birds (e.g. Hinsley et al., 1995; Avery et al., 1995; Hinsley, 2000; Allen and Harper, 2000; Walther and Gosler, 2001). Such attributes must be learnt in a site-specific manner, so I suggest that the position biases and lack of colour biases in blue tits reflects transition from a local to a spatial foraging strategy within the feeder context in blue tits, with site quality information encoded within spatial information. For the colour-biased species, a lack of position biases may also reflect generally equivalent ecological conditions within sites (as I had aimed to achieve). However, a lack of temporal trends within days (coal tits, long tailed tits) or decline in feeder use across the season (great tit, robin) coupled with generally low feeder use in these four species (Table 6-1) suggests that the feeding sites may constitute only a small part of the foraging range of these species. Therefore the colour biases may be a product of greater experience with natural, ephemeral food in the broader environment and hence perhaps a general perception of temporal instability in

food, hence use of local cues. Along with colour cues, another strategy for locating temporally unstable resources is via "public information": social signals from other birds (for review: Krebs and Davis, 1997). That long tailed tits and robins were both more likely to occur with conspecifics and together than expected by chance provides further support for an ephemeral foraging strategy in these species.

The existence of colour biases in wild birds raises a more general point. Most cue selection studies have been performed in captive environments, where careful manipulations to disentangle cue biases and the motivations underlying them are feasible (Healy and Hurly, 2004). However, to be ecologically relevant, cue selection must also be considered in the wild (Humber et al., 2009). In captivity, it is suggested that birds select cues in a context-specific manner, hence become biased toward spatial cues where resources prove temporally stable (Hodgson and Healy, 2002, Chapter 5). That great tits in particular, which have specifically been shown to favour spatial cues under stable foraging conditions in captivity, contradicts captive predictions (Hodgson and Healy, 2002). In wild animals, experience may generate a general rather than context-specific perception of environmental stability. For example, sticklebacks (*Gasterosteus aculeatus*) from both ponds and streams are capable of learning either spatial cues (body orientation) or local cues (a plant situated to one side) when navigating a maze to find food (Odling-Smee and Braithwaite, 2003). However in cue dissociation tasks, pond fish used both cues types whilst stream fish favoured spatial cues. It is suggested that the turbid stream environment rendered local cues unreliable during learning, and hence generated a general perception of unreliability in local cues within stream fish (Odling-Smee and Braithwaite, 2003). Therefore information that is useful (and hence reinforced) in the wild may not be that which we would expect animals to rely on within a given context. There is increasing evidence that environmental variation in early life shapes the kinds of behaviour (Braithwaite and Salvanes, 2005) and propensity toward learning in adulthood (Brydges et al., 2008). Perhaps experience of temporal instability in natural food (beyond the artificial feeding sites) generated general local cue biased searches rather than feeder context specific spatial biases. This supposition is supported by a field study on cue selection in pine siskins (*Carduelis pinus*; Humber et al., 2009). Pine siskins are nomadic foragers in winter (Herbers et al. 2004), but Humber and colleagues (2009) found that they were able to learn spatial cues when foraging at artificial feeders. In general, when the colours were rotated amongst positions, birds assorted used spatial cues (as predicted) to relocate high and avoid low quality feeders. However when the green feeder, which was most rewarding in training, was placed in the least rewarding position, birds

approached the best position and best colour equally. Therefore with general experience that green was rewarding in the majority of the study, such conflicting information may cause some individuals to revert to generally reliable local cues over context-specific spatial cues.

The results of my study should be interpreted with caution. In particular, it suffers two limitations: first, in this experimental design, the four competing colours were presented simultaneously. As such, comparisons between colours were necessary to draw inferences on the mechanisms underlying biases, for example comparing green to red to suggest a conspicuousness bias. To identify colour biases or distinguish colour from contrast biases, pairs of colours or a levels of contrast should be compared explicitly, as for example Schmidt et al. (2004). Second, this study used observation data on unmarked individuals. If colour biases differ between *individuals*, unknown pseudoreplication may suggest generality from the biases of a dominant or resident minority. Worse, if colours are valued or feared to differing extents, colour biases may be obscured by the assortment of individuals of amongst colours of different "quality" on the basis of rank, as occurs in Parid foraging flocks (Lee et al., 2005). However, as results from single records were equivalent (except in coal tits) to total records, this second limitation is unlikely to affect interpretations. Moreover in the context of feeder design, I selected green and blue feeder to match those commonly available to buy. Therefore despite these limitations, I am able to recommend red feeders as the most attractive to wild birds.

CHAPTER 7

GENERAL DISCUSSION

For behavioural ecologists, personality represents a transformation in the way of thinking about adaptation, from an optimality approach (with behavioural variation suboptimal "noise" around an optimal mean response) to variation as the focus of interest and theory (Sih et al., 2004). Initially, the emphasis was on demonstrating the existence of personality in animals, with consistent behaviours described in species as diverse as the dumpling squid (*Euprymna tasmanica*; Sinn et al., 2008) to hyenas (*Crocuta crocuta*; Gosling, 1998), and a huge range of mammals, birds, fish, reptiles, amphibians and invertebrates in-between (Gosling, 2001). Having established its ubiquity, research now focuses on the adaptive significance of personality. Several recent theoretical studies suggest that personality persists by a trade-off between the costs and benefits of maintaining a high energy state (by being bold, active and aggressive to acquire resources at the risk of failing to acquire sufficient resources when food is scarce) versus a low energy state (by being shy, inactive and passive and requiring fewer resources but being outcompeted when food is plentiful) in different environments (Careau et al., 2008, Stamps, 2007, Wolf et al., 2007, Biro and Stamps, 2008, Dall et al., 2004, McNamara et al., 2009). In support of these theories, personality has been linked to variation in life-history traits such as growth and fecundity (for review, see: Biro and Stamps, 2008) and to survival between environments in which this trade-off differs (e.g. food-poor versus food-rich: Dingemanse et al., 2004, predator present versus predator absent: Bell, 2005). However, as outlined in chapter 2, there is a missing link in this study: the behavioural mechanisms underlying these fitness differences are predicted from behaviour in captive studies (e.g. Marchetti and Drent, 2000, Benus et al., 1987, Verbeek et al., 1999), but few studies test whether behaviour in captivity reflects behaviour in the wild (for review, see table 7-1). As outlined in chapter 3, predictions from captive behaviour do not always hold true in field studies (Dingemanse and de Goede, 2004, van Overveld and Matthysen, 2010, Hollander et al., 2008). Therefore a key aim of this thesis was to test captive predictions on personality and also cue selection – my own and from the literature – in the wild.

Table 7-1 A summary of studies relating personality traits to behavioural variation in the wild. Behavioural variation denoted + indicates that the highest values (e.g. fastest, most active, largest) occur at the fast end of the trait axis, - at the slow end and 0 for no relationship. Traits, labelled with source terminology, are denoted [f] when measured in the field (the remainder in the laboratory). Wild behaviours denoted [w] are measured in the wild and denoted [p] are comparisons of individuals in captive drawn from ecologically distinct populations.

Group	Species	Personality trait	Behavioural variation	Source
Birds	*Parus major*	Spatial exploration	+ Post-natal dispersal[wp]	Dingemanse et al. 2002
			+ feeding range[w]	van Overveld & Matthyssen 2009
			0/+ Nest defence[w]	Hollander et al., 2008
			+/- Dominance[w]	Dingemanse & deGoede 2004
	Cyanistes caeruleus	Neophobia	+ neophobia[w]	Herborn et al., 2010
			0 exploration[w]	
		Spatial exploration	0 neophobia[w]	
			+/0 exploration[w]	
		Neophobia	-environmental sensitivity[w]	Chapter 3
		Spatial exploration	+environmental sensitivity[w]	
	Sturnus vulgaris	Spatial exploration	0/- range size[w]	J. Minderman (pers. comm.)
	Anser Anser	Aggression[f]	+ Aggression[w]	Kralj-Fišer et al., 2006
		Socability[f]	+ Sociability[w]	
Fish	*Gasterosteus aculeatus*	Boldness	- Predator experience[p]	Bell 2005, Dingemanse et al. 2007
	Brachyraphis episcope	Boldness	- Predator sensitivity[w]	Brown, 2007
	Pimephales promelas	Boldness	+ boldness[w]	Pellegrini et al., 2010
	Salvelinus fontinalis	Spatial exploration	+ prey-search pattern[w]	Wilson & McLaughlin, 2007
		Boldness[f]	+ foraging movement[w]	Farwell & McLaughlin, 2009
	Rivulus hartii	Boldness	+ dispersal[w]	Fraser et al., 2001
Mammals	*Tamias sibiricus*	Spatial exploration	+ trappability[w]	Boyer et al., 2010
	Tamias striatus	Docility	+ tolerance of humans[w]	Martin & Reale,2008
		Spatial exploration	+ tolerance of humans[w]	
	Tamianscuirus hudsonicus	Docility	+ range size[w]	Boon et al. 2007
		Activity	+trappability[w]	
		Aggressiveness	0 range size[w]	
			+trappability[w]	
	Ovis Canadensis	Boldness[f]	0 range size[w]	Reale et al. 2000
Reptiles	*Lacerta vivipara*	Sociability	- post-natal dispersal[w]	Cote & Clobert 2007
Molluscs	*Pagarus bernadus*	Startle response	+ startle response[w]	Briffa et al. 2008

7.2 Summary of personality results

A key prediction from captive studies is that personality types differ in their ability to cope with environmental change (Benus et al., 1987, Benus et al., 1988, Marchetti and Drent, 2000). It is predicted that slow personality types, i.e. less exploratory, neophobic and passive animals are more sensitive to environmental cues than fast types, and hence cope better in unpredictable environments (Benus et al., 1987, Dingemanse et al., 2004). Surprisingly therefore response to change, termed "environmental sensitivity" (Boyce and Ellis, 2005, Koolhaas et al., 1999), is seldom measured in captivity (Minderman et al., 2009, for review see Sih et al., 2004) let alone the wild (van Overveld and Matthysen, 2010). The aims of chapters 2 and 3 of this thesis were to test whether captive personality traits reflected variation in wild behaviour amongst blue tits (Chapter 2), and consequently to examine whether personality types differed in environmental sensitivity in the wild (Chapter 3).

In Chapter 2, I established a correlation between wild behaviour and two captive traits: neophobia and spatial exploration. One of few studies on the behaviour of personality types in the wild (see table 7-1), and fewer still comparing "like-for-like" by measuring analogous traits in both contexts, this is an important affirmation of captive personality research. Specifically, I found that birds classified as relatively exploratory in captivity were more likely to find new feeding opportunities in the wild and vice versa. Similarly, an individual's neophobia in captivity correlated positively with their latency to approach novel objects (coloured feeder covers) at feeding stations in the wild. This is equivalent to work comparing prey search tactic in the wild to space use within novel aquaria environments in brook char (*Salvelinus fontinalis*) by Wilson and McLaughlin (2007). They found that brook char that used a sit-and-wait tactic in the wild were less exploratory and fish with active search tactics more exploratory in novel captive environments. In this example and my own, exploration behaviour may therefore represent ecologically significant differences in foraging strategy between individuals. My study is also comparable to work by Briffa and colleagues (2008) on hermit crabs (*Pagurus bernhardus*). Although in that case, Briffa and colleagues suggested consistency represented limited behavioural plasticity. By comparison, blue tit foraging strategies appear quite flexible, particularly in neophobic and fast-exploring birds (Chapter 3).

Having validated captive traits in Chapter 2, in Chapter 3 I used blue tits to ask the question: does personality represent systematic variation in environmental sensitivity? I examined responses to changes in two environmental cues: daily maximum temperature and food supply. At the population level, the use of artificial feeding stations declined with increasing air temperature, and visitation to those feeding stations petered out over five days after they were emptied. However, individuals differed in these responses: non-exploratory and neophilic birds tended to use feeders at a fixed level regardless of temperature, and to continue to visit feeders three days after they were emptied. By comparison, exploratory and neophobic birds were flexible in their feeder use and abandoned empty feeding stations more quickly. In both instances therefore, exploratory and neophobic individuals may be regarded most environmentally sensitive. Intriguingly, in great tits (Appendix I) I found very similar results with environmental sensitivity to food supply, suggesting some generality in the ecological significance of the exploration trait amongst Parids.

Beyond validating (or refuting) captive predictions, my findings in Chapter 3 are also important for understanding the maintenance of personality variation within populations. Personality is often thought to represent a trade-off between a generally appropriate response (i.e. consistency across contexts) and the costs of responding with infinite plasticity (DeWitt et al., 1998, Ellis et al., 2006, Briffa et al., 2008). This trade-off may be maintained by differential selection on these relatively fixed personality types across environments (Dingemanse et al., 2004). Recently though, it has been suggested that the balance of this trade-off between consistency and plasticity may differ also between personality types (Biro et al., 2010) with plasticity, represented by environmental sensitivity, equally subject to selection (Nussey, 2005, Wolf et al., 2008). As there are costs to plasticity, for example in time or energy devoted to information gathering (Johnston, 1982), it is predicted environmentally sensitive personality types should be selected in increasingly variable environments (DeWitt and Scheiner, 2004). However, plasticity may also be selected when the current environment differs from that in which a species has evolved, for example a current focus of research is the capacity of populations to adapt to climate change (Visser 2008). Within Parids, the timing of breeding provides an opportunity to estimate the extent of this capacity: birds respond to increasing day length as cue to breed but their larvae prey respond to air temperature as a cue to hatch, therefore if birds cannot adjust their response to toward shorter day length cues or else use other environmental cues to breed, breeding may be mistimed (Both et al., 2009, Visser et al., 2004). Evidence of adaptation to climate change is found in

selection on both stereotyped responses, for example lay date via selection on the *Clock* gene (Liedvogel et al., 2009), but also on "plasticity in breeding behaviour" (Nussey et al., 2005). Birds that are more plastic in breeding behaviour adjust their lay date relation to breeding success and environmental cues in previous breeding attempts, for example higher or lower spring temperatures (Nussey et al., 2005) or food supply (Grieco et al., 2002). Here, I provide evidence that individuals differ in their response to temperature and food supply in winter. As adults were more environmentally sensitive than juveniles, I predict that neophobic and exploratory blue tits may be more sensitive to environmental cues and hence also exhibit plasticity in breeding behaviour. By developing methods to quantify both personality and environmental sensitivity, my study provides a foundation for this further research on the link between personality, environmental sensitivity and adaptation to long term environmental change.

In chapter 2, I found no differences in personality between juvenile and adult (1 year +) blue tits. This was surprising, as juveniles often exhibit lower neophobia than adults, perhaps to equip them to learn more quickly about their new environment (for review, see: Greenberg, 1995). However, in the wild juveniles exhibited less environmental sensitivity to temperature than adults (chapter 3). This is interesting: it suggests that the raw material underlying environmental sensitivity, i.e. the innate level of either sensitivity to environmental cues or behavioural plasticity, is evident in juveniles as well as adults, but that juveniles do not express that variation in the wild. Perhaps the ability to respond to environmental cues is contingent on experience: environmentally sensitive (fast-exploring, neophobic) juveniles may not have learnt to respond to temperature (Komers, 1996). Alternatively, the costs and benefits of responding plastically may differ between age classes. Juvenile Parids are generally subordinate to adults within competitive environments (Dingemanse and de Goede, 2004). Often, subordinate birds within dominance structured flocks carry more fat reserves than adults (Hake, 1996, Polo and Bautista, 2002). As food is less predictable for subordinates than dominants, with the added possibility of competitive exclusion, this may represent an alternative strategy for coping with environmental unpredictability: "insurance" (Dall and Johnstone, 2002). In this case, continued feeding despite warmer temperatures would be a safe strategy for juvenile blue tits. It is notable that age did not affect environmental sensitivity to food supply, where competition (for empty feeders) would be low (Chapter 3). Most importantly, this age effect illustrates the value of considering personality in the wild: from captive studies, where ages did not differ in behaviour (Chapter 2), it may be predicted that age classes would behave the same in the wild.

An interesting observation on age and personality is that personality types often differ in longevity (for review: Biro and Stamps, 2008). As personality types differ also in metabolic rate (Careau et al., 2008) and physiological stress responsiveness (Cockrem, 2007), hence production of pro-oxidants, it is suggested that this may reflect a cumulative physiological cost to personality. Indeed, personality variation may be maintained within populations by linkage to life history traits such as longevity or growth rate that are under selection in different environments (Wolf et al., 2007, Biro and Stamps, 2008). It is surprising therefore that the relationship between personality and oxidative profile has not been studied more extensively, as oxidative stress is often proposed as mediator in life history trade-offs (for review: Monaghan et al., 2009). In chapter 4, I found that greenfinches differing in neophobia and exploration differed also in oxidative profile (Chapter 4). Specifically, birds at the "fast" end of the trait axes, which were fastest to explore novel objects and least neophobic, had lower levels of oxidative stress or oxidative damage than slow types. It is surprising that animal personality and oxidative stress have not been studied more extensively Whilst relationships between personality and oxidative profile have been measured explicitly in only one other study (Costantini et al., 2008), this result fits well into the broader literature on proximate mechanisms for personality. In a range of species, fast types have been shown to have lower gluccocorticoid (stress hormone) levels than their slower counterparts (Veenema et al., 2003; Carere et al., 2003; Martin and Reale, 2008; Cavigelli and McClintock, 2003; Martins et al. 2007; Hoglund et al., 2008). Gluccocorticoids stimulate the metabolism to enable rapid behavioural response to environmental stressors, such as the fight or flight response (Cockrem, 2007). Pro-oxidants, biomolecules that damage the body tissue under oxidative stress, are primarily a by-product of metabolism. Therefore, personality types that have more sensitive or elevated stress responses may produce more pro-oxidants than less responsive types. The relationship between gluccocorticoid variation and oxidative damage has been demonstrated experimentally: supplementing poultry with gluccocorticoids has similar consequences for oxidative damage (Lin et al., 2004b) as observed in my study and that of Costantini and colleagues (2008). To test the hypothesis of oxidative costs as a cumulative cost of personality fully, I would need first to demonstrate that slow greenfinch personality types have higher stress responsiveness. If so, I would predict that slow types may have a shorter lifespan by cumulative costs of oxidative stress. In the wild though, bold types take greater behavioural risks, so this variation may be cancelled out by a higher rate of instantaneous mortality (Natoli et al., 2005). This highlights again the importance of testing predictions in the wild.

A common assumption of personality research is that traits fall along a single-dimensional continuum, with fast and slow individuals at the extremes (Wilson et al., 1993). However in chapter 4, I found that physiological costs of personality in terms of oxidative damage were greatest in intermediate personality types. Whilst this conclusion was drawn from a small sample size (n = 22 greenfinches) of captive-bred animals, I suggest that study of the physiological and indeed behavioural costs to intermediate personality types warrant further investigation. In captive studies, personality traits have often been categorised into fast and slow extremes (e.g. bold and shy, Wilson et al., 1993), or else intermediate personality types have been systematically excluded by experimental design (e.g. Hardcourt et al., 2009) or selective breeding (e.g. in mice: Benus et al., 1987, in great tits, for review: Groothuis and Carere, 2005). Also, by classifying individuals using averages across repeated measures of personality traits (as I have done in chapters 2 and 4, albeit across measures that were positively correlated), it is possible that both inconsistent individuals (fast in one trial, slow in the next) and genuinely intermediate individuals (intermediate in all trials) may be lumped together, leading to misrepresentation of intermediates in even studies where they are included. Animals that are intermediate in a personality trait are sometimes "different" rather than middling in other behaviours, such as foraging strategy within associative learning tasks (Arnold et al., 2007) or ranging behaviour (Boon et al., 2008). Furthermore, comparing the same trait across contexts, variance in behaviour is often lower in intermediates than extremes, with intermediates adjusting their behaviour less in response to changing social context (Vas et al., 2008) or predator presence (Coleman & Wilson 1996; Bourne & Sammons 2008). Indeed in wild great tits (*Parus major*), variance in survival and reproductive success between food-rich and food-poor years are also lowest in intermediates (Dingemanse et al., 2004). Therefore I suggest intermediate personality types, and perhaps comparison of true intermediates to "intermediates" that switch between fast and slow behaviour, may be an important line of inquiry in future studies.

Finally, I found no correlation between personality traits within individuals hence no evidence of a proactive-reactive trait in either blue tits (Chapter 2) or greenfinches (Chapter 4). This was particularly surprising in blue tits, as the closely related great tit is the archetype of the proactive-reactive personality trait (Groothuis and Carere, 2005). In greenfinches, I suggest that the traits may have different underlying physiological correlates, as neophobia and object exploration related differently to oxidative profile (chapter 4). From studies on stress response to novel objects, it appears that presenting a novel object with food elicits a physiological stress response (Richard et al., 2008) but

presenting a novel object alone does not (Mettke-Hofmann et al., 2006, but see Apfelbeck and Raess, 2008). As a novel object presents both a potential threat and an opportunity for learning, it is possible that neophobia is an assay of variation in stress responsiveness between greenfinches but exploration only variation in information-gathering. In blue tits, neophobia and exploration traits appear also to reflect different behavioural strategies. Whilst both neophobic and fast-exploring individuals were most environmentally sensitive to changes in food supply, only fast-explorers appeared to gather information before food was removed (discovering new feeding opportunities: chapter 2). They also inspected feeders when they were first emptied, visiting at a higher rate than slow explorers on day 1 of the manipulation, whilst neophobic birds left immediately (chapter 3). Unlike the trait neophobia, exploration did not predict avoidance of feeders that were novel in appearance (Chapter 2). These differences in the types of information gathered, and possibly also responsiveness toward stressors, may result in very different forms of environmental sensitivity between personality types (van Overveld and Matthysen, 2010). This is particularly important when considering the adaptive significance of behavioural syndromes such as the proactive-reactive trait: with no correlation between neophobia and exploration behaviour, blue tits and greenfinches could have any combination of oxidative profiles and any combination of behavioural strategies. Where behavioural syndromes exist therefore, this may reflect selection not only on particular traits but also particular combinations of traits (Sih et al., 2004).

7.1 Summary of cue selection results

In chapters 5 and 6, I examined cue selection in the relocation of temporally stable food sources. In captive greenfinches (chapter 5), I found that cue preferences changed with increasing experience of an invariant foraging situation. In a simple foraging task, I gave birds the option of re-finding hidden food either using a local cue: a conspicuous coloured cotton wool ball marking food, or a spatial cue: the position of a well on an ice cube tray in which food was hidden. After one encounter with this task ("one-trial test"), returning birds generally favoured the local cue. As discussed in Chapter 5, I cannot distinguish an adaptive bias toward local-cue learning from an artefact of stimulus design as explanations for this bias (LaDage et al., 2009). However, by observing a cue switch within the same scenario after ten encounters, I can eliminate stimulus design as the

reason for biases toward spatial cues in the "repeated-trial test". I attribute this cue-switch to the development of an expectation of temporal stability, acquired through learning.

I then examined in detail the mechanisms underlying cue selection in the one-trial test. I found that greenfinches were able to quickly learn spatial cues in the absence of local cues ("one-trial spatial test"). However, increasing visual distraction by substituting a white distracter for a coloured distracter interfered with this cue selection process. It is possible that birds were better able to learn local cues because the presence of a local cue "overshadowed" spatial cues in the first one-trial test (Cheng, 2008; Cheng et al., 2007; Gray et al., 2005). Over the ten repeated encounters though, birds both overcame any such interference and developed a bias toward spatial cues. As such I concluded that they were able to learn both cue types but actively favoured local or spatial cues dependent on the temporal stability of the context. However, this study utilised a very simple foraging task. Indeed, as discussed in Chapter 5, due to practical limitations it was necessary to further simplify the test from a selection of one option from seven to just one from three. This conclusion required further testing therefore, both using a more complex captive task and, as I attempted in Chapter 6, in the wild.

Based on findings in chapter 5 I predicted that wild birds using feeders, which constitute a temporally stable feeding location, would disregard visual aspects of feeders when relocating them. In the wild, natural ephemeral foods may vary between scattered and clumped distributions. The ability to learn both cue types (as in chapter 5), and switch between them with varying temporal stability in food distribution may be adaptive (Humber et al., 2009). In chapter 6, I established eight artificial feeding sites across urban parks in Glasgow. At each site, there were four feeders, coloured red, blue, green and yellow, and every week I systematically rotated these colours amongst four fixed positions within each site. Over repeated encounters with the feeders, I expected birds would learn that they occurred in fixed positions and hence to disregard colour, feeding instead at random or perhaps exhibiting position biases. I was surprised therefore to find that four out of five species responded to colour, even after six days of exposure.

Three species (the great tit, long tailed tit and robin) favoured red feeders, but particularly when the feeders had newly been rotated. Red feeders are most conspicuous against the predominantly green foliage background, so a conspicuousness-biased search pattern may be the mechanism by which they were relocated. Birds generally exhibit heightened attractions toward familiar palatable foods and aversions toward unpalatable foods when their background contrast is elevated (Osorio et al., 1999, Schmidt et al.,

2004; Gamberale-Stille and Guilford, 2003). A conspicuousness-biased foraging strategy is therefore a highly basal strategy that, unlike spatial cue use, does not require context-specific learning (Schmidt et al., 2004). Coupled with a tendency in great tits and long tailed tits toward using social cues to locate food, I suggest these birds did not learn in a context-specific manner but instead used a more general, ephemeral foraging strategy even within temporally stable contexts. In other studies, experience of enhanced environmental variability in early life has been shown to influence the kinds of behaviour animals display (Braithwaite and Salvanes, 2005), propensity toward learning (Brydges et al., 2008) and indeed to modify cue selection (Odling-Smee and Braithwaite, 2003) in adulthood. Perhaps experience of temporal instability in natural food (beyond the artificial feeding sites) caused wild birds to adopt a general strategy rather than learning specific strategies for each context. This again highlights the importance of testing behavioural predictions in the wild.

7.3 Bird feeders: a link to wild behaviour?

Artificial feeders are a testing ground for captive and theoretical predictions on wild animals. The funnelling of birds from the broader environment to a focal point allows us to quantify for example dominance hierarchies (Dingemanse and de Goede, 2004), energy intake (e.g. Sandlin, 2000) or body mass change (Boisvert and Sherry, 2000). Moreover, feeders provide a site in which to perform manipulations on wild birds, for example to introduce novel objects (Echeverria et al., 2006, Herborn et al., 2010), or to alter perceived predation risk (Macleod et al., 2005), environmental stability (Humber et al., 2009; Chapter 6) or food availability (e.g. van Overveld and Matthysen, 2010; Chapter 3). In Chapters 2 and 3, I used feeding stations to monitor behaviour using PIT tag technology. PIT tags are a very powerful tool when individual presence in a particular place and time can be given meaning. For example, when it reveals with whom an individual associates (Pike et al., 2008) or, in my study, how long it has taken them to approach novel feeding opportunity (Chapter 2). Unlike observational studies or radio-telemetry though, PIT tags cannot tell us about the behaviour of an individual in the broader environment. This poses an interesting question: to what extent does behaviour at feeders represent behaviour in the wild?

Dependency: the transition from natural foods toward reliance on artificial feeders is a key concern, for both research and animal welfare (Brittingham and Temple 1988,

Sterba 2002). For research, the subversion of natural behaviour this would constitute could be a serious misrepresentation of wild behaviour. So far, there is little evidence that wild birds do become dependent on artificial feeding sites however. The diets of wild black-capped chickadees (*Poecile atricapillus*; Brittingham and Temple, 1992) and Australian magpies (*Gimnorina tibicen*; Jones, 2002), and the food provisioned to the nestlings of Australian magpies (O'Leary and Jones, 2006), blue tits (Cowie and Hinsley, 1988) and Florida scrub-jays (*Aphelocoma coerulescens*; Fleisher et al., 2003) for example all have been shown to contain between 70 and 86% natural food despite the availability of artificial feeders. Moreover, at the population level birds appear to use feeders in a flexible manner, reducing feeder use with increasing air temperature (Chamberlain et al., 2005; Chapters 3 and 6) or greater natural food availability (Cannon et al., 2005; Chamberlain et al., 2005; 2007). Brittingham and Temple (1992b) suggest this flexibility reflects the persistence of natural foraging biases toward ephemeral food even within temporally stable contexts. Their suggestion is further supported by findings in chapter 6, in which four species of common garden passerine responded to colour and social cues when relocating feeders: foraging strategies better suited to the location of naturally ephemeral rather than artificially temporally stable food (chapter 5).

Of course, what occurs at the population level does not necessarily reflect the behaviour of the individual (Chapter 3). Systematic variation in feeder use within species could still generate sampling biases in studies of intraspecific variation in behaviour. For example, I trapped 32 female blue tits at feeders but 86 males (Chapter 2). There was no evidence that sexes differed in average feeder use (Chapter 3), yet females are often subordinate to males in Parids (Braillet et al., 2002, Dingemanse and de Goede, 2004). It is possible therefore that I sampled only very competitive females. Feeders are often used to study personality in the wild (van Overveld and Matthysen, 2010, Herborn et al., 2010, Dingemanse and de Goede, 2004, Echeverria et al., 2006). With respect to personality, systematic biases in average feeder use between personality types would be a problem. Fortunately, consistent with studies on great tits (Dingemanse and de Goede, 2004, van Overveld and Matthysen, 2010), in blue tits I found no evidence that personality types differed in their average feeder use (chapter 3). This was contrary to expectation as in captive studies (Benus et al., 1987, Benus et al., 1988, Verbeek et al., 1994, Marchetti and Drent, 2000), neophilic animals are often more prone to routine-formation than neophobic animals: a tendency which I expected to translate into feeder dependency in the wild. For the purposes of comparing captive behaviour to wild therefore, feeder use appears an appropriate method of assaying wild behaviour.

However to fully understand personality traits in blue tits, I would need also to trap and personality test birds away from feeders. I attempted to trap blue tits away from feeders in the 2008-9 field season, but with no success. After mist-netting at each feeding station once, in the second of two rotations I mist-netted simultaneously at a permanent feeding station and a location that was near low scrubs and 50m from the feeding station but otherwise selected at random. On four such mornings of mist-netting, only one re-trap blue tit (captive tested already within the season) and non-focal species were trapped at the random locations. If non-feeder using blue tits exist I may not therefore have described the whole range of personality variation within the blue tit. Wilson and colleagues (1993) for example found that bolder pumpkinseed sunfish (*Lepomis gibbosus*) were more likely to enter novel traps than shier fish, which were caught instead using indiscriminate seine nets. Had they and other researchers (e.g. Minderman et al. 2009; Dingemanse et al., 2002) not employed a range of trapping techniques, the full spectrum of personality traits within those species would not have been described (see also: Boon et al., 2008).

7.3 Final thoughts

In this study, I have studied personality in both greenfinches and blue tits. Whilst greenfinches are a model species for variation in physiology (e.g. Lindstrom et al., 2003, Lindstrom, 2004, Horak et al., 2002), with personality I found consistent differences in oxidative profile that have not previously been accounted for. Moreover blue tits, a model species for research on breeding behaviour (e.g. Tripet et al., 2002, Arnold et al., 2010) differed consistently in their response to environmental cues in the wild. Personality variation may therefore represent a missing link in understanding existing literature in different fields of research (Careau et al., 2008).

In this study, I have also sought to test captive behavioural predictions in the wild. In some cases, I was able to validate long-held beliefs (Chapter 2) but in others my findings have challenged expectations from captive studies (Chapters 3 and 6). In captivity, stickleback respond differently to environmental cues dependent on shoal composition (Pike et al., 2008, Nomakuchi et al., 2009), their experience (Dingemanse et al., 2009) or perception of predation risk (Bell and Sih, 2007) and their experience of water turbidity (Odling-Smee and Braithwaite, 2003). The wild environment is infinitely more complex than these few variables synthesised in captivity, and the variety of

conditions that animals are subject to and learn about in the wild can never truly be replicated. As such, I suggest that studies that find ways to test hypotheses in nature are crucial to understanding the adaptive significance of variation in response to environmental cues when foraging.

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APPENDIX I

CAPTIVE EXPLORATION BEHAVIOUR AND WILD FORAGING BEHAVIOUR IN GREAT TITS

I | ABSTRACT

Personality traits are behavioural responses that vary between individuals but are consistent within individuals over time or contexts. Great tits (*Parus major*) are a model species for personality research, particularly within the trait "exploratory tendency", which describes movement in novel environments. A key prediction from captive studies is that fast exploring great tits form foraging routines whilst slow explorers are more flexible in their foraging behaviour. I tested whether exploratory tendency predicted wild foraging behaviour. Over two winters, 37 great tits were taken into captivity for a short period and their movement in novel versus familiar parts of a small environment measured. Both exploratory tendency (movement in novel minus familiar) and activity in the trial were independent of the weather, day length or body condition at capture, and repeatable in 18 birds tested twice within years. Birds were returned to the wild and their behaviour recorded by an electronic monitoring system at feeding stations. I measured variation in two behaviours. First, "wild exploratory tendency": discovery of short term feeding stations installed 110-260m from eight permanent feeding stations (n = 27 birds). Second, "environmental sensitivity to food supply": latency to abandon experimentally emptied feeding stations (n = 14 birds). Surprisingly, captive exploratory tendency did not predict wild exploratory tendency. Moreover feeder abandonment was quickest in birds that were most exploratory in captivity, and also birds that were most active in the exploration trial. Therefore birds did not differ in foraging behaviour whilst food was available, and exploratory birds broke foraging routines most quickly. Results for wild exploratory tendency contradict those obtained contemporaneously from a sympatric blue tit population (*Cyanistes caeruleus*). However, the relationship between captive exploratory tendency and feeder abandonment is remarkably consistent with findings in blue tits and also an analogous study on another wild population of great tits.

I II INTRODUCTION

Personality traits are behavioural responses that differ consistently between individuals over time or contexts. For example, individuals of the same species, sex and age often differ in exploratory tendency (activity within novel environments, e.g. Verbeek et al., 1994), "boldness" (latency to feed in risky or novel environments, e.g. Wilson et al., 1993), aggression (e.g. Huntingford, 1976), sociability (e.g. Cote and Clobert, 2007) or activity (Sih et al., 2003). These five axes of variation are personality traits that have been described in a broad taxonomic range (for review: Gosling, 2001). The great tit (*Parus major*) has become a model species for research on personality (for review: Groothuis and Carere, 2005), in particular the trait "exploratory tendency". Work on captive bred lines of great tits selected for "fast" and "slow" exploration has contributed significantly to our understanding of the genetic and physiological basis of personality (Groothuis and Carere, 2005). Observing that fast and slow exploring great tits differ in their survival in the wild (Dingemanse et al., 2004), researchers have also drawn upon these captive line studies as a source of predictions on the behavioural mechanisms underlying that variation.

Behavioural comparisons of the fast and slow selection lines (e.g. Marchetti and Drent, 2000, Verbeek et al., 1996, Verbeek et al., 1999, Verbeek et al., 1994) suggest that survival differences may be explained by variation in foraging strategy (Dingemanse et al., 2004). Fast and slow lines differ on three counts. First, they differ in their use of information when first locating food. For example when food is hidden and social cues to its whereabouts available (trained "demonstrators" foraging in the correct locations), only fast birds respond to those cues; slow birds continue to search independently (Marchetti and Drent, 2000). Second, when re-finding food, fast birds appear more reliant on old information than slow birds. For example, on commencing foraging, fast birds tend to target previously rewarding locations within an environment whilst slow birds appear to search anew (Groothuis and Carere, 2005). A consequence of this is the tendency toward routine-formation in fast birds: when previously predictable food sources are removed, fast individuals continue to visit the unrewarding location whilst slow birds quickly extend their search to new areas. Indeed, in a variety of species, "fast" personality types (exploratory but also in this species aggressive, bold, active) are often slower to respond to environmental changes, and hence more routine in their behaviour, than "slow" (passive, shy, inactive) types (Marchetti and Drent, 2000, Jones and Godin, 2010, Benus et al., 1987, Benus et al., 1988). This difference in responsiveness to environmental

change is referred to as “environmental sensitivity” (Boyce and Ellis, 2005, Koolhaas et al., 1999). Finally, in great tits exploratory tendency co-varies with the personality traits aggression and boldness (Carere et al., 2005). Fast or “proactive” (fast exploring, bold and aggressive) great tits are therefore prone to routine-formation but also equipped to monopolise feeding situations that prove to be predictable. Conversely, slow or “reactive” (slow exploring, shy and passive) individuals are more flexible and sensitive to change, hence are expected to cope better with unpredictable food availability (for review: Groothuis and Carere, 2005). These predicted differences in foraging strategy fit the observed variation in survival well: fast exploring females do better in food-rich winters, when monopolisation of clumped, predictable resources would be selected, and slow types do better in food-poor winters when the distribution of food is unpredictable (Dingemanse et al., 2004).

In order to validate this behavioural mechanism, however, it is critical to determine whether fast and slow individuals behave in the wild as predicted. Specifically, whether slow types are less routine than fast types within predictable contexts and fast types more routine than slow types within unpredictable contexts. Studies comparing captive to wild behaviour do not always conform to prediction. For example, in captivity fast exploring great tits are generally subordinate to slow explorers (Verbeek et al., 1999), but in the wild this is only the case in non-territorial juveniles: fast exploring, territorial adults are most likely to dominate at feeders (Dingemanse and de Goede, 2004). Furthermore, third variables that covary with the traits I waspredict will generate variation in fitness may be the source of variation in fitness. For example, observing variation in reproductive success between personality types (Both et al., 2005), (2008) Hollander and colleagues (2008) expected exploratory tendency (activity in a novel hence potentially risky environment) to translate directly into movement during nest defence, i.e. predator mobbing behaviour (activity in a risky environment). Active mobbing behaviour did not differ with personality; instead exploratory tendency predicted level of vocal chiding toward predators, which may also have the consequence of improved fledging success. Routine-breaking has been studied twice in the wild in great tits. With the definition of routine-breaking as leaving a previously rewarding site, in both studies fast birds were actually more prone to routine-breaking than slow birds: fast birds dispersed further following post-natal dispersal (i.e. left familiar natal foraging sites: Dingemanse et al., 2003) and, whilst slow birds remained in the vicinity, moved away from emptied artificial feeding stations (van Overveld and Matthysen, 2010). The aim of my study was to test whether captive exploration behaviour predicted variation between

great tits foraging in the wild. After measuring exploration during a short period in captivity, I returned great tits to the wild where they were accustomed to foraging at artificial feeding stations. The birds were fitted with passive integrated transponders ("PIT tags") so that I could monitor their behaviour at those feeding stations. My aims were as follows: first, to test whether exploration was repeatable within my methods and study population. Second, in 27 birds I tested whether exploration behaviour predicted individual discovery of new short term feeding stations, positioned near to permanent feeding station, which I refer to as "wild exploratory tendency" (Chapter 2). And finally, in 14 birds I measured variation in response to the emptying of those permanent feeding stations, which I refer to as "environmental sensitivity to food supply" (Chapter 3).

I III METHODS

The study site was located on the east bank of Loch Lomond, UK (56°08'N 4°37'W). In October 2008, I established a network 8 feeding stations at approximately 500m intervals through deciduous woodland. These feeding stations were baited with peanut granules until the end of February, thus were a predictable source of food throughout winter. Each consisted of two opaque tubular Defender™ feeders (35cm height, 7cm diameter) hung above one another from an oak trunk at 2m and 3m above ground level. Only one bird could access each feeder at a time, via a small hole. Onto that hole I attached an 8cm x 5cm hoop antenna (TROVAN®, United Kingdom) fitted onto a wooden platform perch (both 8cm x 5cm). Over the two years, I caught 37 birds by mist-netting at these sites. On first capture, each bird was fitted with a leg-ring mounted Passively Integrated Transponder ("PIT" tag; 11.5 mm x 2.1 mm, <0.1g, Trovan Unique™; as Herborn et al., 2010). The PIT tag produces an amplitude modulated code signal within the electromagnetic field of the antenna, thus allowed us to monitor feed use in the wild via an electronic monitoring system (Trovan™ LID665). At capture, I also determined age (juvenile/adult) and sex from plumage traits (Jenni & Winkler 1994). After 2 or (in 2009) 3 days in captivity, the birds were released at their feeding station of capture. All birds were returned to the wild at least 12 days prior to the feeder abandonment manipulation in 2009, when the 8 permanent feeding stations were suddenly emptied at the end of winter.

For husbandry and detailed methods, see Chapter 2. Briefly, the exploration trial was conducted within the home cage of the focal bird (150cm x 50cm x 50cm). On arrival in captivity, they were enclosed within one half of the cage, using an opaque metal divider, and left undisturbed for at least 2 hours. In the exploration trial, I measured behavioural response on gaining access to the "novel" half of the cage (see below). After this trial, birds had access to the entire cage. As part of separate studies, all birds then took part in further behavioural trials on this and one further day, and were also blood sampled on the second day in captivity. In the 2008-9 season, I kept the birds in captivity for a third morning, in order to re-run the exploration trial to test repeatability of individual behaviour. After two (2007-8) or three (2008-9) days in captivity, all birds were released at their feeding station of capture, at least one hour before sunset.

By enclosing the birds into one half of the home cage for over two hours on arrival into captivity, I anticipated that they would become familiar with that part of the cage and hence behind the cage divider would be a novel environment. The familiar and novel cage halves were both similarly enriched: each contained three perches covered with plastic plant vines. My aim was to assay exploration independently of neophobia, and hence create an environment that was novel only in that it was unexplored. To motivate birds toward foraging activity, I removed the food bowl for 1hr and water bowl for 30 min prior to the trial. To start the trial, I removed the cage divider, stepped behind a screen, and observed the focal bird through a small hole for 10 minutes. In other exploration trials on great tits, only movement in novel environments is measured, and individuals are often forced to enter those environments to start the trial (e.g. Verbeek et al. 1994). Here, I allowed the birds the option of remaining within the familiar environment throughout the trial. As the birds had been in captivity for only a short while prior to testing, I allowed this option to help distinguish exploration from activity due to escape behaviours in the novel environment (Mettke-Hofmann et al. 2009). I scored birds for the number of movements in the trial, defining a movement as a hop/flight between two perches and/or the floor, the cage wall or the front and rear of the cage. I also recorded the endpoint of each movement: novel or familiar. After the test, I returned the food and water bowl and the bird was allowed free access to the entire cage.

In 2008-9, I conducted a second exploration trial for each bird, on day 3 in captivity. In that season, on arrival into captivity all birds were randomly allotted to a

cage lined either with white paper (as in 2007-8) or brown paper. After collecting a blood sample on the afternoon of day 2 (when birds in 2007-8 were released), I moved each bird to one half of a new home cage, of the other cage type. They were left undisturbed until the following morning, when exploration trials began one hour after the lights were switched on. The arrangement of perches/artificial plant material was similar between these white-lined and brown-lined cage types, but the artificial leaf shapes differed slightly. My aim was to create two cage types that were sufficiently distinct to re-motivate birds toward exploration but not so distinct that they stimulated different levels of exploration across replicates. This appeared successful: controlling for cage order and bird identity, there was no difference in movements in the familiar (linear mixed model, LME: $t_{1, 17} = -1.12$, $P = 0.28$) or novel environments (LME: $t_{1, 17} = -1.43$, $P = 0.17$) between brown versus white-lined cage types.

To investigate whether activity specifically in the novel environment or activity in general correlated with behaviour in the wild, I drew two measures from the movement scores. "Exploratory tendency" related specifically to movement in the novel environment; I controlled for variation in activity level between birds by deducting the number of movements in the familiar environment from the number in the novel environment. "Activity in the exploration trial" was the movements in the novel and familiar environments summed. As these variables were not independent, they were subsequently analysed separately.

I III B Between-individual sources of variation

To measure repeatability of behaviour in the exploration trial, we must first identify and control for other sources of variation between individuals entering captivity that may also generate consistent individual differences in behaviour. Birds caught on colder, wetter or shorter days for example may have a higher perceived starvation risk than individuals caught on warm, dry or long days, which may in turn systematically alter their propensity toward exploration in captivity. Therefore I recorded day length and collated weather data on rainfall (mm), minimum and maximum temperature on the day of and day prior to capture from Met office records from nearby Glasgow Bishopton (www.metoffice.gov.uk). I also calculated body condition at capture, as the residual of body mass regressed on tarsus length (Linden et al., 1992). Finally, in Parids smaller, juvenile and female birds are generally subordinate at feeders (Braillet et al., 2002).

Coming from the wild where they may recently have experienced competitive exclusion, I predicted that these birds may also have greater motivation to explore on entering captivity than larger, adult or male birds. I used wing length as a measure of overall body size. Age (juvenile/adult) and sex were determined from plumage traits (Jenni & Winkler 1994); there were 18 juveniles and 19 adults, and 25 males and 12 females.

I III C WILD EXPLORATORY TENDENCY

Birds were scored for whether or not they discovered new, short term feeding stations. For each replicate of this trial I installed one new feeder, on a 1.5m pole, within the study site an average of 160 meters (range: 110m-260m) from one of the eight established feeding stations. It was installed before sunrise, left undisturbed for three days, and then removed after sunset. The feeder had one perch fitted with a PIT tag antenna, to identify and score birds 1 or 0 for discovering the feeder within that time. There were nine consecutive replicates of this trial in the 2007-8 season, and 16 consecutive replicates in the 2008-9 season. As such replication was uneven across years but also, as birds were added to the study as the seasons progressed, between individuals within seasons. Therefore feeder discovery was analysed using the number of new feeders an individual did discover relative to the number it could have discovered (i.e. the number of replicates in which it participated).

Independent of exploratory behaviour, an individual's likelihood of discovering new feeders would be greater in birds using regularly using more of the permanent feeding stations, hence covering a larger area of the study site. At the end of the field season, from PIT tag records I deduced which permanent feeding stations each bird had used. On average, birds used on average 1.64 permanent feeding stations (range: 1-3). The likelihood of feeder discovery would also increase as the distance between an individual's permanent feeding stations and the new feeder decreased. I therefore calculated the distance between the nearest permanent feeding station and the new feeder in each replicate for each bird. These variables were controlled for in the analyses of feeder discovery (see statistical methods).

I III D ENVIRONMENTAL SENSITIVITY TO FOOD SUPPLY

Environmental sensitivity to food supply was defined as the tendency to abandon feeding stations within three days of emptying. To investigate feeder abandonment in more detail, I also compared feeder visitation on the day of feeder emptying and after five days. At the end of the 2008-9 season I emptied the 8 permanent feeding stations and then recorded visitation to the empty feeders on that day and after 3 and 5 days. Two days prior to that manipulation, I used PIT tag records to identify birds present at each feeding stations. Those birds were only then included in the experiment if they were recorded again at that feeding station on the day of the manipulation at least once prior to and once after emptying the feeders, hence experienced the change in food supply. Fourteen birds fitted these criteria. I conducted the manipulation in two blocks, emptying 4 feeding stations on 02/03/09 and the remaining 4 on 03/03/09, between 9.20am and 10:40am each day. I installed PIT-tag readers at each site within 30 minutes of sunrise and removed them after sunset on the day of the manipulation, after 3 days and after 5 days. From these records, I extracted the number of visits by each bird on each day. Birds that visited least on day 3 were classified as most environmentally sensitive.

I III E ETHICAL NOTE

All work was licensed by the UK Home Office, with permission for taking birds in captivity and for using PIT Tags obtained from Scottish Natural Heritage and the British Trust for Ornithology respectively. Studies were carried out in accordance with ASAB/ABS's guidelines for the treatment of animals in research, and subject to ethical review by WALTHAM® Centre for Pet Nutrition and the University of Glasgow. I captive tested 37 great tits between 2007 and 2009 for this study. Whilst in captivity, no birds lost more than 10% body mass there was on average a body mass gain (0.52% ± 5.12%). Following release, 31 of the 37 great tits were later identified in the wild within the same season via PIT tag records or re-trapping.

I iii f Statistical Methods

Analyses were carried out using R 2.9.1 (R development core team, 2009). There were no differences in behavioural data between years so data was pooled across years. Exploratory tendency and activity in the exploration trial were extracted from the same data (movement in the exploration trial), thus were not independent. As such, models were run separately for each of these measures and where both were significant I referred to Akaike's Information Criterion (AIC) to determine which measure better described the data.

I iii f i Defining captive exploration behaviour

I first determined whether between-individual variation at capture explained a significant proportion of the variation in behaviour during the exploration trial. Captive personality traits were not normally distributed so I used nonparametric Mann-Whitney U-tests or Kendall rank sum correlations. I applied a Bonferonni correction for multiple comparisons, with a p-value of less than 0.004 for significance. I tested consistency across days using a linear mixed model, with trial order as a random effect. I then calculated repeatability of exploratory tendency and activity in the exploration trial using the mean squares from an ANOVA, with the repeated measures of exploratory tendency/activity in the exploration trial as the dependent variable and individual identity as the independent variable, following Lessells & Boag (1987).

I iii f ii Relationships between captive and wild exploratory tendency

Feeder discovery was measured up to 16 times for each individual. I accounted for repeated measures with a Generalized Linear Mixed Model (GLMM), specifying feeder discovery as the dependent variable and individual identity as a random factor. Feeder discovery was binary (discovered versus not discovered) so I used a binomial error structure. To control for experimental variation between replicates, I included the number of permanent feeding stations an individual regularly used (i.e. site coverage) and also the distance between an individual's nearest permanent feeding station and the new feeder in each replicate as covariates. Finally, I specified either exploratory tendency or activity in the exploration trial as an independent variable. To determine whether these exploration

measures explained a significant proportion of variation in feeder discovery, I compared this GLMM to a GLMM excluding the exploration measure using a likelihood ratio test (LRT).

I iii f iii Relationships between captive exploratory tendency and environmental sensitivity to food supply

To first relate prior feeder use (visitation two days prior to the manipulation) to exploration behaviour, I used generalized linear models (GLMs). Prior feeder use was a count: I used a quasi-Poisson error structure to account for this and for over-dispersion. To investigate relationships between exploration behaviour and feeder visitation on days 1 and 3, I used generalized linear mixed models (GLMMs). The dependent variable, feeder visitation, was a count so models had a Poisson error structure. Day (day 1 or day 3) and either exploratory tendency or activity in the exploration trial were my main independent variables. I included age (adult or juvenile) as a fixed factor to control for variation in foraging experience and, as juveniles are subordinate to adults in Parids (Dingemanse and de Goede, 2004), feeder access. I also included prior feeder use as a covariate, to control for individual differences in feeder dependency. Finally, as the manipulation was staggered over two days, I specified rotation (first or second) as fixed factor. To avoid over-parameterising the models I included only the interaction between day and exploration measure, as I was interested in changes in visitation behaviour between personality types over days. To test specifically whether captive behaviour affected feeder visitation on days 1 or 3, for each day I constructed a general linear model (GLM) with visitation as the dependent variable and age, prior feeder use, rotation and either captive exploratory tendency or activity in the captive exploration trial as the independent variables. For day 1, I specified quasi-Poisson error structure to account for overdispersion. For day 3, as visitation was very low (see results), I specified visitation as a binary variable: visited versus did not visit. To test the significance of either captive exploratory tendency or activity in the exploration trial I performed a likelihood ratio test (LRT) between a model including the captive behavioural measure and a model excluding the captive behavioural measure. On day 5, most birds did not visit the emptied feeders or visited only once (see results). As such, I treated feeder visitation on day 5 as a binary variable (visited, did not), and used GLMs with a binomial error structure to identify relationships between visitation and exploration measures.

I IV RESULTS

I IV A DEFINING CAPTIVE EXPLORATION BEHAVIOUR

The number of movements in the first exploration trial ranged from zero to 672, but the median was 12 and the mean 100 (IQR = 163), indicating that some individuals were highly active and others relatively inactive. Ten birds did not move at all in the first exploration trial, and 3 of these (of the 18 birds included in the repeatability analysis) also failed to move in the second exploration trial.

Exploration behaviour did not differ between sexes or ages (exploratory tendency: all $P > 0.54$; activity in the exploration trial: all $p > 0.06$) so data were pooled to analyse other sources of between-individual variation. All other morphometric and environmental variables were non-significant (exploratory tendency: all $P > 0.11$; activity in the exploration trial all $P > 0.08$). Therefore consistency and repeatability were calculated on actual scores. Controlling for trial order, exploratory tendency (LME with trial order random: $F_{1, 17}$ 7.4, $p = 0.0001$) and activity in the exploration trial ($F_{1, 17}$ 3.83, $p = 0.004$) were both consistent within the 18 great tits tested twice in the 2008-9 season. Exploratory tendency across days 1 and 3 was significantly repeatable (ANOVA: $F_{1, 17}$ 6.61, $p = 0.0001$, $r = 0.74$), as was activity in the exploration trial ($F_{1, 17}$ 3.83, $p = 0.004$, $r = 0.57$).

I IV B RELATIONSHIPS BETWEEN CAPTIVE AND WILD EXPLORATORY TENDENCY

Wild exploratory tendency (i.e. feeder discovery) was independent of both exploratory tendency (GLMM: LRT $\chi^2 = 2.63$, N = 27 birds, $p = 0.11$) and activity in the exploration trial (GLMM: LRT $\chi^2 = 0.77$, N = 27 birds, $p = 0.38$).

I IV C RELATIONSHIPS BETWEEN CAPTIVE EXPLORATORY TENDENCY AND ENVIRONMENTAL SENSITIVITY TO FOOD SUPPLY

Feeder use prior to food removal did not vary with exploratory tendency (GLM with quasi-Poisson error structure: $t_{1,13} = 1.35$, $P = 0.2$, effect ± s.e. 0.004 ± 0.003) or activity

in the exploration trial ($t_{1,13}$ = 1.58, P = 0.14, effect ± s.e. 0.003 ± 0.002). Following emptying of the feeders, feeder visitation declined from a median of 30 visits (range 1 – 87) on day 1 to zero (range 0 – 13) on day 3. In GLMMs of both exploratory tendency (Table I-1, Fig. I-1a) and activity in the exploration trial (Table I-2, Fig. I-1b), a significant interaction between exploratory tendency and day indicates that exploratory individuals visited the emptied feeding stations more frequently than less exploratory individuals on day 1, but this relationship was reversed on day 3. Age was significant within both models, with juvenile birds visiting the emptied feeding stations more often than adults (Tables I-1 and I-2). Rotation (date on which feeder manipulations were conducted) explained variation in post-manipulation feeder visitation in GLMMs with exploratory tendency as an independent variable (Table I-1) but not in GLMMs with activity in the exploration trial as an independent variable (Table I-2). Prior feeder use did not explain variation in either GLMM (Tables I-1 and I-2). Comparison of AIC values suggests exploratory tendency (AIC 153.2) described the variation in feeder visitation on days 1 and 3 better than activity in the captive exploration trial (AIC 158.5). In GLMs constructed to investigate feeder visitation on day 1 or day 3 separately, on day 1 feeder visitation was significantly greater in exploratory than non-exploratory birds (LRT deviance = -96.21, F = 5.87, n birds = 14, P = 0.03) but no significant differences were identified with activity in the exploration trial (LRT deviance = -71.75, F = 3.66, n birds = 14, P = 0.08). On day 3, exploratory birds were significantly less likely to visit than less exploratory birds (LRT deviance = -14.97, n = 14 birds, P = 0.0001) but no significances were identified with activity in the exploration trial (LRT deviance = -0.31, n birds = 14, P = 0.58). On day 5, only 4 of the 14 birds visited the emptied feeding stations. Visitation on day 5 was independent of exploratory tendency (GLM with binomial errors: $z_{1,13}$ = -0.9, p = 0.37, effect ± s.e. = -0.012 ± 0.013) or activity in the exploration trial ($z_{1,13}$ = -0.84, p = 0.4, effect ± s.e. = 0.003 ± 0.004).

Table I-1 Results from GLMM on visitation of the emptied feeding stations by great tits in relation to the independent variable: exploratory tendency

Predictors	*z (d.f.)*	*P-value*	*R (S.E).*
Prior feeder use	0.28 (9)	0.776	0.001 (0.003)
Rotation	2.44 (9)	0.014	1.195 (0.488)
Age	2.08 (9)	0.037	1.334 (0.641)
Exploratory tendency x day	-3.72 (12)	0.0002	-0.02 (0.005)

The results are of a model with individual identity specified as a random effect, and a Poisson error structure; n visitation records = 28 and n great tits = 14

Table I-2 Results from GLMM on visitation of the emptied feeding stations by great tits in relation to the independent variable: activity in the exploration trial

Predictors	*z (d.f.)*	*P-value*	*R (S.E.).*
Prior feeder use	0.33 (9)	0.74	0.001 (0.003)
Rotation	1.9 (9)	0.057	1.061 (0.558)
Age	2.0 (9)	0.046	1.301 (0.652)
Activity in exploration trial x day	-4.9 (12)	< 0.0001	-0.008 (0.002)

The results are of a model with individual identity specified as a random effect, and a Poisson error structure; n visitation records = 28 and n great tits = 14

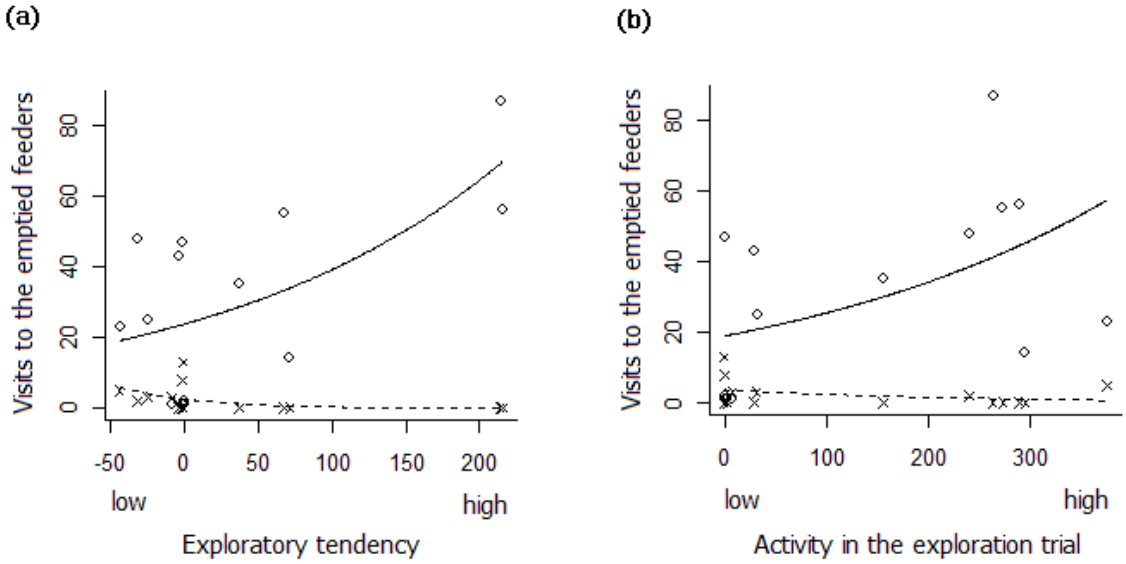

Figure I-1 Relationships between (a) exploratory tendency and (b) activity in the exploration trial and the number of visits to the emptied feeders 1 and 3 days after the feeders were emptied. Visits on day 1 are shown with open circles and relationships with lines; visits on day 2 are shown with crosses and relationships with dashed lines

I v DISCUSSION

Captive exploration behaviour did not predict exploratory tendency in the wild (i.e. feeder discovery) but did predict environmental sensitivity in the wild (i.e. feeder abandonment after three days). In the environmental sensitivity to food supply trial, there was a positive relationship between exploratory tendency (also, but less powerfully, activity in the exploration trial) and feeder visitation immediately after the loss of the food supply (day 1). This represents a change in behaviour as personality types did not differ in feeder use prior to the manipulation. However, by day 3, only the less exploratory individuals continued to visit the feeders. Therefore results were contrary to predictions from captive studies (for review: Groothuis and Carere, 2005): I expected less exploratory birds, analogous to the "slow" exploration lines, to remain flexible in their foraging behaviour and hence to discover new feeding opportunities in spite of available predictable food sources. I also expected fast explorers to remain longer at emptied permanent feeding stations than slow explorers, due to formation of foraging routines. Less exploratory birds also abandoned the emptied feeding stations, but over a longer time scale, by day 5. This suggests that exploratory tendency relates positively to the rate at which unprofitable feeding opportunities are abandoned.

From captive studies, it was predicted that exploratory birds would form foraging routines, hence discover fewer new feeding opportunities and take longer to abandon unprofitable feeding sites than less exploratory birds (Marchetti and Drent, 2000). Whilst contrary to captive studies, this study draws striking parallels to work on another population of great tits by van Overveld and Matthyssen (2010). Overveld and Matthyssen found that the abrupt removal of an artificial feeding site stimulated fast explorers to move to new foraging areas whilst slow explorers remained within the vicinity of the old feeding site (Overveld & Matthysen, 2009). As the great tits in that study did not differ in feeding range size after that manipulation, the authors suggest the movement reflected variation in the way individuals used information. As predicted by studies on the captive lines (for review: Groothuis and Carere, 2005), it appeared that fast explorers relied on old information, returning to formerly encountered feeding sites (thus explaining the sudden distant movement). In contrast, slow explorers relied on current information, remaining within the area to continue updating their information on the currently unprofitable site. Perhaps therefore the prolonged visitation to the emptied feeding stations in my study represents updating of information on food availability by less exploratory birds, rather than expectation of food *per se*. Intriguingly, Overveld and

Matthysen (2010) also found no difference in range size between fast and slow individuals prior to the manipulation. This corroborates the absence of correlation between personality and feeder discovery in this study: perhaps all birds maintained a similar range size about the permanent feeding stations, thus were equally likely to encounter the new feeders. Moreover, the similarity between these studies suggests some generality in the expression of the exploration trait across populations of great tits.

The same studies were conducted simultaneously on a sympatric population of blue tits (*Cyanistes caeruleus*; Chapter 6). I found striking similarity between species in the relationship between exploratory tendency and environmental sensitivity to food supply: exploratory blue tits also visited feeders at a higher rate immediately following feeder emptying on day 1, but visited less often than less exploratory blue tits on day 3. However in blue tits, exploratory tendency also positively predicted wild exploratory tendency (i.e. feeder discovery). The blue tit and great tit are closely related species that share a similar ecological niche and indeed use similar space, often foraging together in mixed flocks during winter (Cramp and Perrins, 1994). As such, blue tits and great tits are often in direct competition for food. At nearly twice the mass of the blue tit, the great tit it is the dominant species in these mixed flocks (Cramp and Perrins, 1994). Where food is predictable to great tits therefore, their monopolisation of those sites may render the permanent feeding stations relatively unpredictable to blue tits. Moreover, where food availability permits, great tits are territorial in winter, thus the feeding stations may support territorial behaviour in great tits (Dingemanse and de Goede, 2004). Therefore, I suggest the expression of exploratory behaviour was either not stimulated in great tits, with low requirement to find alternate feeding opportunities (i.e. competitive dominance) and/or suppressed by pressure to remain near the feeding station (i.e. territoriality). By this reasoning, we may expect juvenile great tits, which are subordinate to adults (Dingemanse and de Goede, 2004), to be more exploratory, but this was not the case. However, within the community, mist-netting efforts at the permanent feeding stations in both years suggest that blue tits and coal tits out-numbered great tits, by a ratio of around 8:2 in the 2007-8 (blue tit: coal tit: great tit = 113:50:61) and 8:1 in 2008-9 (58:41:19). Therefore even juvenile great tits may have relatively high status within predominantly blue tit and coal tit mixed flocks.

It is suggested that personality variation is maintained within great tits by a trade-off between selection against fast types in unpredictable environments, where they are unable to find enough food to sustain their level of activity/aggression/exploration (or take excessive risks to do so), and against slow personality types in predictable

environments where food availability and the opportunity to monopolise food selects for a higher (more competitive) levels of activity/aggression/exploration (Dingemanse et al., 2004). My results provide mixed support for the predictions on coping behaviour within predictable versus unpredictable environments, as personality types differed in their response to environmental change (new feeding opportunities or altered food availability), but not as traditionally expected from captive studies.

I VI REFERENCES

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APPENDIX II

LONG TERM CONSISTENCY IN NEOPHOBIA IN THE EUROPEAN GREENFINCH

II I INTRODUCTION

I measured neophobia in 17 male captive-bred greenfinches (*Carduelis chloris*) in spring 2008. The same individuals contributed to the study for chapter 4, four months later, in which neophobia was re-measured by similar methods. Here, I describe the methods used to measure neophobia in spring 2008 and compare the scores obtained for the same individuals in spring 2008 and autumn 2008. My aim was to examine temporal consistency in the neophobia trait.

II II METHODS

In spring 2008, from the 30/04/08 to 1/5/08, each bird took part in two neophobia trials, one on each day. Each trial consisted of two phases: a novel object phase and a disturbance phase. Phases were conducted around 1.5 hrs apart within each day, with the order randomised on day 1 and counter-swapped on day 2. Prior to a phase, the food bowl was removed to motivate birds toward foraging activity. After a further 30 min the water bowl was also removed. After 1h total without food, the food bowl was returned to the cage and the latency to approach recorded. In the novel object phase, the food bowl also contained one of four similarly sized (c. $3cm^3$) novel plastic objects, one red, one white, one yellow and one silver. Unlike the trial described in chapter 2, along with the regular seed mix the food bowl in both phases contained three fresh spinach leaves, a food type with which the birds were familiar. The spinach appeared to stimulate the birds to approach more quickly in this trial than that described in chapter 2, as mean disturbance latencies were significantly shorter in this trial (paired Wilcoxon rank sum test: $V_{16} = 28$,

P = 0.02). Therefore birds were only observed for 10 min, and birds that did not approach within that time given a maximum latency of 600 seconds.

To calculate neophobia scores, I took into account two issues. First, each individual was exposed to only 2 of the 4 novel objects, although there were no differences in novel object phase latency between objects (LME, with individual as a random effect: $F_{3,14} = 0.33$, P = 0.8). Second, independent of response toward the novel object, individuals may differ in their feeding motivation or tolerance of disturbance by the observer. Therefore for each trial I constructed a general linear model (GLM) between novel object phase latency as the dependent variable and disturbance phase latency and object identity as the independent variables, 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 two measure of neophobia for each bird, one per object (as Boogert et al., 2006). This is the method by which neophobia scores were calculated in autumn 2008 (chapter 4).

II III RESULTS AND CONCLUSIONS

Mean latency to approach the food bowl was significantly greater when a novel object was present (paired Wilcoxon rank sum test: V = 0, $N_1 = N_2 = 17$, P < 0.0001), thus the object elicited a neophobic response. Bird identity explained a significant proportion of the variation in the disturbance phases (LME, with trial order as random factor: $F_{1, 15} = 18.16$, P < 0.0001) and novel object phases (LME, with object identity nested in trial order as a random factor: $F_{1, 10} = 3.91$, P = 0.01). As birds were consistently fast or slow within phases I used a mean z value per bird as the neophobia score to compare to neophobia in autumn 2008. Individual neophobia was significantly repeatable (ANOVA: $r = 0.53$, $F_{1, 16} = 3.28$, $P = 0.01$) in spring 2008. Finally, individual neophobia measured in spring 2008 correlated positively with their neophobia measured in autumn 2008 (GLM: $t_{1, 16} = 3.2$, P = 0.006; see Fig. II-1). Therefore, neophobia was a temporally stable personality trait in greenfinches.

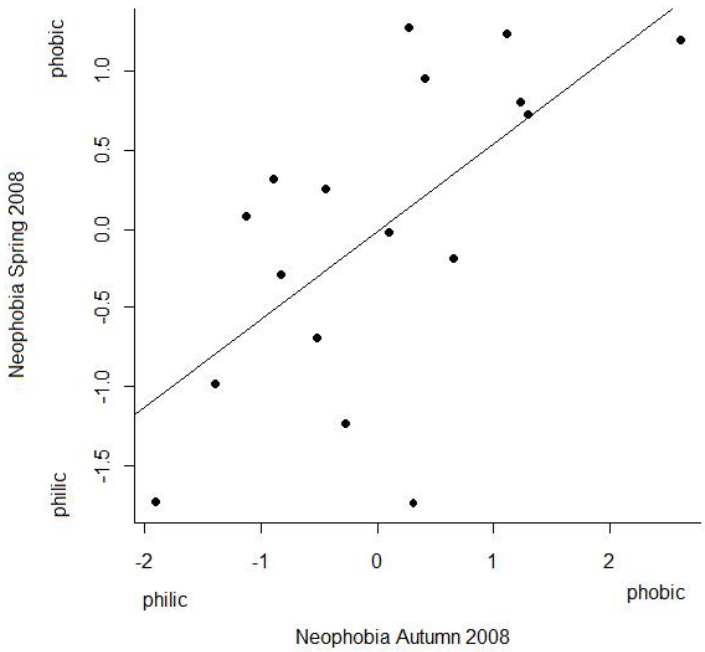

Figure II-1 The relationship between neophobia measured in spring 2008 and neophobia measured in autumn 2008 (chapter 4) for 17 male greenfinches.

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