Personality traits in the blue tit

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Academic Dissertation

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Articles

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I
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II
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III
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IV
Brommer, J.E. & Kluen, E. 2012. Exploring the genetics of nestling personality traits in a wild passerine bird: Testing the phenotypic gambit. Ecology and Evolution. DOI: 10.1002/ece3.412

V
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Abstract

In order to adapt their behaviour optimally and to be able to increase fitness, individuals are assumed to respond flexibly to environmental variation they encounter. Contrasting with this classical behavioural ecological point of view is the concept of animal personality. The latter focuses on understanding the mechanisms underlying and evolutionary processes maintaining variation in the expression of a behavioural trait over time and across situations or contexts. Originating in human psychology, personality studies have recently been integrated into the fields of ecology and evolution. Studies on consistent variation in behaviour within and between individuals (personality) have resulted in numerous insights and these are still expanding.

In the first chapter of this thesis I research underlying factors and possible consequences of the response (delayed hatching) of blue tits (Cyanistes caeruleus) to encountered climatic variation. I find that hatching delay (i.e. number of days hatching was delayed) is associated with early laying dates and low mean temperatures during the egg-laying phase. In addition hatching delay is negatively associated with clutch hatchability and female body condition. Using a reciprocal cross-fostering protocol on a large number of broods, I find that hatching delay may also negatively affect developmental parameters in offspring, in particular body mass of nestlings at fledging. Results from this study demonstrate that environmental conditions during egg laying can have effects lasting throughout the breeding and nestling period.

In chapters II to V I investigate variation in behaviour among individuals. The focus in these four chapters is on personality traits in blue tits. I first design an experimental setup, using a bird cage, in which several behavioural traits can be measured in a quick and non-invasive manner and which can be applied in both winter and breeding season. In addition several behavioural traits are measured during handling of both adult and nestling birds. All these behavioural measures are then used to test several aspects of behaviour in a personality context in the blue tit. The behavioural traits derived from the bird cage are repeatable over time and qualify as personality traits in this species. In addition I find an association between one of the measured personality traits in the cage and a single nucleotide polymorphism in the 3rd exon

of the dopamine receptor (D4) gene (DRD4), similar to what has been found in recent research on great tits (Parus major). This suggests that there is a genetic basis underlying this personality trait and that this genomic region might be involved in animal personality.

I apply a reaction norm framework to assess context specificity of the traits measured in the bird cage, using measures from (partly) the same birds measured in two distinct contexts (winter and breeding season). I show that one needs to carefully consider the context under which individuals are assayed and that a recorded behaviour may or may not be repeatable in another context.

Furthermore I use data from a cross-foster protocol on nestling blue tits in combination with quantitative genetics. I assess the heritability of three behavioural traits and show that these traits form a behavioural syndrome at both the phenotypic and genetic level. In addition, from the applied animal model analysis I can conclude that environmental factors, encountered by nestlings during the rearing period, may have a considerable impact on a nestling's personality. Thus, taken into account findings from the first chapter in this thesis, the development of both physical and behavioural traits in an individual seems to find its origin already in the earliest phases of life.

Finally I test whether three personality traits and two immunological traits in the blue tit covary and form a syndrome which includes behavioural and immunological traits. I find that there are intrinsic correlations between behavioural and immunological traits; however there is no strong evidence for the existence of a syndrome of these traits in the blue tit.

Tiivistelmä

Perinteisen käyttäytymisekologisen näkökulman mukaan käyttäytyäkseen optimaalisella tavalla ja parantaakseen kelpoisuuttaan yksilöiden oletetaan reagoivan elin ympäristönsä vaihteluihin joustavasti. Uusi tutkimusala, eläinten persoonallisuustutkimus, tarkastelee asia eri tavalla. Persoonallisuustutkimus tarkastelee mekanismeja ja evolutiivisia prosesseja, joiden seurauksena tutkituissa ominaisuuksissa esiintyy ajasta ja tilanteesta riippuen yksilöllistä vaihtelua. Ekologiaan ja evoluutiobiologiaan juurtuneen eläinten persoonallisuustutkimuksen perusta on psykologiantutkimusperinteessä. Eläinten persoonallisuustutkimus on tuottanut nopeasti uusia oivalluksia ja tutkimusala laajenee jatkuvasti.

Väitöskirjan ensimmäisessä osassa tutkin säätekijöiden vaikutusta sinitiaisen (Cyanistes caeruleus) hautomiskäyttäytymisen alun myöhästymiseen. Hautominen myöhästyi jos pesye oli suuri ja jos muninnan aikana oli viileää. Hyväkuntoiset naaraat pystyivät aloittamaan haudonnan viiveettä. Kun poikasia siirrettiin pesyeestä toiseen, haudonnan viivästymisen havaittiin aiheuttavan poikasten kasvun ja kehityksen huononemista. Tutkimuksen tulokset osoittavat, että muninnan aikaisilla ympäristöolosuhteilla voi olla pesäpoikasajan yli ulottuvia vaikutuksia.

Väitöskirjan muissa osatöissä (II – V) tutkin käyttäytymisen vaihtelua yksilöiden välillä. Näiden neljän työn keskiössä ovat sinitiaisen persoonallisuuspiirteet. Ensimmäisessä osatyössä esittelen häkkikokeen, jota voidaan käyttää monien persoonallisuuspiirteiden nopeaan ja häiriötä tuottamattomaan tutkimiseen niin kesällä kuin talvella. Koe sopii pesivien aikuisten ja pesäpoikasten tutkimiseen. Häkkikokeessa havaittavat ominaisuudet ovat yksilöllisesti säilyviä eli toistuvia, eli niitä voidaan pitää sinitiaisen persoonallisuuspiirteinä. Kuten talitiaisellakin (Parus major), eräs tutkituista ominaisuuksista on yhteydessä dopamiinireseptorigeenin (DRD4) kolmannessa eksonissa esiintyvään yhden emäksen polymoprfismiin (SNP). Tuloksen perusteella voidaan olettaa, että persoonallisuuspiirre on perinnöllinen ja että kyseinen genomin alue on yhteydessä eläinten persoonallisuuteen.

Sovellan rektionormin käsitettä tutkiessani osittain samojen yksilöiden

käyttäytymistä kesällä ja talvella. Tulosten perustella voi osoittaa, että tutkimusajankohta vaikuttaa tuloksiin, ja että käyttäytymispiirteestä riippuen havainnot voivat olla toistettavissa tai ne voivat olla toistumatta.

Siirtämällä poikasia pesästä toiseen ja käyttämällä kvantitatiivisen genetiikan menetelmiä arvioin käyttäytymispiirteiden periytyvyyttä. Osoitan, että käyttäytymispiirteet muodostavat ns. käyttäytymissyndrooman, joka on sekä fenotyyppinen että geneettinen ominaisuus. Siirrettyjen poikasten kehityksen perusteella voi osoittaa, että poikasten kasvuympäristöllä on suuri vaikutus pesäpoikasten persoonallisuuteen. Poikasen kasvuympäristö vaikuttaa siten sekä poikasen fyysiseen kehitykseen että sen persoonallisuuteen.

Viimeisessä osatyössä tutkin, vaihtelevatko sinitiaisen kolme persoonallisuuspiirrettä ja kaksi immunologista ominaisuutta samansuuntaisesti. Tulokset eivät kuitenkaan tue ajatusta, että sinitiaisella olisi persoonallisuuspiirteistä ja immunologisista ominaisuuksista koostuva käytäytymissyndrooma.
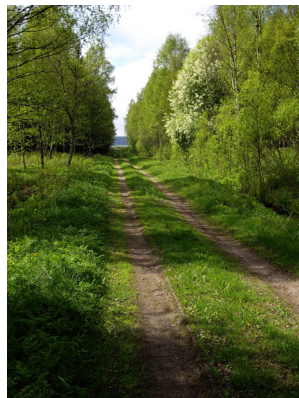
Thanks to Hannu Pietiainen for the translation of the abstract into Finnish

Summary

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Summary

1   Introduction

1.1   Variation in environment and behaviour

Individuals encounter varying circumstances throughout their lifetime. For instance, variation in their direct living environment (e.g. habitat changes, variation in food availability) or variation in weather conditions (e.g. annual weather cycles and extreme weather events). Traditional behavioural ecological theory assumes that individuals are highly plastic and an individuals' behaviour should match the requirements of its environment and be reversible (Sih et al 2004a, Bell 2007). Natural selection is assumed to act on variation in phenotypes, such that those which are best adapted to their encountered environment will have highest fitness (e.g. reproductive output). Thus, when environmental conditions are unpredictable or rapidly changing, one would expect individuals to be able to rapidly adjust (be plastic) to optimize their fitness (Roff 2002, Sih 2004a). The possibility of animals to adapt their behavioural phenotype (plasticity) to deal (adaptively) with changing environmental conditions during their lifetime has been the focus of much research (e.g. Schlichting & Pigliucci 1998, Piersma & Drent 2003, Charmantier et al. 2008).

One such plastic response that has been the focus of many studies recently is the adaptation of individuals to climate change (Brommer et al. 2005, Reed et al. 2006, Gienapp et al. 2008), and in particular the annual start of the breeding season in wild birds (Charmantier et al. 2008, Visser et al. 2004, Visser 2008). For instance, in some small passerines, in which timing of their breeding onset partly depends on a peak in food availability several weeks after laying the first

egg. In this case timing is of key importance to be able to optimally feed the nestlings and thus achieve a higher reproductive output. Over the past three decades, climate change has induced a global increase in the mean annual temperature. In particular higher temperatures in spring have advanced the phenology of reproduction (Walther et al. 2002). Individuals are able to respond to these changes by adjusting their timing of breeding (Charmantier et al. 2008). However, evidence is accumulating that the change in phenology of endotherms, such as birds, may not match the change observed in their ectothermic prey, leading to a mismatch with a possibly severe decrease in avian reproductive output (Visser et al 1998, Visser 2008).

In northern Europe, temperatures have mainly increased in winter and early spring, resulting in so called asymmetric climate change (IPCC 2007). Birds have responded to this asymmetric climate change by starting to breed earlier. However, advancing the onset of breeding might confront individuals with adverse weather conditions during the early phase of the breeding season. In chapter **I** I study the response to such adverse weather situations during the early breeding stages of the birds, and show that individuals have certain flexibility in dealing with these circumstances, by adjusting the hatching date of the eggs. However, this flexibility may have negative consequences later on, both for the incubating parent (female) and its offspring.

1.2   Individual based variation in behaviour

The interest of traditional behavioural ecological studies was on the population mean of a focal behaviour, and individual variation in the expression of behaviour was merely regarded as noise (Wilson 1998). More recently the focus of animal

behavioural studies has shifted from variation in behaviour at population level to variation in behaviour at the individual level (Groothuis & Carere 2005). In addition, the concept of personality and its methods to study these were adopted from human psychology and implemented into ecological studies on non-human animals. There are multiple definitions of animal personality found in the literature (Gosling 1999, Koolhaas et al. 1999, Sih et al. 2004a, Réale et al. 2007, Dingemanse et al. 2010). In animal behaviour, the concept of personality broadly refers to individual consistency in behaviour over time, across situations or contexts, within which individuals can differ along a behavioural continuum, for instance boldness-shyness (Wilson et al. 1994, Gosling and John 1999, Sih et al. 2004a,b, Dingemanse & Reale 2005, Groothuis & Carere 2005, Réale et al. 2007). In addition there are multiple analogues to personality used in the literature; personality can be referred to as temperament, coping styles/strategies or behavioural syndromes. Each of these can be traced back to the focal point of interest in variation in behaviour between individuals. Personality and temperament link to the classification of behaviours used in studies on human personalities (Wilson 1998). Coping styles and strategies have mainly been used in the context of stress physiology in animals and typically describe an individual's ability and strategy to deal with a stimulus or challenge (Koolhaas et al. 1999). Behavioural syndromes refer to a package (suite) of correlated behaviours, which are consistent over time (Sih et al. 2004a). One of the main findings in the field of personality is that there is a large variation in individual behaviour. Individual animals are differing consistently in their aggressiveness, activity, exploration, risk-taking, fearfulness and reactivity (Gosling and John 1999, Sih et al. 2004). This variation in individual behaviour is likely to have both ecological and evolutionary consequences (Sih et al. 2004) and thus be a focus for selection.

The main focus of animal personality research is understanding the mechanisms underlying and the evolutionary processes maintaining variation in the expression of a behavioural trait over time and across situations or contexts. A first step in animal personality research is quantifying personality traits, which is testing that a focal trait is indeed an intrinsic property of an individual. Often personality traits are quantified in an experimental setup in captivity, in which the individuals are subject to a stimulus or varying conditions. For instance, individuals were tested in a novel environment room to test their explorative behaviour (Verbeek et al. 1994, Dingemanse et al. 2002) or in a cage with a novel object to test differences in neophobia (Verbeek et al. 1994, Nilsson et al. 2010, this thesis chapter **II** and **IV**). Also studies have been done where a focal behaviour was quantified under natural circumstances in an individuals' natural environment (e.g. Garamszegi et al. 2008). Measuring behavioural traits under laboratory or natural circumstances each have their advantages and disadvantages which will be discussed further on (paragraph 2.7.1). Nowadays personality traits have been quantified in numerous species of vertebrates and invertebrates in the animal kingdom; amphibians (e.g. Sih et al. 2003, Koprivnikar et al. 2012), birds (e.g. Verbeek et al. 1994, Quinn and Creswell 2005, Herborn et al. 2010), fish (e.g. Bell 2005, Brown et al. 2005), invertebrates (e.g. Riechert and Hedrick 1993, Johnson and Sih 2007, Briffa et al. 2008) mammals (e.g. Réale et al. 2000, Martin & Réale 2008) and reptiles (e.g. Cote and Clobert 2007, Carter et al. 2010). Examples of quantified personality traits are: activity (e.g. Chappell et al. 2007), aggression (e.g. Natarajan et al. 2009), boldness - shyness (e.g. Coleman & Wilson 1998) and exploration - avoidance

(e.g. Dingemanse & de Goede 2004). And several studies have shown correlations between the above described behavioural traits. In birds for example it was found that individual great tits that are fast in exploring novel environments are also aggressive to conspecifics, bold to novel objects, risk-taking and stay relatively calm in a stressful situation (Dingemanse et al. 2002, Carere & van Oers 2004, Carere et al. 2005, van Oers et al. 2004, van Oers et al. 2005a).

1.3 Consistent variation in behaviour between individuals

Consistency in behavioural traits is typically quantified by the repeatability of a trait. In a review by Bell et al (2009) the average repeatability value for a behavioural trait was 37%, which was calculated for a large selection of behavioural traits. This indicates that a considerable part of the variance displayed in a behavioural trait is a result of environmental (non-genetic, residual) factors (e.g. Bell and Sih 2007). Repeatability reflects the amount of variation between individuals in a trait relative to the total phenotypic variation (i.e. the sum of between individual and within individual variation (Lessells & Boag 1987). Repeatability is also an indication of the upper limit of heritability of a trait, because it includes variation from both genetic and environmental sources, whereas heritability includes only between individual genetic differences (Boake 1989, Falconer & Mackay 1996). To be able to estimate heritability values for traits measured in natural populations one needs to be able to partition the different variance components of the focal trait. By using for instance a reciprocal cross-foster design (such as used in chapter **IV**) in a population with a known pedigree, one is able to partition the phenotypic variance of a focal trait into additive genetic, nest-of-origin, nest-of-rearing and residual variance components using an animal model. This type of analysis allows comparing the genetic (heritable) versus the environmental sources of variance in a (behavioural) trait.

There are multiple hypotheses (genetic and non-genetic) as to why individual consistency can be maintained in nature (e.g. Dall et al. 2004, Sih et al. 2004a, Wolf et al. 2008). One way how consistent differences among individuals can be maintained is because of genetic differences. For instance research on human behaviour has revealed several candidate genes underlying human behavioural traits (e.g. dopamine receptor D4 gene (DRD4) and the serotonin transporter protein (SERT); reviewed in Savitz & Ramesar 2004). More recently polymorphisms in the DRD4 gene have been associated with novelty seeking in mammals bred in captivity (horses, Equus caballus: Momozawa et al. 2005; monkeys, Cercopithecus aethiops: Bailey et al. 2007; dogs, Canis familiaris: Hejjas et al. 2007) and in birds in selection lines for explorative behaviour (fast vs. slow) of great tits, Parus major (Fidler et al. 2007). In this thesis (chapter **II**) we find that a single nucleotide polymorphism on the dopamine receptor gene (DRD4) is associated with 'time to escape' from a bird cage. Possibly this gene can affect multiple traits, which could lead to genetic correlations of these traits (van Oers et al. 2005b). Personality traits may be correlated to each other or to other traits such as physiological ones in an individual. When trait correlations are found on the genetic level, evolution of the correlated traits can be restrained; selection on one of the correlated traits will affect selection of the other and independent evolution of the traits is hampered (Lynch & Walsh 1998, Sih et al. 2004 a,b).

1.4 Aims of the thesis

The blue tit (Cyanistes caeruleus) is a species which is ideal to study both life history traits as well as personality traits. The birds are readily using nest boxes to breed in, which make studying them relatively easy. In addition this species has relatively large clutch sizes (i.e. many nestlings) and is robust to handling. Among bird ringers this species is infamous for its 'ferociousness' when being trapped in mist-nets and when being handled. Often birds struggle and bite/peck during handling with bleeding hands as a result. Not only when birds are handled, but also in their defence at the nest do the birds show aggressive behaviour which is quite remarkable considering its size. All these factors together stimulated me together with Jon Brommer, to study this birds' behaviour in a both in a life history and a personality context. The study area was established in 2003 by Jon Brommer. All data collected and experiments done in this thesis are from a blue tit population living in the study area.

In chapter **I** I focussed on underlying factors and possible consequences of hatching delay. Hatching delay here is a deviation from the 'general' egg laying and incubation pattern (i.e. continuously laying an egg per day after the start of egg-laying and incubating 13 days). Especially under the recent advancement of spring arrival (i.e. higher temperatures earlier in spring) and advanced spring phenology, individuals attempt to start breeding earlier. However, the advancement of spring is not without the occasional set back in temperature increase. Sudden cold spells, lasting for several days, may put individuals that have already started their breeding activity in jeopardy; cold might affect hatchability of the eggs directly, energetics during egg laying or incubation may exceed energy resources in readily available food resources for the

incubating female or future food availability (for offspring) may be low due to delayed phenology. Female blue tits have to some extent control over the timing of hatching of their eggs in response to environmental variables such as climate and food availability. I investigated the association between hatching delay (i.e. number of days hatching was delayed), clutch hatchability and female body condition. By using a reciprocal cross-fostering protocol, on a large number of broods irrespective of their experienced hatching delay, I addressed possible downstream effects of hatching delay on developmental parameters in offspring.

In chapters **II-V** the focus was on personality traits in blue tits. First an experimental setup was designed (using a bird cage) in which adult blue tits could be tested. The main goals of the setup were: 1) to be able to measure behavioural traits that were repeatable. 2) to be able to apply the setup both in winter and in the breeding season (in varying outdoor locations). 3) to have a setup that allows rapid testing such that multiple individuals could be tested in a relatively short period of time (in winter day length is short and it is not desirable to have individuals for a long time in captivity, in the breeding season individuals need to return to their nestlings). This setup is extensively described and discussed in chapter **II**. In addition to the behavioural traits measured in the cage setup, 'simple' behavioural measures were taken during handling (measuring) of the birds, on both adult and nestlings just prior to fledging. The behavioural measures were used to test several aspects of behaviour in a personality context in the blue tit, which is specified briefly in the aims per chapter below.

For all the behavioural traits measured in adults repeatability was calculated and association between the focal behavioural trait and variables such as sex

and age were tested. In chapter **II** the associations between behavioural traits measured in the cage in the winter season and two single nucleotide polymorphisms on the dopamine receptor gene (DRD4) were tested. In chapter **III** I use data on the behavioural traits measured in the cage over two seasonal contexts from 3 consecutive breeding and winter seasons. Repeatability within and across seasons for each of the traits is calculated using a reaction norm approach. In chapter **IV** heritability of three behavioural traits measured on blue tit nestlings were estimated. Using data from the reciprocal cross-foster experiment from three breeding seasons (including 2896 nestlings), in combination with pedigree information on the nestlings, we test whether the behavioural traits form a syndrome on both the phenotypic and the genetic level and discuss whether the phenotypic correlations correctly capture the genetic correlations. In chapter **V** I test whether three behavioural traits (one from the cage and two measured during handling) and two immunological traits (IgG-level and haematocrit) covary and form a syndrome which includes covarying behavioural and physiological traits, consisting with a common axis of variation in adult blue tits. I partition the covariances between the traits, obtained from a multivariate analysis, into between-individual and within-individual (i.e. residual) covariances. This way I can test whether a phenotypic correlation captures truly intrinsic covariances (at the between-individual level) or captures merely noise.

2 Methods

2.1 Study species

The blue tit is a small hole-breeding passerine, from the family Paridae. Blue tits are common throughout the whole western Palearctic and occupy habitats consisting of broadleaf forests and mixed spruce birch forests. The birds breed in small natural cavities (e.g. in trees), but also in nest boxes and cavity-like locations in human built structures (e.g. under roofs of houses). In the study population nests build in nest boxes consist of a basic moss layer lined with hair (e.g. moose, horse, dog), feathers, plant material (aromatic plants, moss' spore capsules and stems, tree bark (Juniper) and grass) and other often insulating materials (e.g. in this population; vole skin and man-made insulating material from clothing or construction sites). Nest building typically starts towards the end of April, and the laying-date of the first egg in this population typically is around the 1st of May (mean laying date (2005-2009) =31.5 (in April days), SD =4.52 (days), N = 456 (breeding pairs); figure 1). The clutch size of a blue tit typically consists of 8-14 eggs (in this population (2005-2009) mean clutch size = 10.7, SD = 1.37, N = 456; figure 1). Females incubate the eggs for about 13 days, whilst being provisioned by the male, and both
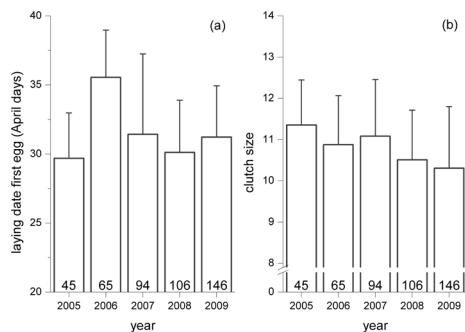

Figure 1 Mean laying date of the first egg (a) and mean clutch size (b) in the breeding seasons 2005-2009, for first layed clutches by blue tits in the Tammisaari population. The whiskers indicate the standard deviation and the numbers in the bars show the number of first broods in the particular year. Laying date is in April days; 20 = 20th of April, 35 = 5th of May etc.

parents take care of the nestlings. Nestling food mainly consists of caterpillars. Adverse weather conditions (cold (frost) and substantial periods of rainfall) during the egg laying and incubation period and early nestling phase can have negative effects on hatchability of the eggs (chapter **I**) and cause increased mortality among nestlings. Recruitment of the nestlings into this breeding population is around 6% (unpublished data). Blue tits in Finland are partial migrants, where a part of the population (mainly first year individuals with a female bias) migrates away from their natal area in autumn (end September-October). Resident birds in the population form together with migrants from elsewhere and other Paridae winter flocks, in which they move around during the winter period. From February onwards males start singing and perform territorial behaviour (personal observation).

2.2 Study area

All studies in this thesis were performed on blue tits from a population breeding in nest boxes in the years 2005–2010, near the city of Tammisaari in south western Finland (60°01′ N, 23°31′ E). The study site is about 10 km2 in size and has been established in 2003 and has gradually expanded until 2005 when approximately 400 nest boxes were available. The number of nest boxes has somewhat fluctuated in the years mainly due to destruction by forestry practices, woodpeckers, moose, pine martens or climatic events. In the event that a nest box had disappeared or was otherwise not available for birds to breed in, these were replaced, such that the total number of nest boxes in the area was always more or less the same. Nest-boxes used for this study had a 26 mm entrance-hole diameter, allowing preferentially blue and coal tit into the nest boxes (Dhondt & Eyckerman 1980). The number of blue tits breed-

ing in the nest boxes increased (figure 1) over the study period covered in this thesis and levelled off in the 3 breeding seasons thereafter (data not shown). The nest boxes are attached to trees with rope at about 1.6m height, in a forest area that consists of continuous mixed boreal forest interspaced by arable land. The main tree species composition consists of Scots pine (Pinus sylvestris), Norway spruce (Picea abies), downy and silver birch (Betula pubescens and Betula pendula). This study area is quite different in tree species composition from studies done on Paridae in for instance middle and western Europe, where one of the important species is oak (Quercus robur and Quercus petraea), especially with regards to food availability during the nestling stage in the breeding season. Oak in Finland is growing at its northern most limits and with the exception of one location (Ruissalo, Turku), oak forests do not occur on the mainland of Finland and in our study area oak is only sparsely present.

2.3 Basic protocol for the breeding season and winter

From the start of the breeding season, the last week of April, all nest boxes are checked for breeding activity at weekly intervals. To establish laying date (date of laying the first egg in a clutch) and clutch size in nest boxes occupied by blue tits, we visited each nest box every 5–8 days. The laying date was calculated by back dating from the incomplete clutch assuming a female lays one egg per day (Perrins 1979). When a clutch was completed we calculated the expected hatching date assuming that (1) one egg per day was laid, (2) incubation started after laying the penultimate egg and (3) eggs needed to be incubated for 13 days in order to hatch. Hence, expected hatching date = laying date + clutch size + 12. Near the hatching date nests were visited daily in the afternoon, starting from 1 day be-

fore the expected hatching date, to establish the exact hatching date (date of first nestling = day 0). In case a brood hatched hatchlings were weighed to obtain the average mass per nestling, a metric we used in the cross-fostering protocol to establish suitable cross-foster matches (see paragraph 2.5).

When the nestlings were 9 days old the birds were weighed and ringed for lifetime identification. At 16 days old the nestlings were subject to a full 'adult' measurement protocol described in paragraph 2.4. Blue tit nestlings in our population typically fledged at the age of 18-22 days, depending on their condition.

Only in some years a small number of pairs established a second brood. The protocol described above was not used in the second broods. Instead a simplified protocol was applied; to establish the social parents adults were trapped and nestlings of the second broods were ringed for life time identification. In this thesis all data on nestlings that has been used has solely come from first broods. After each breeding season all nest boxes were cleaned of nesting material.

Adult birds were caught in the winters 2007/2008, 2008/2009 and 2009/2010, at a feeding station which was established more or less in the centre of the study area, using mist nets. The feeding station was equipped with 3 feeders with continuous food supply (peanuts, sunflower seeds and fat balls). After catching adults were measured (paragraph 2.4). And in case the individual was a known bird (a ringed individual) it was subject to blood sampling and behavioural testing (paragraph 2.6 & 2.7).

2.4   Measurements taken on adults

Adults were caught with box traps or mist-nests during the nestling phase of the breeding season when they were feeding their nestlings. Catching was done typically after the nestlings were 6 days old. After capturing, birds were ringed (if unringed), tarsus was measured by holding the tarsometatarsus in a low angle to the tibiotarsus and folding the foot inward to be in line with the tarsometatarsus (accuracy, 0.1 mm) using a sliding calliper. Head length was measured from the tip of the beak to the back of the skull (accuracy, 0.1 mm) using a sliding calliper, wing and tail length were measured using a ruler and body mass was measured (accuracy, 0.1 g) using a 20 g Pesola spring balance. Age (2nd calendar year or older) was estimated based on plumage characteristics (Svensson 1992). Sex was determined based on presence or absence of a brooding spot in the breeding season and based on plumage colouration in the winter (Svensson 1992). The latter was retrospectively corrected (a few cases) in case a sex was wrongly assigned in the winter period and the bird was breeding in the study area.

2.5   Cross fostering of nestlings

In avian quantitative genetic studies, cross-fostering is frequently used (Merilä & Sheldon 2001). In this thesis I apply a reciprocal cross-fostering technique (chapter **I** & **IV**). In reciprocal cross-fostering a part of the nestlings from brood 'A' are being fostered by parents from brood 'B' and vice versa. Blue tits are robust to handling and disturbance and have relatively large brood sizes which make them an ideal species to perform this kind of experiment. Cross-fostering was carried out in the breeding seasons 2005 – 2009 on nestlings from first broods at the age of 2 days (day 2). Nest pairs were matched for hatch date and average mass of hatchlings, and –when possible– brood size. An equal number of nestlings were reciprocally swapped between two nests. The pair of families

between which nestlings were swapped were termed 'dyad', the brood in which a nestling hatched was termed 'nest of origin' and the one in which it was reared 'nest of rearing'. The decision on which nestlings were swapped was made random-systematically. In the first nest of a dyad, nestlings were weighed and individually marked by clipping a unique combination of their toe nails. By the toss of a coin it was decided whether the heaviest nestling stayed in its nest of origin or was moved to another nest of rearing. In dyads where broods were of contrasting sizes, the number of offspring cross-fostered was approximately half the smaller brood size of the dyad, and swapped young were matched for similar body size in the other (larger) brood of the dyad. Thus, the focus of the cross-fostering was always to swap approximately equal sized offspring, thereby minimizing any pre cross-fostering effects.

The data from the cross-fostering technique allows separating genetic (origin) from environmental (rearing) effects. In chapter **I** I tested whether hatching delay had long-term consequences for the nestlings, by using data from broods that were cross-fostered irrespective of their experienced hatching delay. In chapter **IV** I used cross-foster data on the broods together with pedigree data of the nestlings in the broods to be able to partition the phenotypic (co)variances into additive genetic, nest-of-origin, nest-of-rearing and residual components. This was done to establish the relation of genetic versus other sources of variance in offspring personality traits.

2.6 Sexing offspring, blood sampling and blood based analyses

Sex determination of the offspring was done by DNA analysis on feathers sampled when nestlings were at the age of 9 days. Two to five feathers were sampled from the back of the nestling and stored in 95% ethanol. DNA was extracted from one small feather using the protocol of Elphinstone et al. (2003). Sexing was based on a polymerase chain reaction (PCR) with sex-chromosome specific primers (P2 and P8; Griffiths et al. 1998) using GE Healthcare "ready-to-go" PCR beads following the manufacturer's instructions. The product was then visualized on an agarose gel stained with ethidium bromide.

Blood sampling of adult birds was done in each season (breeding or winter). In case a bird was caught multiple times in a season, a blood sample was taken only once (first time caught). Blood was drawn (ca. 50-100 μl) from the brachial vein by venipuncture (see figure 2). Blood samples were stored into heparinised haematocrit capillary tubes (75μl) and sealed with wax on one side and kept in a cool bag until further analysis. Within 12 hours after blood sampling the samples were centrifuged for three minutes at 10 000 r.p.m. after which the haematocrit (relative amount of red blood cells in the total blood volume) was measured with a digital sliding calliper (to near-

Figure 2 Blood sampling done on an adult blue tit, picture taken by J. Brommer.

est 0.1 mm). Subsequently blood cells and plasma were separated by cutting the capillary tube and the plasma was stored in a marked 1.5 ml storage tube at -20°C until immunoglobulin (IgG) analysis (see chapter **V** for details). The red blood cell part of haematocrit blood samples were stored in ethanol and were used for extraction of blue tit genomic DNA for SNP-analysis (see chapter **II** for details).

2.7 Behavioural measurements

2.7.1 Measurements in captivity or in the wild

Most studies on personalities have been carried out in captivity. Either animals were caught in the wild and subsequently raised/ given time to adapt to captivity before the personality assays started (e.g. Butler et al. 2006) or animals were completely reared in captivity (e.g. Verbeek et al. 1999). Few studies directly measured personality traits on wild animals (Coleman and Wilson 1998, Réale et al. 2000, Réale and Festa-Bianchet 2003, Briffa et al. 2008, Hollander et al. 2008, Herborn et al. 2010). There are several advantages and disadvantages of studying animal behaviour in either situation (captivity vs. wild). Ultimately one could combine both and test whether behaviour tested in captivity reflects behaviour in the wild (e.g. Herborn et al. 2010). Frequently studies measure behaviour in captivity and test the fitness of these individuals in the wild thereafter (e.g. Dingemanse et al. 2004).

Studying individual behaviour in the wild has some difficulties with continuously changing parameters such as time and weather that might have confounding effects on the trait measured. Furthermore, there are several parameters which are more difficult to control when testing animals in the wild. Naturally varying conditions can have far less obvious impact on the recorded behaviours. For example,

measurement of an individual's behaviour in the wild can be affected by conspecifics in its immediate surroundings (e.g. on a feeding table in winter due to the presence of a dominance structure; Lambrechts & Dhondt 1986), by an encounter with a predator previous to the measurement or due to bad physiological condition (hunger or disease).

To help contrast individuals' behaviours, one can measure behaviour traits in an artificial, standard environment, by taking the individuals temporarily out of their natural environment. This allows the researcher to control many testing conditions (Campbell et al. 2009). For example by keeping the animals captive for a longer period and feeding them ad libitum, one can control the possible effect of hunger on the measured behaviour. However, it requires that the animals need to be kept in captivity for a significant period of time, which is not always desired or possible in some situations (e.g. in the breeding season) or might be harmful in others (e.g. taking birds out of freezing temperatures and house them inside (warmer) before releasing them (into cold), which causes physiological stress, Newton 1998).

In this thesis I apply an approach (explained in chapter **II**) where birds are tested in a bird cage under outdoor circumstances while minimizing the time an individual is in captivity. This allowed rapid testing of individuals in situ in the field and in contexts in which testing time is limited (breeding season). From the analysis of the videos that were taken during the test, three behavioural traits were derived; activity in the cage (number of movements through the cage), neophobia related behaviour (response of the birds to a novel object: pink plastic toy pig) and time to escape from the cage (for details on these measures, see chapter **II**).

Besides the cage test I also measured behaviours of the birds whilst they were being handled. Since the protocol that was used to measure morphological traits in individuals (adults and nestlings at day 16; paragraph 2.4) was standardized (i.e. every measurement is always carried out in the same way in the same order) behavioural measures were taken during this procedure. Aggression displayed during handling (on a scale 1 - 5) and breath rate (time it took a bird to breath 30 breaths) at a fixed point in the measuring procedure were scored for each bird handled (in nestlings see chapter **IV**, adults see chapter **V**), in addition in nestlings a docility measure (number of struggles per second) was done prior to the start of the morphological measurements (see chapter **IV**)

2.8   Analyses

One of the key points in a measurement of a behavioural trait is that there is variation between individuals in the response measured in a given context. Lack of variation can have multiple reasons. There can be a too small sample size to obtain sufficient differences between the individuals. Or individuals might respond all in a similar way, for example due to a lack of precision of the measurement. There may also be situations where variation cannot be detected because it is not present in the population that is studied. For instance when selection regimes have eroded genetic and phenotypic variation in a focal trait in a certain environment, variation among individuals in the particular trait may be absent.

2.8.1   Repeatability

Consistent individual differences in animal behaviour have been quantified in many studies (for an example of studies quantifying repeatability of animal behavioural traits see table 1 in Bell et al.

2009), by using repeated measurements on the same individuals. Repeatability R is one of the cornerstones of animal behaviour and is defined as the variance that occurs between individuals VI over the total phenotypic variance VP (VP = VI + VR, where VR is the residual or within-individual variance and R = VI / VP; Falconer and Mackay 1996, Hayes and Jenkins 1997, Lynch and Walsh 1998). Repeatability is quantified by taking repeated measures of a (behavioural) trait on a set of individuals at different points in time in order to separate VI from VR. Low repeatability can be found when for instance all individuals respond more or less similarly to the response measured and this lack of (behavioural) variation then results in a low repeatability. Low repeatability can also be the result of high within individual variation relative to the between individual variation. Typically repeatability values of personality traits range from 0.20 to 0.50 (average repeatability of behavioural traits from a set of studies is 0.37, reviewed in Bell et al. 2009). In all chapters in the thesis dealing with quantification of behavioural traits (chapters **II-V**) first a repeatability value has been calculated to see whether the focal trait is an intrinsic property of an individual and thus the focal trait would qualify as a personality trait. Repeatability of the behavioural traits was calculated using linear mixed-effects models (LMM) with the trait measure as the dependent variable, the population intercept as fixed effect and bird ID as a random effect. Following the recommendations of Nakagawa & Schielzeth (2010), information on individuals with only one measure was retained in the model. Repeatability values calculated in this thesis are so called 'raw phenotypic repeatability values', meaning that in the calculation of repeatability no other fixed effects (that control for possible effects of these on the behaviour displayed) than the population mean are included in the LMM. The variance com-

Table 1. An indication of the raw phenotypic repeatability of the five behavioural traits measured on adult blue tits in this thesis and their statistics such as they were calculated in their context of the thesis chapter. The last two columns display the between season correlation in the traits and its p-value In bold are the repeatability values which were significantly greater than zero and their LRT statistics and the the correlations significantly different from 0. The values are obtained from chapter III & V.

Behavioural trait	R	χ2	P	across-season correlation	P (corr.)
10 based log Escape				-0.013	0.92
- all data	**0.11**	**2.74**	**0.05**		
- breeding season	0.12	1.54	0.11		
- winter season	**0.32**	**9.23**	**0.001**		
Square-root Activity				**0.424**	**<0.001**
- all data	**0.25**	**19.70**	**<0.001**		
- breeding season	**0.24**	**7.99**	**0.002**		
- winter season	**0.18**	**3.05**	**0.04**		
Difference upper zone				0.021	0.86
- all data	0.07	1.62	0.10		
- breeding season	0	<0.01	0.50		
- winter season	**0.46**	**10.26**	**<0.001**		
Handling aggression				**0.50**	**0.007**
- all data	**0.40**	**65.7**	**<0.001**		
Breath rate				**0.72**	**0.003**
- all data	**0.18**	**14.0**	**<0.001**		

ponents (estimated with Restricted Maximum Likelihood) were extracted from the LMM and we calculated the raw phenotypic repeatability of the personality trait following Nakagawa and Schielzeth (2010). Statistical significance of the repeatability was tested by likelihood ratio test (LRT) of the log-likelihood of models with and without the random effect (bird identity).

2.8.2 Heritability

Finding a (behavioural) trait to be repeatable is the first evidence that variation between individuals is determined by elements intrinsic to the individual. A repeatability measure, however, does not allow a separation between genetic or non-gentic variance components of the focal trait (Réale et al. 2007). Repeatability and individual consistency may originate from several (non-genetic) sources such as: maternal effects, common envi-

ronmental effects, learning and environmental effects specific to each individual (Falconer and Mackay 1996). Only when the phenotypic variation of the focal trait is heritable can evolution act on this (Endler 1986).The heritability (h^2) indicates the proportion of total variance of the behavioural trait that is attributed to the effect of genes. This is defined as the ratio of genetic variance (VG) to the total phenotypic variance (VP), where h^2 = VG / VP (Falconer and Mackay 1996, Roff 1997, Lynch and Walsh 1998), this value represents the evolutionary potential of a focal trait. Heritability of animal personality traits has been calculated in several studies under laboratory conditions, using selection lines (e.g. Drent et al. 2003). To be able to study heritability of traits in the wild one needs to study a natural system, where selective processes are not artificial. Recent studies of heritability on behavioural traits in the wild have exploited a statistical method called 'animal model' (Réale et al. 1999, Kruuk 2004, Schaeffer 2004), which can tackle complex pedigrees. The number of studies of heritable personality traits in the wild is relatively low, often because to be able to get sufficient statistical power to analyse the pedigree, a large sample size is needed with sufficient genetic links (half and full sibs) between the individuals. In this thesis heritability estimates of personality traits were calculated in chapter **IV** using an animal model, in the other chapters dealing with personality traits, not enough related individuals were tested to be able to calculate a heritability estimate.

2.8.3 Reaction norms

Individual consistency in behaviour does not need to imply that the focal behavioural trait is invariant. For instance, over an environmental gradient the behavioural response (trait measured) of an individual might vary. Consistency in a reaction norm framework implies that

all individuals behaviourally respond in the same manner to variation in environmental context such that their ranking is maintained, for example the most active individuals in a context (environment) of 'no predators present' are also displaying the highest activity levels in the context 'predators present', although the general level of activity displayed in the latter context can be different (e.g. lower) than in the other context(s). In recent years, the concept of reaction norms has been applied to repeated measures gathered on individuals in the wild to provide a framework to describe the variation across individuals and link this variation to individual performance (e.g. Brommer et al. 2003, Brommer et al. 2005, Nussey et al. 2005, Wilson et al. 2005, Brommer et al. 2012). The key aspect when applying the reaction-norm framework to repeated measures in individuals is that there may be variation across individuals in the extent they adjust the trait under consideration in response to environmental conditions. This variation in plasticity across individuals is termed 'I x E' (individual x environment) and can be further partitioned into a genetic and a non-genetic component 'G x E' (Nussey et al. 2007). Calls for applying the reaction-norm concept to the study of personality have been made (Martin and Réale 2008, Stamps and Groothuis 2010a) and several studies on animal personality have now implemented the approach (reviewed by Dingemanse et al. 2010).

Reaction norms are modelled using random regression models, where the reaction norm terms are modelled as random effects (Nussey et al. 2007). I apply this method in this thesis in chapter **III** where the individual response in the behavioural traits measured in the cage experiment over two distinct contexts (environments) is used, i.e. measures done in the breeding season and in winter. A (linear) reaction norm consists of two terms; elevation and slope. Where ele-

vation is the individual response in the trait in the 'baseline' environment (in this thesis: breeding season), the slope of each reaction norm (line) displays the response of each individual in the focal trait to the environment (Schlichting & Pigliucci 1998, Roff 2002, Nussey et al. 2007). In case there is significant consistent variation between individuals in the trait response and individuals respond in a similar fashion in their trait response as a function of the environment (rank orders stay the same), the random regression part of the model will have a significant elevation term and a non-significant slope term. When the slope term of the random regression model is significant, this indicates that individuals differ in their change in response of the trait (plasticity) over the environment. This means that each individual responds flexibly and in an individual-specific manner to the environmental context it experiences (Dall et al. 2004, Sih et al. 2004a, Dingemanse and Réale 2005). This pattern may arise because, in one context, it is beneficial (adaptive) to behave differently from conspecifics whereas in another context there is no benefit.

2.8.4 Statistical Software

All statistics in chapter **I**, **II** & **III** were performed using statistical program R (R Development Core Team 2010), which is freely available. For each of the chapters the packages used for the specific analyses are indicated in the material and methods section and statistical literature that deals with the specific topic is referred to. In the chapters **IV** (animal model) & **V** (variance partitioning) statistical software ASReml (VSN international, U.K.) was used.

3 Main results and discussion

3.1 Response to environmental variation

A delay in hatching is generally considered beneficial (Monrós et al. 1998, Naef-Daenzer et al. 2004), when leading to an improved match between offspring food demands and the peak in food availability. However, in chapter **I** I show that there are also costs of hatching delay. I identify that temperatures during the early stages (egg laying and incubation) of the breeding phase are negatively correlated with the amount of days that hatching is delayed, and in particular low temperatures during the egg-laying phase are correlated with length of hatching delay ($r = -0.52$). Hatchability of the clutch, that is the percentage of eggs hatched from the total clutch size, is impaired when hatching is delayed long (see figure 3). From a cross-foster experiment on the broods, I conclude that delayed hatching impairs nestling growth, resulting in a lower (residual) body mass
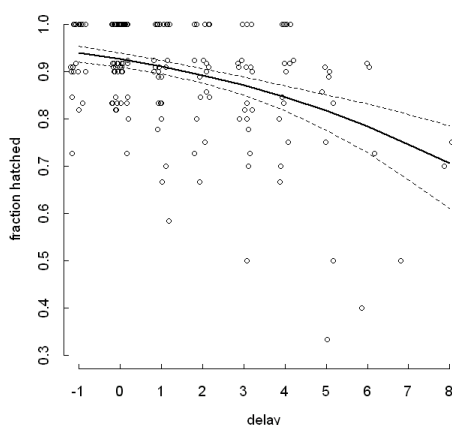
Figure 3. Fraction of hatched eggs plotted against hatching delay (dots). The solid line displays the fitted values, and the dashed lines are the 95% confidence bands for a quasi-binomial GLM model. The model describes the fraction of hatched eggs as a function of hatching delay (see Appendix S2, chapter I). The values for hatching delay are 'jittered' for graphical purposes.

at fledging and these effects are mediated maternally (through the nest of origin). This means that nestlings that have hatched from an egg that encountered hatching delay had lower body mass at fledging. Possibly stress of the female during the egg-laying phase may have caused higher deposition of corticosteroids in the eggs, which has shown to have negative effects on growth of nestlings (Hayward & Wingfield 2004; Saino et al. 2005; DuRant et al. 2010). Females that delay their hatching tend to produce smaller clutch sizes. In addition females body mass near (at hatching or within 2 days thereafter) the date of hatching of the eggs is lower with increased hatching delay of the clutch. Both results are independent of the temperatures encountered during the egg laying and incubation phase. Therefore these results do not seem to be driven by the environmental conditions but instead signal that energetic constraints act on the breeding female. These constraints may act in two non-mutually exclusive pathways. (1) A female in poor somatic condition may have insufficient energy to deal with the cold spell and has to delay the hatching of her offspring. Such females are still in poor condition at the time their offspring hatch. (2) Delaying hatching is energetically costly and causes a low body condition for a female at hatching. Finally, hatching delay did not seem to affect survival of both females and nestlings back into the breeding population. Nestling survival of individuals that have experienced delay may be offset by positive survival effects of having hatched early in the season (Verhulst and Tinbergen 1991), since hatching delay mainly occurs in those broods that were started early in the breeding season.

3.2 Variation in behaviour and repeatability

When the focus of a study is to examine consistent differences in the response

to a stimulus between individuals, one generally aims at quantifying variation of this response for each individual and tries to capture this for instance via an experimental setup. To be able to standardize the behavioural measurement as much as possible, it is inevitable that the focal individual needs to be tested in captivity, since under natural circumstances the conditions surrounding the measurements are not under control of the researcher and could therefore vary per measurement and confound measurements taken (see paragraph 2.7.1 for a discussion). The designed experimental setup in chapter **II** adapted parts of earlier successfully employed tests on a related bird (great tit; Verbeek et al. 1994) and in the same species, blue tit (Nilsson et al 2010). Three behavioural traits were successfully quantified with the experimental setup: (1) neophobia related behaviour, (2) activity (movements through the cage during a fixed time period) and (3) time to escape from the cage. Birds that were tested in the cage showed between individual variation in the expression of the three behaviours and these were consistent over time (repeatable). In addition adults and nestlings consistently differed between individuals in their expression of the behavioural traits measured during the standardized morphometric measurements protocol (handling aggression breath rate and docility (nestlings); chapter **IV** & **V**).

3.3   Repeatability of personality traits

In this thesis repeatability was calculated for all behavioural traits measured on adults (i.e. behaviours from the bird cage and those measured in the hand). Table 1 shows the values off the repeatability of the different behavioural traits. All behavioural traits are repeatable at least over time and in most cases also over context. The values of repeatability fall within the commonly found range of repeatability values of behavioural traits (0.20 – 0.50; Bell et al. 2009). Because all traits are repeatable over time the measured behavioural traits qualify as personality traits. However, the traits neophobia related behaviour and escape time, both measured in the cage, were not repeatable when measured in the breeding season context (as opposed to the winter season when they were repeatable). Repeatable traits may still be considerably plastic across contexts. In case behaviour is adjusted in an individual-specific manner to the context (Nussey et al. 2007), consistency across contexts may be low. A reaction norm concept was applied in chapter **III** to investigate the change in repeatability over the two contexts for neophobia related behaviour and escape. The reaction-norm concept implies that repeatability of a behavioural trait over time may depend on the context under which it is quantified and the correlation of a behavioural trait between different contexts may be low or absent. From this analysis it becomes clear that a lack of repeatability over the contexts is mainly because of a strong reduction in the variance among individuals in the breeding season (for neophobia related behaviour) and because of changes in the ranking of individual-specific behaviours across the seasonal contexts. Thus the reaction norms are crossing in these behaviours over the two seasonal contexts. In this chapter (**III**) evidence for both patterns of context-specific repeatability predicted by the reaction-norm concept in behaviours measured in an artificial setup on individuals from the wild is found. If the behaviours measured are under selection in a direction consistent across seasons and in case the pattern of crossing reaction norms has a genetic basis, it could present one way to maintain variation in behaviour. This is because it implies that selection would favour different individuals in different contexts.

3.4 Heritability of personality traits

A personality trait can be target of selection only if it is heritable. After repeatability, heritability is often the next step in research done on a the trait (van Oers et al. 2005b). Personality traits have, in general, a modest heritability (e.g. Réale et al. 2007, van Oers & Sinn 2011), of around 0.3 or lower. In chapter **IV** heritability of three personality traits (docility, aggression and breath rate) measured in nestling blue tits was calculated using quantitative genetic methods on data collected from a reciprocal cross-foster design. The three personality traits had a modest but clearly significant heritability. The additive genetic variance component contributed 16 – 28% of the phenotypic variance of the personality traits. For the traits handling aggression and docility the 'nest-of-origin' variance component, which can be interpreted as the maximal contribution females can have on phenotypic variance (via maternal effects), explained only a small portion (< 5%) of the phenotypic variance. Nevertheless, 'nest of origin' variance contributed 7% of the phenotypic variance in breathing rate, illustrating that non-additive genetic and/or other sources of early-environmental variance can make a clear (i.e. >5%) contribution to the phenotypic variance in a nestling personality trait. Environmental factors, captured in the 'nest of rearing' part of the variance, may have a considerable impact on a nestling's personality which was shown in the 'nest-of-rearing' variance. This variance part explains approximately 16% of phenotypic variance in breathing rates (compared to $h2 = 17\%$), 10% of docility ($h2 = 16\%$), and 14% of the variance in handling aggression ($h2 = 28\%$). Possibly parents of offspring can, through rearing, affect the personality of the offspring. Similar rearing effects have been found in morphological traits such as nestling tarsus length and body mass (Kruuk et al. 2001, Merilä et al. 2001). Results here contribute to the knowledge that conditions during the early parts of a lifetime in an individual are very important to an individual's development, both physical and behavioural.

In evolutionary biology one of the main interests is identifying genes that underlie variation in traits displayed in natural populations. For personality traits a promising candidate gene has been found in humans; the dopamine receptor gene (DRD4; Savitz & Ramesar 2004). Recently, polymorphisms of this gene have been associated with novelty seeking and exploration in set of domesticated or captively bred animal species (Fidler et al. 2007) and in one population (out of four) of wild living great tits (Korsten et al. 2010). In chapter **II** a genetic basis underlying the repeatable cage behaviours measured in winter was tested, by investigating the association of the personality traits with polymorphisms in the DRD4 gene. In particular the focus was on polymorphisms on exon 3 of the DRD4 gene, which was the same location in which the polymorphisms of this gene associated with exploration (Fidler et al. 2007, Korsten et al. 2010) in the closely related great tit were found. One of the two genotyped polymorphisms (DRD4-SNP905) was found to be associated with escape behaviour from the cage (see figure 4). This association suggests a possible functional link between the DRD4 gene polymorphism and behavioural phenotype. The observed association does not allow for a direct, causal relationship between the DRD4 and escape behaviour, because the SNP905 polymorphism is synonymous (not leading to a difference in protein structure). However, the significant association of escape behaviour with DRD4- SNP905 suggests that the trait has a genetic basis in this species, independent from whether DRD4 is causally involved with escape behaviour or not.
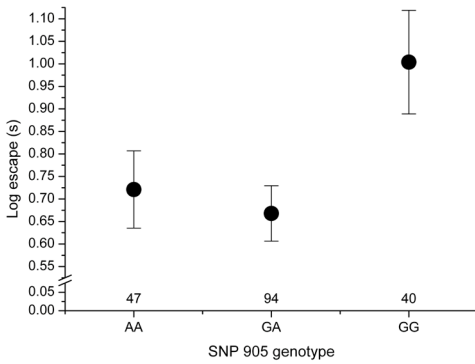
Figure 4 Escape times (log transformed) of birds genotyped for the DRD4-SNP905. The means of escape time values ± SEM are shown; sample sizes of the genotype groups are indicated above the X-axis. Data include a group of unrelated birds measured in the cage for the first time (from chapter II).

3.5   *Personality trait correlations*

From an evolutionary perspective, trait heritability and (genetic) correlations between traits are important. Under varying selection regimes these may explain the persistence of behavioural traits and suites of correlated traits over (evolutionary) time (Sih et al. 2004a). By specifically studying the co-variation of behavioural, physiological and life-history traits we can investigate how various aspects of an individual's life can coevolve, for example through correlational selection (Sinervo & Svensson 2002). Studies on behavioural trait correlations have revealed multiple behavioural traits that are co-varying over time and context, also referred to as behavioural syndromes (Sih et al.2004a). In general, a genetic correlation can arise because of pleiotropic effects (same genes affect more than one behaviour) or because genes are in linkage disequilibrium. However, we know little about the genetics of personality traits (e.g. van Oers et al. 2005b, Réale et al. 2007, Dochtermann & Roff 2010), and especially genetic correlations between personality traits are poorly studied (Dochtermann 2011, van Oers & Sinn 2011). When studying genetic correlations of traits in nat-

ural populations one needs pedigree information on the individuals tested and a large sample size (Wilson et al. 2010). Because these requirements are difficult to meet, research on animal personality is currently primarily based on phenotypic measures of personality traits and phenotypic correlations between these (Dochtermann & Roff 2010, van Oers & Sinn 2011). While phenotypic-level analyses allow for valuable insights, they have restricted relevance for answering evolutionary questions. However, phenotypic correlations still harbour a genetic component and it has been suggested that phenotypic correlation values may be a suitable surrogate for genetic ones (Cheverud 1988, Roff 1996, 1997). This is also termed as the phenotypic gambit (Graven 1984). In two reviews by Roff (1996) and Kruuk et al. (2008) the phenotypic gambit was tested mainly on morphological and life history traits, and indeed it was found that phenotypic and genetic correlations were highly correlated. Dochtermann (2011) tested the phenotypic gambit for behavioural traits and concluded that the sign of phenotypic and genetic correlations agreed, but the magnitude of the genetic correlation between behavioural traits was not necessarily captured well by its phenotypic correlation, as was also found in the earlier reviews. In chapter **IV** the phenotypic gambit was tested for three behavioural traits in blue tit nestlings. For the syndrome as a whole (the three behavioural traits together) the phenotypic correlation matrix approximates the genetic one sufficiently. Strikingly the correlations for all variance components are in the same direction and of roughly the same strength. However, one of the pairwise correlations tested (between handling-aggression and docility) shows that the strong genetic correlation was not captured sufficiently by the phenotypic correlation. The findings in chapter **IV** mirror the conclusion based on meta-analysis by Dochtermann (2011).

To date many studies have investigated correlations among behavioural traits in animals and correlations of behavioural traits with other traits such as physiological traits (for example see table 4 in Réale et al. 2007). One of the findings is that differences in hormone levels are likely to be linked to the behavioural differences displayed (Sih et al. 2004b, Réale et al. 2007). Thus far few studies (e.g. Sild et al. 2011) have investigated the relation between behavioural traits and immunological traits in wild animals. In chapter **V** correlations between three behavioural (activity in the cage and breath rate and aggression measured in the hand) and two immunological measures (from blood; IgG-level and haematocrit) taken in wild adult blue tits were tested. All traits considered were repeatable within and across seasonal contexts. The covariances (derived from a multivariate analysis) between the five variables were partitioned into between-individual and within-individual (i.e. residual) covariances, to allow testing of the correlations at these levels. There was little evidence for the personality traits to be correlated. In addition there was no compelling evidence that the personality traits covaried with the two physiological traits. However, there was an exception, more active individuals in the cage had a slower breath rate (marginally significant) and breath rate was furthermore significantly negatively correlated with haematocrit. These correlations were on the between individual level, and thus capture covariance due to some intrinsic differences between the individuals. In general, these differences consist of additive genetic differences and of so called permanent environmental differences between individuals (Lynch & Walsh 1998). In case the between-individual correlations have a heritable basis, they could play a role in the evolution of these traits in this species.

4   General conclusions and future directions

The results from chapter **I** highlight the importance of 'decisions' made by females with respect to the start of the breeding season and how these may affect fitness. After the start of egg laying females may respond to encountered environmental variability (adverse climatic events), by adjusting the timing of hatching. However, this may have effects that last throughout the breeding season. Delayed hatching reduces hatchability of the clutch, can affect clutch size and reduces female body condition (possibly through energetic trade-offs) close to hatching as well as lowers offspring body mass at fledging. Results from this chapter emphasize the importance of the energetic trade-offs breeding blue tit females need to make during the breeding phase, to manage reproductive costs.

Results found in chapter **I** & **IV** indicate that conditions during the early parts of a lifetime of an individual are important. The response to environmental variation during egg laying and incubation of the female can negatively affect body mass of her fledglings (chapter **I**), resulting in lower nestling weight prior to fledging. And although in this chapter I do not find evidence that the lower body weight near fledging affects survival of these nestlings, from previous research it is known that body weight at fledging does have an important role in survival in nestlings (e.g. Naef-Daenzer et al. 2001). In chapter **IV** I find that environmental factors, encountered by nestlings during the rearing period, may have a considerable impact on a nestling's personality. Thus the development of both physical and behavioural traits in individual seems to find its origin already in the earliest phases of life.

In chapter **III** I found that repeatability of a behavioural trait over time may

depend on the context under which it is quantified. Lack of repeatability over the contexts in this chapter was mainly because of a strong reduction in the variance among individuals in the breeding season (for neophobia related behaviour) and because of changes in the ranking of individual-specific behaviours across the seasonal contexts in the escape behaviour. These findings suggest one needs to carefully consider the context under which individuals are assayed and that a recorded behaviour may or may not be repeatable in another context.

Due to a lack of sufficient pedigree information on all the adults measured, heritability of all personality traits in adults in this thesis could not be estimated. However, I did find an association between the escape behaviour measured in the cage (chapter **II**) and a polymorphism in the 3rd exon of the dopamine receptor (D4) gene (DRD4). Similar to what has been found in the great tit research on the association of exploratory behaviour and the DRD4, the polymorphism is synonymous and therefore a causal link between the polymorphism and the escape behaviour cannot be established. Still this association indicates that there is a genetic basis underlying this adult personality trait. In addition, these results underline the possible involvement of this genomic region in behavioural traits in (wild) animals (see also Momozawa et al. 2005, Bailey et al. 2007, Hejjas et al.2007, Fidler et al. 2007 and Korsten et al. 2010).

I show in chapters **II-V** that with relatively simple methodological design it is possible to quantify behavioural traits in the blue tit, both in adults and in nestlings. The quantified behaviours were repeatable and thus qualify as personality traits. However, for personality traits measured under artificial conditions (the cage behaviours), we do not, at present, understand the ecological relevance of

the observed behaviours, and in particular of escape time. As recommended by Réale et al. (2007), more work is needed to put the aspects of personality I quantified here into an ecological context. Although chapter **V** already sheds some light on for instance the trait 'activity in the cage'. This trait was correlated (at between individual level) with breath rate, possibly indicating that stress (hormones) is linked to the behaviour displayed in the cage. Other tests should be performed to test whether the behaviours measured in the cage correlate with behaviours measured in the wild (e.g. Hollander et al. 2008; Herborn et al. 2010). And ultimately linking the personality traits to fitness components of the individuals, as has previously been done in great tits (reviewed in Groothuis & Carere 2005).

In chapter **IV** the field-based assays of nestling personality traits capture a genetic signal, both in terms of estimating heritability and in terms of estimating statistically significant genetic correlations between personality traits. Working with offspring facilitates obtaining the large sample sizes required for quantitative genetic estimates to have reasonably narrow confidence intervals. It also facilitates the implementation of an experimental design in a wild population, such as reciprocal cross-fostering, which further aids in estimation of quantitative genetic parameters. Knowledge of correlated personality in offspring opens up the possibility to study ontogenetic changes in behavioural syndromes (Stamps & Groothuis 2010b) and allows properly integrating natural selection into our understanding of how variation in animal personality is maintained in the wild.

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