# On the Genetics of Avian Personalities:

mechanism and structure of behavioural strategies in the great tit *(Parus major)*

van Oers, Cornelis Hendrikus Johannes

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# On the Genetics of Avian Personalities:

## mechanism and structure of behavioural strategies in the great tit (*Parus major*)

Over de genetica van persoonlijkheden bij vogels: mechanisme en structuur van gedragsstrategiën in de koolmees (*Parus major*)

(Met een samenvatting in het Nederlands en Duits)

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**Cornelis Hendrikus Johannes van Oers**

geboren op 26 februari 1971, te Wieringen

**Promotor:** Prof. Dr. A.J. van Noordwijk

Nederlands Instituut voor Ecologie (KNAW), Heteren

Faculteit Biologie, Universiteit Utrecht

**Co-promotores:** Dr. G. de Jong

Faculteit Biologie, Universiteit Utrecht

Dr. P.J. Drent

Nederlands Instituut voor Ecologie (KNAW), Heteren

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**Beoordelingscommissie:** Prof. Dr. D. Houle

Prof. Dr. Th. Bakker

Prof. Dr. J.M.M. van Damme

Prof. Dr. M. van Aken

Voor Björn en Torben

# CONTENTS

CHAPTER 1

# General introduction

**Kees van Oers**[1,2]

1. Department of Population Biology of Animals, Netherlands Institute of Ecology, PO Box 40, 6666 ZG, Heteren, The Netherlands
2. Evolutionary Population Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherland

## EVOLUTION

The diversity of organisms is a fascinating subject, and many volumes have been written on the huge number of animal and plant species. In *The Origin of Species*, Darwin (1859) implied that all species stem from one or several primitive ancestors. He also argued that the variation present on earth is generated by evolution through natural selection and through the separation of populations, so that each can evolve separately and become different species. Evolution is the process of change in living populations. Darwin recognised four basic premises to the theory of natural selection. The first two premises assert that because of number regulation in populations, many individuals have to die and the important point is therefore which individuals die. (i) "A struggle for existence inevitably follows from the high rate at which all organic beings tend to increase". (ii) "Hence, as more individuals are produced than can possibly survive, there must be in every case a struggle for existence… " (Chapter 3 Origin of Species). The third premise states that differences in fitness due to differences in traits exist. (iii) "…….can we doubt (remembering that many more individuals are born than can possibly survive) that individuals having any advantage, however slight, over others, would have the best chance of surviving and of procreating their kind?" The fourth premise is about heredity, and already implied in the previous quoted sentence, but stated specifically: (iv) "……..the endless number of slight variations and individual differences, …., as well as the strength of the hereditary tendency" (Chapter 4).

Endler (1986) set out the theory of natural selection in a strict and formal way, separating clearly conditions and logical consequences. Endler defines natural selection as a process in which:

*If a population has* (i) variation among individuals in some trait; (ii) a consistent relationship between trait differences and fitness differences; and (iii) a consistent relationship in trait values between parents and offspring, that is, inheritance;

*Then*: (a) the trait frequency distribution will differ between age classes; and (b) the trait frequency distribution will be predictably different from that of the parents (if the population is not at selective equilibrium).

## VARIATION, HERITABILITY AND FITNESS

A simple explanation for the existence of variation within populations is the following. Virtually every organ and function shows operational variation and this variation forms the basis for differences between individuals over a large range of traits. Essential features to induce evolutionary change in these traits are that this variation is heritable and that at least a part of this variation may affect the likelihood of an organism's survival and reproduction (i.e. lifetime reproductive success or fitness).

Darwin had no convincing mechanism for the inheritance of variation. Only with the rediscovery of Mendel's laws in 1900, a solution for the inheritance of traits was found: each organism receives a definite portion of its genes from each parent, and consequently each parent transmits only a corresponding portion to each of its offspring. At first, Mendel's laws of inheritance seemed only applicable to major or qualitative variation in traits. Quantitative variation was reconciled with Mendel's laws only later (Fisher 1918). Another crucial addition to our knowledge of the evolutionary process is that of Johannsen's (1909) introduction of the distinction between genotype and phenotype. He produced pure lines of self-fertilizing beans. By producing offspring in each of these lines for many generations, he showed that variation in size can be ascribed to either variation in genotype or variation in rearing environment: variation between the lines was constant and caused by genotypic variation, variation within the lines is caused by environmental variation. Ever since, a large debate has been going on which part of the differences between organisms is caused by differences between the genes in the fertilized eggs from which they develop (Nature) or due to the differences in the environment they are raised in (Nurture)(Box 1). The present day consensus is that such a dichotomy is misleading, as the expression of genes crucially depends upon the individual's environment (Pigliucci 2001).

## Box 1. Nature vs Nurture

" The question whether the nature or nurture, the genotype or the environment, is more important in shaping man's physique and his personality is simply fallacious and misleading. The genotype and the environment are equally important, because both are indispensable. …The question about the roles of the genotype and the environment in human development must be posed thus: To what extent are the differences observed among people conditioned by the differences of their genotypes and by the differences between the environments in which people were born, grew and were brought up? "

Th. Dobzhansky (1964)

We now know that "complex traits" (not only in humans, but in all organisms) are influenced by many factors, both genetic and environmental, and these are present simultaneously. Often these genetic and environmental factors interact. The genetic variability in how individuals respond to their environment, we call phenotypic plasticity (Roff 1997). Phenotypic plasticity is often described by a reaction norm. The reaction norm as coded for by a genotype is the systematic change in mean expression of a phenotypic character that occurs in response to a systematic change in an environmental variable (De Jong 1990).

Fitness differences between individuals caused by variation in heritable traits will lead to a change in frequency distribution of the genes or alleles responsible for this variation in the next generation and thus evolutionary change. In relation to this it has proved useful to only include differences in survival and reproduction due to reproducible differences and exclude all differences due to chance events. In technical terms one uses the expectation of fitness over individuals with the same phenotype, and hopes this represents the expectation of fitness over individuals with the same genotype (De Jong 1994).

## GENETIC VARIATION

Although cultural and/or maternal transmission of environmental effects plays an important role forming the phenotypes of next generations, evolution is primarily a genetic process. The presence of genetic variation in a trait and selection on this trait are enough to cause evolutionary change in the composition of a population (Dobzhansky *et al.* 1977; Minkoff 1984; Stearns 1992). Genetic variation comes as a result of meiosis, chromosome mutation and gene mutation, where gene mutations are changes in the chemical makeup of the genes. They occur at random and are generally deleterious. However, a few result in changes that are beneficial under its specific living conditions, which provide the individual an evolutionary advantage.

The existence and maintenance of genetic variation has been of great interest in biological research. But, given that we cannot study the whole animal in its complexity, we usually split animals up in several groups of characteristics or traits according to the nature of the traits. Through this we are able to study processes that define these traits and their relative importance for evolutionary ecology.

## BEHAVIOURAL TRAITS VS. LIFE-HISTORY TRAITS

Classically traits are split up in more or less strict categories: physiological, morphological, behavioural and life-history traits. A question one could ask is, if the difference between these categories is real or whether another partitioning should be made. Differences in life-history traits are nothing but an outcome of variation in morphological, physiological and behavioural traits and should therefore be seen as a higher level than the other categories.

Morphological and life-history traits have had most attention, since they are easily measurable and their variation is very obvious. Morphological traits comprise characteristics that influence the appearance of an individual (e.g. colour or bone size) where life-history traits are traits that play a direct part in reproduction and survival (e.g. sex ratio or clutch size, Stearns 1992).

The strength of the relation between fitness and a particular trait category is often assumed to be negatively correlated with its heritability (Merilä & Sheldon 2000) since selection is assumed to erode additive genetic variation (Houle 1992; Stirling *et al.* 2002a). Empirical studies have shown that life-history traits, which one assumes are closely related to fitness, have lower heritabilities than e.g. morphological traits (Mousseau & Roff 1987; Houle 1992; Merilä & Sheldon 2000), which seems to confirm this hypothesis. Although behavioural ecologists consider many behavioural traits to

be closely related to fitness, the link between fitness and behavioural traits is often unclear (Houle 1992). A recent study of Stirling *et al.* (2002a) showed that heritabilities of behavioural traits are not different from heritabilities of life-history traits, but smaller than heritabilities of morphological traits, suggesting that behavioural traits are as closely related to fitness as life-history traits. One major problem is that these low heritabilities could be caused by an erosion of genetic variation, by selection (elimination - selection hypothesis Houle 1992) or by a disproportional increase in residual variation (disproportional compounding hypothesis, Houle 1992; Merilä & Sheldon 2000; Stirling *et al.* 2002a). The residual variance equals the remaining variance that cannot be explained by the regression when calculating the heritability (Lynch & Walsh 1998). In most studies where estimates of the magnitude of components exist, lower heritability is not due to lower genetic variance, but to a high residual variation.

To get a better understanding of the link between heritabilities and fitness consequences and therefore the evolution of a trait, a good knowledge of the structure of its genetic variation, selection pressures and their interaction is needed. A major advantage of using behavioural traits for these kind of studies, is the possibility to measure them relatively early in life. Where often life-history traits are measurable only later during life, many differences in behavioural traits arise already soon after birth. This makes it feasible to test (i.e. measure the phenotype) of relatively large numbers of individuals and only retain the selected ones to the next breeding season.

## BEHAVIOUR AND EVOLUTION

Since the time of Charles Darwin it has been shown in various ways that the manner an animal behaves contributes to its survival and reproduction, and thus its fitness. The link between variation in behavioural traits and fitness is relatively unclear when comparing this to the link between morphological, physiological and life-history traits and fitness (Houle 1992; Stirling *et al.* 2002a). Studies on fitness and natural selection in any trait require models that incorporate explicit genetic mechanisms. Although genetic approaches have been proven to be very essential to answer questions about adaptive significance and the evolution of developmental and life-history traits, the genetic basis of behavioural traits in studies with an ecological or evolutionary context has been neglected (Boake *et al.* 2002).

Behaviour has the complexity of being both subject to selection and being a major agent of selection within the same species (Boake *et al.* 2002). The way behaviour contributes to the fitness of individuals depends on the interaction of their phenotype and the environment. The phenotype is the combination of the genetics, and ontogenetic development of the behavioural trait as a consequence of the interaction between genes and the local environment. This environment is thereby not necessarily identical to the environment where selection takes place (Figure 1). Therefore, any complete study on behaviour that tries to explain the observed variation in an evolutionary way requires knowledge of both proximate and ultimate factors, covering three main subjects. First information is needed on the genetic structure, second the ontogeny of the trait of interest must be studied and third knowledge is needed on the

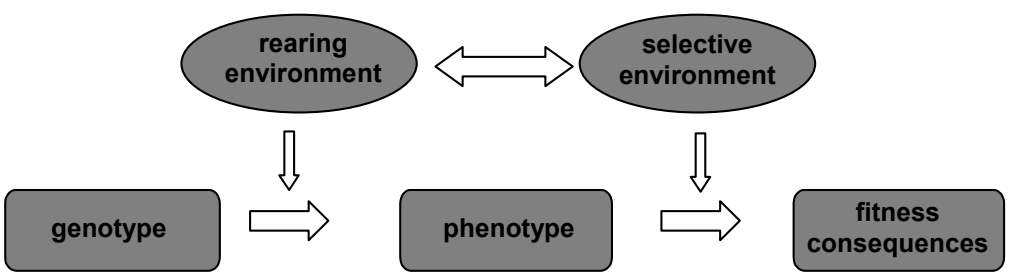


**Figure 1.** A schematic overview of the relation between genotype and fitness. The phenotype is the combination of genotype and environment, as a consequence of the interaction between genes and the rearing environment. The selective environment determines the conditions under which natural selection takes place. This scheme shows that the environment during development is not necessarily the same environment as the fitness-determining environment (after Van Noordwijk & Müller 1994).

selection pressures that drive natural selection at different stages of an individual's life (McFarland 1989).

It is increasingly possible to use genetic approaches to answer questions about the adaptive significance and evolution in natural systems. However, much is still unknown about the mechanisms of inheritance of polymorphic behavioural traits, and even less about how they are shaped by evolutionary processes.

When we want to study inherent differences of the genotype-environment relation in behaviour, we encounter a major problem. Since each individual develops in its unique way it can therefore be measured only once. For each individual the route from genotype to phenotype is unique. One crucial condition when carrying out science is that the phenomena we want to measure have to be repeatable. One way out of this impasse is to use known relationships between relatives. Fundamentally all methods are based on the same logic; we know that relatives both share copies of the same genes, and are different from each other because sibs may get different sets of alleles from the same parents. We thereby change the experimental approach: we do not start from the genotype and end with the phenotypic variation, but we look at the relative importance of genes in relation to the observed phenotypic variation. A prerequisite of studying the genetic influence on phenotypic variation is to exclude other causes of similarity between relatives, such as common environment effects, or culturally or maternally transferred environmental influences. Quantitative genetics (Box 2) provides the concept and the methods for this approach (Lynch & Walsh 1998).

Behavioural differences can be present between species, between populations of a single species and between individuals of the same population. The increase of multiple studies on single species over several populations gave rise to an increasing interest in variation within species, after an initial phase where the interest in behavioural ecology was mainly focussed on the existence of interspecific behavioural variation.

## BOX 2. Quantitative genetics

The inheritance of individual differences in behaviour is of fundamental significance in the evolutionary study of behaviour. With the recognition that multiple genes and multiple environmental factors influence expression of most traits, quantitative genetics is the ideal concept for the analysis of phenotypic variation and evolution (Lynch & Walsh 1998). Quantitative genetics is concerned with the inheritance of individual differences that are gradual rather than categorical (Falconer & Mackay 1996). Most studies in behaviour, just as in other disciplines in biology can be classified in a "how" and a "why" dichotomy. In biology, most "how" questions are concerned with proximate causes of observations, "why" questions deal with ultimate causes. Quantitative genetics provides us with a technique to answer "why" questions with a "how" approach. Automatically it forces us to change back and forth from a reductionistic view, associated with the "how" questions and a more organismal view. Quantitative genetic methods use the resemblance among relatives due to shared genotypes to study the structure of inheritance (Falconer & Mackay 1996; Lynch & Walsh 1998). By estimating which proportion of phenotypic variances is contributed by genetic effects, heritabilities and genetic correlations can be estimated. These are tools for investigating past and current selection pressures and allow us to predict and reconstruct evolution, and characterise constraints of evolution. Quantitative genetic techniques are the basis of this thesis.

## INTRASPECIFIC VARIATION IN BEHAVIOUR

### Individual differences in behavioural traits within populations

To explain differences between closely related species using natural selection was controversial during the 1940s but had become commonplace by the 1960s. Similarly, the adaptive nature of differences between populations was initially controversial (Wilson 1998). Kluijver (1951) was one of the first to state that individual differences in behaviour had an important link to the population structure and dynamics. More studies that looked at the link between evolution and individual differences in behaviour arose in the late 1970s and early 1980s. At that time two categories of behavioural studies could be distinguished: on one hand studies that looked at between population differences in life-history and thus in selection pressures (e.g. Krebs & Perrins 1978; Lomnicki 1978) and on the other hand studies that looked at variation in behaviour within populations (for a review see Lott 1984). The first category mainly focussed on local behavioural adaptation, the latter mainly on evolutionary stable strategies in social systems such as mating systems (Caro & Bateson 1986) and foraging tactics (Barnard & Sibley 1981).

Early studies in both categories considered the existence of alternative phenotypes as being environmentally defined characteristics with constrained flexibility (referred to as tactics Gross 1996). This constraint was expected to be caused by the psychological complexity to shift from one strategy to another, by environmental differences in space and time between populations (multiple-niche polymorphisms; Robinson & Wilson 1994) or by interactions with the individual's life history (particularly during ontogeny), rather than by genetic differences (referred to as strategies; Gross 1996).

Gradually the idea that measured individual differences were only the raw material natural selection acted on, changed to the idea of adaptive individual differences: behavioural traits are not characterised only by an adaptive mean flanked by non-adaptive variation, but the variation in itself can also be maintained by natural selection (Barnard & Sibley 1981; Lott 1984; Wilson 1998).

## Adaptive individual differences

Individuals of many species show consistent individual differences within natural populations (e.g. Verbeek *et al.* 1994; Wilson *et al.* 1994; Hayes & Jenkins 1997; Budaev *et al.* 1999; Brick & Jakobsson 2002) and some studies have shown that these differences play an important role in the life-history decisions of individual animals (e.g. Armitage 1986; Verbeek *et al.* 1999; Fraser *et al.* 2001; Dingemanse 2003).

Individual differences in a range of behavioural traits have been labelled as temperament, coping strategies, styles or syndromes (Wechsler 1995; Boissy 1995; Koolhaas *et al.* 1999), comparable with human personalities (Eysenck & Eysenck 1985; John 1990; Zuckerman 1991). Already in the 1960s, several animal psychologists used the methods from human emotionality and personality research, mainly on dolphins and several primate species (for refs see Buirski *et al.* 1978). In spite of the obviousness of personality differences within animal species, very little work was carried out in evolutionarily based research due to the fear of being accused of anthropomorphism. Evidence is now accumulating that the personality construct exists in most vertebrates and some invertebrates (Gosling & Vazire 2002) and that it may have consequences for many current ecological models.

## Personalities and their genetics

Most information available on the structure of inheritance of personality traits comes from either human or rodent studies. Although in genetic studies on human personalities twin studies are immensely valuable, they have some methodological limitations since natural experiments in twin studies do not permit full experimental control. Animal models have proven to be a useful tool in getting a better grip on the underlying mechanisms of quantitative traits (Koolhaas *et al.* 2001; Wehner *et al.* 2001), in both physiological and behavioural traits. These studies, mainly on rodents were all on captive-bred populations and therefore gave no insight into the evolutionary processes that have shaped these traits (Merilä & Sheldon 2001).

In our project we study personality traits from an evolutionary point of view. Since natural selection can only work on genetic differences, behaviour can only evolve in a

predictable fashion when there are behavioural alternatives in the population and when this phenotypic variation is heritable. In this thesis I study the genetic structure that underlies the inheritance of personality traits in great tits (*Parus major*). I thereby try to answer the following main questions:

- Do polygenic behavioural traits have an additive genetic component, and if yes, what part of the phenotypic variation does it explain?
- What other genetic effects are playing a role in the inheritance of these traits?
- Are these behavioural traits part of a functional syndrome, and if yes, which part of the coherence of these traits is caused by common genes or linkage and which part by environmental circumstances?

## THIS THESIS

This thesis presents the first genetic study on personality traits in a wild, non-human species. For several reasons (see later) we have chosen to use personality traits in the great tit (*Parus major*) as our model system.

### Model species

The great tit (Parus major) is a very common monogamous territorial passerine, which breeds in secondary holes and artificial nest-boxes in all types of wooded areas throughout Europe and parts of Asia and North Africa (Kluijver 1951; Perrins 1965). The social and non-social environment varies enormously in time and place. Males are territorial throughout the annual cycle, when foraging conditions in and around the territory allow. Females compete for males with a strong preference for males with territorial status. During autumn and winter however, the spatial intolerance is often replaced by hierarchical intolerance. Territory owners flock together with neighbouring territory owners and non-territorial birds, particularly when food is locally unpredictable, scarce or difficult to find and/or clumped outside the territory (Drent 1983). Low ranking birds often disperse from flock to flock and thereby between areas.

Great tits lay clutches of 5-16 eggs that hatch after 12-14 days of incubation (Kluijver 1951). Between 16 and 18 days after hatching the chicks fledge from their nest, but are still fed by both parents until complete independence (Drent 1984). After independence, at about 35 days after hatching, the young form flocks in which social hierarchies develop. In this period juveniles disperse between flocks and areas, first caused by the earlier experiences by the parents and later in time by food availability and distribution, and density (Goodbody 1952; Dhondt 1979; Drent 1984). From September of the year of fledging onwards, young males start to claim territories or individual dominance areas on vacant ground between the still existing territories or on less attractive parts of large territories occupied by elder birds. Early territory-ownership strongly increases survival and reproduction (Drent 1983).

Because of the large amounts of data available on pedigreed natural populations of great tits many heritability estimates of life-history traits are available (e.g. Van Noordwijk *et al.* 1981; Van Noordwijk *et al.* 1988).

Besides the broad knowledge we have of the ecology and life-history of the great tit, there is one more good reason to use great tits as model species: great tits can be kept and bred under laboratory conditions. Their breeding activity can be synchronised with natural populations, which is necessary for cross fostering. Moreover, nestling great tits can be hand-reared under standard conditions without influencing the behavioural differences, which is a requirement for a quantitative genetic study of avian personality traits.

## Personalities in the great tit

Social dominance plays an important role in the life of individual great tits (Krebs & Perrins 1978; Drent 1983) and individuals frequently have to cope with temporal and spatial variation in their social and non-social environment. Verbeek (1997) started her study to see whether early aggressive and explorative behaviour in great tits is consistent within individuals and could be used to predict later dominance relations and social structure. Verbeek *et al.* (1994) showed that male great tits consistently differed in exploratory behaviour in a novel environment (test room) and in boldness towards a novel object in their home cage. These differences in early exploratory behaviour extend to feeding behaviour (Drent & Marchetti 1999; Marchetti & Drent 2000) and aggressive behaviour (Verbeek *et al.* 1996), thereby predicting dominance (Drent & Marchetti 1999; Verbeek *et al.* 1999). These behavioural differences reflect differences in personalities, behavioural strategies or coping styles as were reported in several other animal species (Wilson *et al.* 1994; Koolhaas *et al.* 1999). To be able to study personality traits in an evolutionary context these traits must be at least partly heritable. To investigate the genetic basis of early exploratory behaviour, Piet J. Drent (see chapter 2) started a two-way selection experiment for 'fast' and 'slow' explorative behaviour in 1994. Although the experiment stopped after four generations of selection and the data have been present before the start of this PhD study, new knowledge and extra data allowed us to analyse the data in a more proper way. With the addition of a complementary experiment in my PhD project the decision was made to include this selection experiment in my thesis as the start for further analyses.

The selection lines on early exploratory behaviour are the starting point of the NWO program "Functional significance, heritability and plasticity in coping styles in a free living bird". In this project we study the structure of inheritance (this thesis), the ontogeny and plasticity (Carere 2003), and the fitness consequences of avian personalities (C. Both, Post-doc, University of Groningen; Dingemanse 2003). This program aims to integrate both proximate and ultimate factors, and tries to incorporate at least part of all the three subjects that are necessary for a complete study of the evolutionary background of behavioural traits. We thereby combine both controlled laboratory set-ups with descriptive and experimental research in natural populations.

## Thesis outline

Verbeek *et al.* (1994) found that individual male great tits consistently differ in early exploratory behaviour. These differences can have several causes. In Chapter 2 (P.J. Drent with K. van Oers and A.J. van Noordwijk) and chapter 4 (with P.J Drent and A.J. van Noordwijk) we will address the question whether common genes cause the resemblance between parents and offspring in early exploratory behaviour (Chapter 2) and risk-taking behaviour (Chapter 4). For this purpose two artificial selection experiments were set up.

The realised heritability of a trait only gives information on the amount of additive genetic variation relative to the observed phenotypic variation. The inheritance of a trait however, may be dependent on other genetic effects than additive genetic effects only. We used the lines artificially selected for early exploratory behaviour to produce F1 crossings and crossed these back with the original lines. In Chapter 3 we present the additive and non-additive genetic effects calculated from these lines and crosses with the use of a matrix model.

A discussion within the studies of consistent individual differences in behaviour is whether these personalities are domain specific or domain general. In Chapter 5 the question is studied whether the relative differences between individuals in risk-taking behaviour are dependent on whether it is measured in a social or a non-social context.

Much behaviour is phenotypically correlated, and behavioural traits are therefore not expected to inherit independently of each other. Adaptive individual differences in behaviour are interesting to study on their own, but they serve as examples of a more general process, and cannot be seen without the context of the whole organism. Therefore, studying single behaviours will only make sense when considered in the context of the whole phenotype (Price & Langen 1992). We therefore study a range of ecologically important behaviours. Besides the tests for measuring boldness and exploration (Chapter 2 and Chapter 3), we developed a test to measure risk-taking behaviour (Chapter 4), which is assumed to be an independent personality dimension (Zuckerman 1991). In Chapter 6 we used the results of the selection experiments of Chapters 2 and 4 to analyse the genetic coherence of these traits.

In the final Chapter 7 I will summarize our findings and try to answer our main questions. I will thereby use the results of the other participants of the program to view our results from an evolutionary point of view.

CHAPTER 2

# Realized heritability of personalities in the great tit

**Pieter J. Drent[1], Kees van Oers[1,2] and Arie J. van Noordwijk[1]**

1. Department of Population Biology of Animals, Netherlands Institute of Ecology, PO Box 40, 6666 ZG, Heteren, The Netherlands
2. Evolutionary Population Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

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## ABSTRACT

Behaviour under conditions of mild stress shows consistent patterns in all vertebrates: exploratory behaviour, boldness, aggressiveness covary in the same way. The existence of highly consistent individual variation in these behavioural strategies, also referred to as personalities or coping styles, allows us to measure the behaviour under standardized conditions on birds bred in captivity, link the standardized measurements to the behaviour under natural conditions and measure natural selection in the field. We have bred the great tit (Parus major), a classical model species for the study of behaviour under natural conditions, in captivity. Here, we report a realized heritability of 54 ± 5% for early exploratory behaviour, based on four generations of bi-directional artificial selection. In addition to this, we measured hand-reared juveniles and their wild-caught parents in the laboratory. The heritability found in the mid-offspring–mid-parent regression was significantly different from zero. We have thus established the presence of considerable amounts of genetic variation for personality types in a wild bird.


## INTRODUCTION

Individual animals often face an enormous temporal and spatial variation in their social and non-social environment. The ability to cope with this variation is an important determinant of fitness. Rapidly accumulating evidence for many vertebrates, including man, shows that individuals react to mildly stressful events in different ways. This behavioural variation is often highly consistent within individuals and independent of sex, age or social status. Different behavioural and physiological reactions are correlated (Mendl & Paul 1991), indicating that they are a fundamental aspect of behavioural organization comparable with variation in human personalities (Buss 1991). The reactions of individuals can be quantified on main axes such as 'shyness–boldness' (Wilson *et al.* 1994; Greenberg 1995), 'exploration' (Clark & Ehlinger 1987) or aggressiveness' (Benus *et al.* 1991; Sluyter *et al.* 1996a). Individuals on the extremes of the main axes can be characterized as having different strategies or coping styles (review, Koolhaas *et al.* 1999). At one end of the range we find an 'active' strategy, characterized by rapid decisions, manipulating stressful events, relatively insensitive to external stimuli, ready to form routines, a high level of aggressiveness, boldness, a high level of testosterone and a high reactivity of the sympathetic nervous system.

At the other end of the range, we find a 'passive' strategy, characterized by caution in decisions, relatively highly sensitive and readily adjustable to the external situation, a relatively low level of aggressiveness, shyness and a high reactivity of the hypophyse–pituitary adrenal axis and the parasympathetic nervous system. These animals generally adapt themselves to the environment (Bohus *et al.* 1987; Benus *et al.* 1991; Hessing *et al.* 1994). This system can be captured in several terms: 'coping styles', 'behavioural strategies', 'neophobia' (review, Greenberg & Mettke-Hofmann 2001) and 'A- or B-personalities'. Artificial selection in several domesticated mammalian species has shown that this behavioural variation has an important heritable component (e.g. Van Oortmerssen & Bakker 1981; Sluyter *et al.* 1995). In

house mice, the existence of gene-environment interactions has been shown to some degree (Benus *et al.* 1987), indicating that early experiences can induce persistent behavioural changes in stress-response in adulthood (review, De Kloet *et al.* 1998). Many behavioural traits are potentially affected by many gene loci. However, only a subset of these loci is variable within populations. Among the key questions in describing different behavioural traits within natural populations is how this variation is caused and maintained, and what consequences this has for the individual fitness. For these questions, it is a prerequisite to assess the role and the structure of a genetic component in these behavioural traits in natural populations (Brodie & Russel 1999). A population is able to react in an adaptive way, only in the presence of genetic variation; at what rate this happens is dependent on the amount of genetic variation (Falconer & Mackay 1996).

In our model species, the great tit Parus major, hand-reared individuals of both sexes consistently differ in the way they explore a new environment ('fast' versus 'slow'). This is strongly correlated with differences in behaviour towards novel objects (Verbeek *et al.* 1994; Drent & Marchetti 1999). There were significant differences among sib-groups in the outcomes of these tests (Verbeek *et al.* 1994; Drent & Marchetti 1999), indicating either a determination early in life or genetic effects. These individual differences in exploration and boldness have predictive value for differences in aggressiveness (Verbeek *et al.* 1996), recovery time and behaviour after lost contests (Verbeek *et al.* 1999), foraging behaviour (Marchetti & Drent 2000).

A bi-directional selection experiment was started with wild-caught great tits hand reared in the laboratory. We used a combined score of the exploration of an unknown environment and the reaction to a novel object in the familiar environment. Both tests were performed approximately 40 days after hatching. Individuals with high and low scores were mated assortatively to become the parents of the F1 generation. We used a design with cross fostering and split broods to separate genetic effects from a possible parent-offspring resemblance caused by common environment effects. We report the results from the first four generations of selection on this score. Heritability estimates obtained in the laboratory do not automatically predict responses to selection in the wild. The expression of exploratory behaviour in birds raised in the laboratory could be context dependent (Lambrechts *et al.* 1999). Therefore, we assessed estimates of heritability of exploration of a novel environment in a natural population by collecting wild adult great tits and their nestlings from the field.

## MATERIAL AND METHODS

### Study species

The great tit is a common monogamous territorial passerine, which breeds in secondary holes and artificial nest-boxes in all types of wooded areas throughout Europe and parts of Asia and North Africa (Perrins 1965). Individuals frequently have to cope with temporal and spatial variation in their social and non-social environment. Areas differ in the presence and distribution of resources such as food, roosting and

breeding holes in both time and space (Gibb 1954; Betts 1955; Van Balen 1973; Van Balen 1980). Males are territorial throughout the annual cycle, when foraging conditions in and around the territory allow. However, during autumn and winter, the spatial intolerance is often replaced by hierarchical intolerance during flocking behaviour with other neighbouring territory owners and their mates and non-territorial birds, particularly when food is locally unpredictable, scarce or difficult to find (Drent 1984). Females compete for males with a strong preference for males with territorial status. The hierarchical organisation in flocks is similar to the classic study of jays by Brown (1963; De Laet 1976; Drent 1983) and resembled the scrounger-producer system as pointed out by Barnard & Sibly (1981). Low-ranking birds often disperse from flock to flock and thereby between areas. After independence of the parents, that is ca. 35 days after hatching, the young form flocks in which social hierarchies develop. In this period juveniles disperse between flocks and areas, first caused by the earlier experiences by the parents and, later, by density and food availability and distribution (Goodbody 1952; Dhondt 1979; Drent 1984). From September of the year of fledging onwards, young males start to claim a territory or individual dominance area on vacant ground between the still-existing territories of adult males or on less attractive parts of large territories. Early territory ownership strongly increases survival, dispersal and reproduction and thus fitness (Drent 1983; Drent 1984).

## Animal collection from the field

We collected nestlings from two wild populations at an age of 10 days after hatching. In 1993, we collected 81 nestlings from 11 broods. In 1998, 15 pairs were caught in the breeding boxes with spring traps and transported to the laboratory together with their 102 nestlings. These birds were taken from the same populations as those from 1998. In the laboratory, the adults were housed individually in standard cages of 0.9 m x 0.4 m x 0.5 m with solid bottom, top and rear walls and a wire-mesh front and three perches. They were tested for exploration of a standard novel environment 10 days after capture. After testing, the parents were released at their capture site.

We only collected broods without nestling mortality and with a normal nestling growth (weight on day 10: 13.0 g or higher) (see Van Balen 1973). For the later generations the nestlings were weighed at the age of 5 and 10 days. The tarsus was measured at day 10. If at an age of 5 days the weight of the young stayed behind the expectation of well-grown broods (less than 7.0 g) artificial food (frozen mealworms and larvae of the wax moth) was offered daily in a small cup inside the respective nestbox. Almost all these parents used this food, which resulted in a mean brood weight greater than 13 g on day 10 after hatching.

## Rearing of the young

Ten-day-old nestlings, collected from the field, were divided into groups of 4-5 siblings. These groups were placed in natural nests in cardboard boxes. The young were hand reared on a mixed diet (Verbeek *et al.* 1994). Survival during hand rearing was 95%, and 17-20 days after hatching, the normal fledging age, the young start to

leave the nests. The fledglings were then housed in small wire cages (0.5 m x 0.4 m x 0.4 m) with two perches, maintaining the sibling groups from the nestling phase. At day 20, small cups containing a beef heart mixture, supplemented with insect food and water were placed in the cages. Within a few days after the first young started to exploit this food, hand feeding was gradually withdrawn. At day 35 after hatching birds were housed individually in standard cages. At this age, juveniles in the field also normally become independent of their parents. Birds were kept under natural light conditions with acoustic and visual contact with each other. Juveniles of parents with different scores were housed in the same room. Each cage was connected to a light-tight observation room (4.2 m x 2.5 m x 2.3 m) via a sliding door (20 cm x 20 cm) in the rear wall.

## Standard tests

Two different tests were performed to all hand-reared juveniles. A novel environment test was conducted in a standard observation room (analogous to an open field test; for details see Verbeek *et al.* (1994) two days after individual housing. The time needed to visit four of the five artificial wooden trees was converted linearly to a scale of 0–10. A score of 10 ('fast') means that the bird reached the fourth tree within 1 min and a score of 0 means that the bird did not reach the fourth tree within 10 min ('slow'). Respectively, 10 and 11 days later, this was followed by tests of the reaction to two different novel objects conducted in their individual cage (see also Verbeek *et al.* 1994). In these tests, a novel object was introduced on one of the outer perches. For this, a penlight battery was used on the first day and an 8 cm bendable pink rubber toy ('pink panther') on the second day. The latency to approach this object (in seconds) and the shortest distance to this object within 120 s were scored.

## Bi-directional artificial selection of early exploratory behaviour

For the parental generation, we selected those juveniles from the birds collected in 1993 that had the highest and lowest summed scores. Both fast and slow lines were started and maintained with nine pairs. Pairs were kept in aviaries (2.0 m x 4.0 m x 2.5 m) from December onwards. In spring, eggs were collected daily and exchanged with dummy eggs. Clutches of eight eggs from the same pair were brought to the field and incubated by foster females. One day after hatching, nestlings were exchanged to form mixed broods of, at most, eight young. As far as possible, each foster brood consisted of equal numbers of offspring from both selection lines. Nestlings were collected at an age of 10 days and then hand reared in mixed groups in the laboratory. For later generations we formed pairs from the offspring by selecting the individuals with the highest scores for the 'fast' line and lowest scores for the 'slow' line, avoiding full-sib and first-cousin mating. Hand rearing was identical to that carried out on the parental generation.

## Statistical analysis

The narrow sense heritability ($h^2$) measures the proportion of total variance that is attributed to the effect of genes and is defined as the ratio of additive genetic variance

($V_A$) to total phenotypic variance ($V_P$), with $h^2 = V_A / V_P$ (Falconer & Mackay 1996; Lynch & Walsh 1998). We estimated the within-family in relation to between-family variance (also referred to as broad sense heritability) using a Kruskal-Wallis test with nest as a grouping variable. For this analysis, the data from both 1993 and 1998 were used. The resemblance of offspring to their wild-caught parents was calculated from weighted and non-weighted mid-offspring-mid-parent regression on the exploration of a novel room. For the analyses of the bi-directional selection lines, we used the mid-parent values and the mean of sib groups per guest pair. For the analysis of the foster parent effect in 1995 and 1996, we used a Poisson regression with juvenile exploration score, corrected for over-dispersion (for details see Crawley 1993), as the dependent variable and foster parent and biological parent as factors in the full model. For this analysis, we used GLIM 4.0 for ecologists (Crawley 1993). For all other analyses, we used SPSS 10.1 software.

## RESULTS

### Realized heritability of early exploratory behaviour

In table 1 the population measures of the exploration score for the juvenile populations of 1993-1997 are given. Although there were fluctuations in the response to selection, the artificial selection experiment showed strong effects in four generations (figure 1a). In the up- and down-selection the mean score changed respectively from 1.78 to 21.31 units per generation. In figure 1b the cumulative response to selection (response compared with the starting population) has been plotted against the cumulative selection differential (the deviation of the individuals used as parents from the mean value in their generation). The realized heritability in the base population is the proportion of the total observed variance in the starting population that can be attributed to genetic factors, which in this case is calculated from the regression coefficient of the cumulative response to selection over the cumulative directional selection differential: $0.545 \pm 0.046$ (linear regression; $r^2 = 0.95$, $F_{1,8} = 139.32$ and $p < 0.0001$).

Behavioural traits are usually quite sensitive to the environment in which individuals have grown up. A careful inspection of figure 1a shows that both lines have relatively low scores in the F3 and relatively high scores in the F4 generations. It is therefore necessary to investigate whether the effects of the microenvironment cause a parent-offspring resemblance. Our cross-fostering design, in which a large proportion of the offspring in the F2 (1995) and F3 (1996) generations from both selection directions were raised together in mixed broods by foster pairs in the field until 10 days after hatching, made it possible to analyse this in more detail. Maternal effects transmitted through egg characteristics however are, although unlikely, still possible. In figure 2, the mean exploration score of full sibs that were raised together in one foster nest are plotted against the mid-parent scores of their biological parents, for the F2 (figure 2a) and the F3 (figure 2b) generation. A line connects the

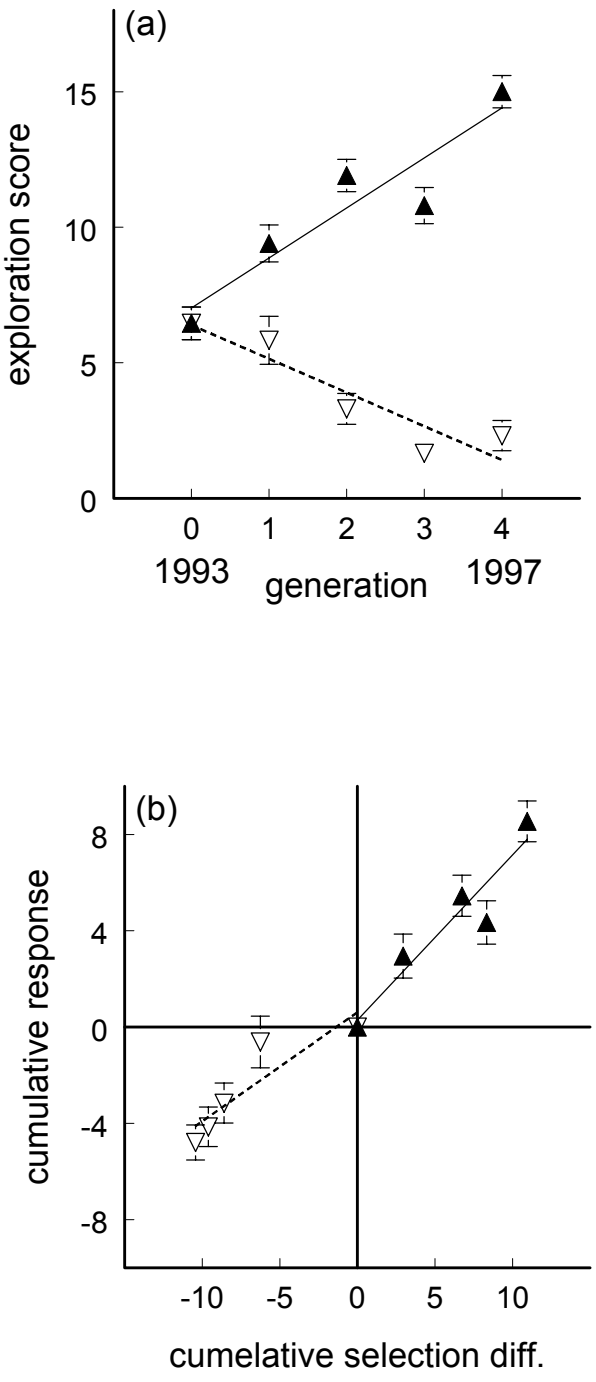


**Figure 1.** Response to artificial selection per generation (a) and relative to the cumulative selection differential (b) for both up-selection (filled triangle) and down-selection (inverted open triangle) with s.e.m. Lines represent regression lines for up- and down-selection. The slopes for up- and down-selection separately are 0.69 (linear regression; r2 = 0.90, F1,4 = 26.03 and p = 0.015) and 0.45 (linear regression; r2 = 0.80, F1,4 = 12.27 and p = 0.039), respectively.

two sib groups from the different selection lines that were raised together in one foster nest. In the absence of a genetic component, we would expect horizontal lines. In the case of solely genetic effects, the regression coefficient of the lines would approach one. Using the mean values of sib groups raised together in one foster nest, the regression coefficient of offspring values on parental values is 0.75 (range: 0.62–

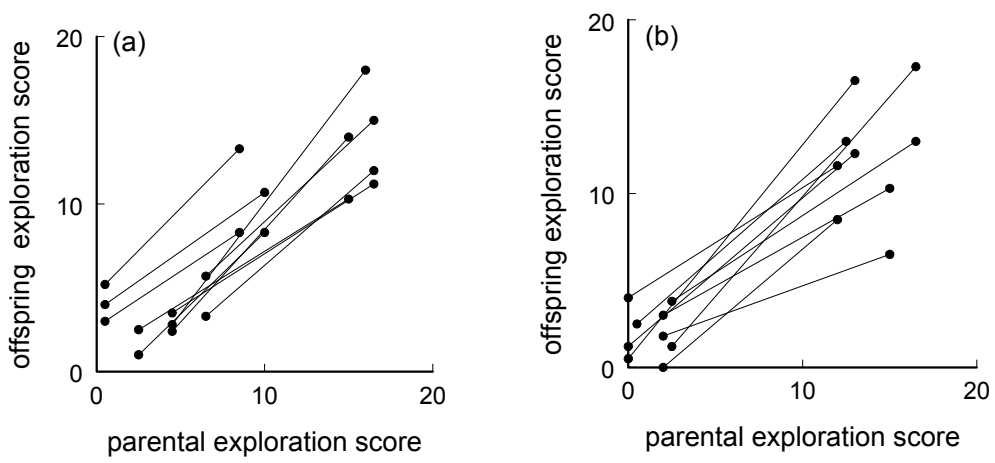


**Figure 2.** Effect of cross fostering for the F2 generation in 1995 (a) and the F3 generation in 1996 (b). Each line connects two dots, which represent one fast and one slow sib group, which were raised together in one foster group. Both graphs show data on nine foster groups.

1.32; linear regression: $R^2 = 0.70$, $F_{1,18} = 42.99$ and $p < 0.0001$; figure 2a) in the F2 generation and 0.73 (range 0.36–1.23; linear regression: $R^2 = 0.68$, $F_{1,21} = 45.14$ and $p < 0.0001$; see figure 2b) for the F3 generation. Because eggs were brought to the field with minimum delay, and we maximized the number of chicks raised in mixed broods, combinations are often not symmetrical, which complicates statistical analysis. However, the results of a Poisson regression of juvenile exploration score with biological, and foster, parents as factors are clear. Both for the F2 and F3 data, there is a highly significant effect of biological parents (1995: $\chi^2_8 = 56.48$ and $p < 0.0001$; 1996: $\chi^2_{10} = 72.11$ and $p < 0.0001$) and no effect of foster parents (1995: $\chi^2_{12} = 5.47$ and $p = 0.94$; 1996: $\chi^2_{17} = 8.33$ and $p = 0.96$). Interactions were not significant (1995: $\chi^2_2 = 0.12$ and $p = 0.94$; 1996: $\chi^2_3 = 1.94$ and $p = 0.58$). Analysis of the data for both years combined confirms a significant effect of the biological parents, but no interaction and no effect of guest pairs. This implies that the effect of raising conditions within our set-up is small and that parent-offspring resemblance is not due to parental influences on the juvenile environment.

## Heritability of wild-caught parents and their hand reared offspring

To confirm whether the variation in our laboratory lines is related to the variation under natural conditions, we estimated the resemblance of exploration scores of adult great tits collected from the field and their laboratory-raised nestlings. We collected a second group of wild adult birds together with their 10-day-old offspring in 1998. There was no difference in mean exploration scores per nest between these juveniles and those collected in 1993 (t-test, $t_{24} = 0.46$ and $p = 0.65$). The within-nest variance was again smaller than the among-nest variance (Kruskal-Wallis test, $\chi^2_{25} = 46.0$ and $p = 0.006$). This demonstrates that young from the same brood show more resemblance to each other than to offspring of other broods. Exploration scores of the juveniles from this group were lower than those of their parents, which were measured as adults. This was probably due to a mixture of age and seasonal effects (Dingemanse *et al.* 2002).

**Table 1.** Population measures of the exploration score for the juvenile populations of 1993–1997. (Abbreviations: $S_{cum}$, the cumulative selection differential; n, total number of offspring tested; m, mean; s.e.m., standard error of mean; $h^2$, heritability; s.e., standard error of heritability; $V_P$, phenotypic variance; $V_A$, additive genetic variance.)

| type | year | $S_{cum.}$ | n | m | s.e.m. | $h^2$ | s.e. | $V_P$ | $V_A$ |
|---|---|---|---|---|---|---|---|---|---|
| down-selection | 1993 | | 85 | 6.45 | 0.60 | | | 31.01 | |
| | 1994 | -6.26 | 24 | 5.83 | 0.89 | 0.10 | 0.00 | 18.84 | 1.88 |
| | 1995 | -8.59 | 37 | 3.30 | 0.57 | 0.37 | 0.21 | 12.10 | 4.45 |
| | 1996 | -10.43 | 47 | 1.62 | 0.42 | 0.46 | 0.16 | 8.15 | 3.75 |
| | 1997 | -9.61 | 35 | 2.31 | 0.56 | 0.43 | 0.13 | 10.93 | 4.71 |
| Up-selection | 1994 | 2.94 | 52 | 9.40 | 0.68 | 1.00 | 0.00 | 24.29 | 24.29 |
| | 1995 | 6.74 | 43 | 11.91 | 0.60 | 0.81 | 0.10 | 15.28 | 12.35 |
| | 1996 | 8.33 | 60 | 10.80 | 0.67 | 0.52 | 0.18 | 26.81 | 13.97 |
| | 1997 | 10.95 | 31 | 15.00 | 0.59 | 0.78 | 0.13 | 10.80 | 8.43 |

We estimated the resemblance in this group as if it was heritability, although the measurements in parents and offspring were made at quite different ages. Both the weighted ($h^2 = 0.247 \pm 0.101$ and $p = 0.017$) and the non-weighted ($h^2 = 0.331 \pm 0.114$ and $p = 0.018$) mid-offspring –mid-parent regression were significantly different from zero, but not from each other (t-test, $t_{107} = 0.181$ and $p < 0.05$). Because these two methods produce the most extreme estimates of heritability, this indicates that family size had no major effect.

## DISCUSSION

We have shown that variation in coping behaviour is heritable in a wild bird population by performing a bi-directional selection experiment in captivity. We found a strong response to selection after four generations of selection. Laboratory conditions might overestimate natural heritabilities, owing to a reduction in environmental variability (Riska *et al.* 1989). Our heritability measures from the mid-offspring–mid-parent regression of wild-caught parents and their hand-reared offspring and the selection experiment in the great tit also show this. This result is also confirmed by a parallel study, where adults from a natural population were taken to the laboratory, tested and released within 24 h (Dingemanse *et al.* 2002). Using known family relationships, broad sense heritability estimates of ca. 30% for the behaviour in an unfamiliar room are similar to this resemblance of adult parents and juvenile offspring. This is in agreement with the result from several other comparative studies on the similarity between heritability estimates in the laboratory with those in the field (Riska *et al.* 1989; Weigensberg & Roff 1996). Laboratory estimates of heritability tend to be somewhat higher, but not significantly different. Furthermore, we cannot completely

exclude environmental maternal effects. In a model study Riska *et al.* (1985) pointed out that their influence will diminish after one generation of selection, which in our case would mean that the response to selection would have decreased or even been absent after the second generation of selection (for more details see Reznick & Bryga 1987).

The question of the evolutionary origin and persistence of phenotypic variation in behavioural traits within populations is a central topic in biology. The genetics underlying individual variation in behaviour in natural populations is often not well known owing to the difficulties in distinguishing between environmental and genetic effects (Griffith *et al.* 1999). The extent to which environmental effects or genes determine individual variation in behaviour is essential for the explanation of coexistence of different phenotypes and thereby for the population dynamics and evolution of the system. The three key questions in understanding the presence of variation in behavioural traits within one natural population are: how the variation is caused, what the consequences are for the individual fitness and how the variation is maintained. Behavioural strategies with restricted plasticity are suites of correlated behaviours that reflect within-individual consistency in reactions to cope with environmental challenges across context. In other words, an individual's reaction in one context is linked to its reaction in another context. The different traits of these strategies have not evolved in isolation but as a package (Price & Langen 1992; Lynch & Walsh 1998). The within-individual correlations between traits generate trade-offs in reaction norms across context, which can have a major role in evolution. In understanding the evolution towards behavioural strategies a useful analogy with consistent variation in life history could be made (Stearns 1992). Owing to the tradeoffs optimally in one context and have to pay the cost in another context. Therefore, the combination of spatial and temporal-social and non-social variation in the environment resulting in different selection regimes, and the trade-offs between different traits, can explain the maintenance of the different strategies (Mangel & Stamps 2001).

Genetic differences between behavioural strategies could have critical implications for ecology and evolution. Animals have to cope with an enormous spatial and temporal variation in their social and non-social environment. Under the non-social environment, man-caused changes and variability in the environment become increasingly important. The differences in ability to cope with challenges are an important determinant of differences in local survival, dispersal and reproduction (lifetime reproductive success, fitness). These in their turn determine differences in density and in the genetic structure of populations in time including the frequency distribution of behavioural strategies. Density and frequency distribution are a part of the social environment resulting in competition for resources and in frequency-dependent competition and/or cooperation in flocks and breeding couples, thereby influencing the fitness of their individual members.

Without knowledge of the context and the individual consistent behavioural strategies, it is not surprisingly that conflicting ecological results could arise. Different behaviours, which are part of the strategy, and different consistent behavioural

strategies, should not be studied in isolation in one context, as is often done by behavioural ecologists. Understanding the outcome in any given context could require an understanding of the implications of their correlated behaviours over context. This implies that the relative success, in fitness terms, of individuals with different strategies often changes with the context, probably resulting in major consequences for differences in population size and structure (Drent & Marchetti 1999; Drent *et al.* 2002).

However, such knowledge is almost always lacking in studies on vertebrate species under natural conditions. Density-dependent selection could also be a mechanism for the cause of this variation (Chitty 1958). It was shown to be responsible for varying selection pressures, thereby accounting for maintenance of variation in throat colour in lizards (Sinervo *et al.* 2000). To our knowledge, only one study in birds was able to couple genetic variation in behaviour to fitness consequences (Pulido *et al.* 2001). In respect of behaviour to cope with environmental challenges, only one vertebrate study in a population of wild house mice indicated that the relative frequency of coping strategies changes in the different population-dynamic phases, suggesting that differential selection on these heritable strategies occurs in the wild (Benus *et al.* 1987). Our results, and the extensive knowledge of the ethology and ecology of the great tit, indicate that this species is a suitable model to carry out such research in the wild, and our demonstration of considerable amounts of genetic variation is a major step forward.

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CHAPTER 3

# Additive and nonadditive genetic variation in avian personality traits

**Kees van Oers[1,2], Pieter J. Drent[1], Gerdien de Jong[1,2] and Arie J. van Noordwijk[1]**

1. Department of Population Biology of Animals, Netherlands Institute of Ecology, PO Box 40, 6666 ZG, Heteren, The Netherlands
2. Evolutionary Population Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

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## ABSTRACT

Individuals of all vertebrate species differ consistently in their reactions to mildly stressful challenges. These typical reactions, described as personalities or coping strategies, have a clear genetic basis, but the structure of inheritance in natural populations is almost unknown. We carried out a quantitative genetic analysis of two personality traits (exploration and boldness) and the combination of these two traits (early exploratory behaviour). This study was carried out on the lines resulting from a 2-directional artificial selection experiment on early exploratory behaviour (EEB) of great tits (*Parus major*) originating from a wild population. In analyses using the original lines, reciprocal $F_1$ and reciprocal first backcross generations, additive, dominance, maternal effects ands sex-dependent expression of exploration, boldness and EEB were estimated. Both additive and dominant genetic effects were important determinants of phenotypic variation in exploratory behaviour and boldness. However, no sex-dependent expression was observed in either of these personality traits. These results are discussed with respect to the maintenance of genetic variation in personality traits, and the expected genetic structure of other behavioural and life-history traits in general.


## INTRODUCTION

Individuals within populations, differ consistently in how they react to mildly stressful challenges (Gosling 2001). Although dependent on the environmental context, the same range of reactions is found independent of sex, age or social status (Wilson *et al.* 1994). Such behavioural differences are quantified on axes such as the "big five" (openness to experience, conscientiousness, extraversion, agreeableness and neuroticism) in humans (John 1990), and aggressiveness (Hessing *et al.* 1993), reactivity (Benus *et al.* 1991), boldness/shyness (Wilson *et al.* 1994), temperament (Réale *et al.* 2000), neophobia (Greenberg & Mettke-Hofmann 2001) and exploration (Benus *et al.* 1987) in other animals. Different behavioural, physiological and pharmacological reactions are correlated, indicating that these are fundamental aspects of variation in behavioural organisation. In humans this is referred to as variation in human personality, in other taxa also as behavioural tendencies, temperaments, syndromes, constructs, styles or strategies (Wilson *et al.* 1994).

In our model species, the great tit *Parus major,* hand-reared individuals of both sexes consistently differ in the way they explore a novel environment, and these differences are strongly correlated with differences in behaviour towards novel objects (Verbeek *et al.* 1994; Drent & Marchetti 1999; Drent *et al.* 2002). A heritable component was shown to exist for exploration and boldness in a directional selection experiment (chapter 2) and in a natural population (Dingemanse *et al.* 2002 ;chapter 2). These individual differences in exploration and boldness have predictive value for differences in aggressiveness (Verbeek *et al.* 1996), recovery-time and behaviour after lost contests (Verbeek *et al.* 1999), foraging behaviour (Drent & Marchetti 1999; Marchetti & Drent 2000) and reactions to stress (Carere *et al.* 2001; Carere 2003).

Much is still unknown about the structure and mechanisms of inheritance of polymorphic behavioural traits in general (Merilä & Sheldon 2001), personalities in

particular. Even less is known about how they are shaped by evolutionary processes (Réale & Festa-Bianchet 2002). Knowledge about the genetic structure and mechanisms of behavioural traits, is however crucial to understand the evolution of life-history traits in natural populations (Van Noordwijk 1990; Merilä & Sheldon 1999; Réale & Festa-Bianchet 2000).

In this study we performed a crossing experiment to estimate additive and non-additive genetic components, maternal effects and sex-dependent expression of exploration and boldness. Great tits of two lines resulting from an two-directional artificial selection experiment for the extremes of the combination of these traits ('fast' and 'slow' explorers; chapter 2), were crossed to produce hybrid $F_1$ and their first backcross generations. By using the two original lines (2 groups) and the reciprocals of the $F_1$ (2 groups) and first generation backcrosses (4 groups), we have phenotypic means of 8 groups. This provides enough data to test the adequacy of genetic models of expected group means containing additive, dominance, maternal effects and sex-dependent expression (Mather & Jinks 1971; Houle 1991).

Our aims are (i) to get a better insight in the structure of inheritance of exploration and boldness in a wild bird species, and (ii) to see whether the expression of exploration and boldness depends on offspring sex. We will discuss how our results fit the current theories of the genetic structure and the maintenance of genetic variation in life-history traits.

## MATERIALS AND METHODS

### Study system

The great tit is a very common monogamous territorial passerine, which breeds in secondary holes and artificial nest-boxes in all types of wooded areas throughout Europe and parts of Asia and North Africa (Perrins 1965). From September of the year of fledging onwards, young males start to claim a territory or individual dominance area on vacant ground between the still existing territories of adult males or on less attractive parts of large territories. Early territory-ownership is strongly related to survival, reproduction thus fitness (Drent 1983). Males are territorial throughout the annual cycle. During autumn and winter, the spatial intolerance is often replaced by hierarchical intolerance during flocking behaviour with other neighbouring territory owners and their mates and non-territorial birds, particularly when food is locally unpredictable, scarce or difficult to find.

We breed great tits in semi-open aviaries of 2.0 by 4.0 by 2.5 m. Birds are paired up in December and breeding pairs are kept in aviaries from December until the end of the breeding season. From September until December birds are kept in groups of 6 to 8 individuals per aviary, to mimic natural winter flocking. Juveniles are housed individually in standard cages of 0.9x0.4x0.5 m with a wooden bottom, top, sides and rear walls, a wire-mesh front and three perches, as soon as they reach independence. All birds are kept under natural light conditions and have auditory and visual contact with other individuals. We feed the captive great tits with a protein rich mixture, and a

commercial seed mixture, supplemented daily with mealworms (*Tenebrio molitor*) or sunflower seeds, while water is provided *ad libitum*.

## Lines and crosses

All genetic groups (lines and crosses) and their sources used in the analyses are shown in Table 1. The parental groups, $P_1$ and $P_2$, were birds from the fourth generation of selection lines for 'fast' and 'slow' early exploration respectively (chapter 2). To obtain the reciprocal $F_1$ crosses we mated birds from both lines ($P_1$ x $P_2$ and $P_2$ x $P_1$; in which the female is always the first in the combination) with a total of 9 pairs each. Of the available F1 offspring, 36 birds were mated with both P lines, forming two backcross combinations and their reciprocals.

**Table 1.** Sources of the groups (genetic lines and crosses) used in the analyses. n = number of individuals per group.

| GROUPS | n | SOURCE |
|---|---|---|
| **lines** | | |
| $P_1$ | 31 | fourth generation of the 'fast' line |
| $P_2$ | 35 | fourth generation of the 'slow' line |
| **crosses** | | **offspring from:** |
| $F_1$ | 44 | $P_1$ females x $P_2$ males |
| $F_1$m | 22 | $P_1$ females x $P_2$ males (male offspring |
| $F_1$f | 15 | $P_1$ females x $P_2$ males (female offspring) |
| $F_1$R | 12 | $P_2$ females x $P_1$ males |
| $F_1$Rm | 4 | $P_2$ females x $P_1$ males (males offspring) |
| $F_1$Rf | 4 | $P_2$ females x $P_1$ males (female offspring) |
| $F_1$● | 56 | $F_1$ and $F_1$R combined |
| $B_1$ | 0 | $P_1$ females x $F_1$● males |
| $B_1$R | 6 | $F_1$● females x $P_1$ males |
| $B_2$ | 7 | $P_2$ females x $F_1$● males |
| $B_2$R | 17 | $F_1$● females x $P_2$ males |

To avoid effects caused by the parental environment as much as possible, eggs were collected daily before 9 am, and replaced with dummy eggs. Eggs were stored in a separate room, in a machine that turned the eggs every two hours. Full clutches were exchanged with clutches of wild females in natural field populations. Breeding in the aviaries is synchronous to breeding in the filed populations. Nestlings were collected from the foster nests at an age of 10 days and then hand reared until independence in the lab (for details on hand rearing see chapter 2). We tested birds of all groups 35 days after hatching, as described below.

## Tests

To measure the early exploratory behaviour score (exploration and boldness) we performed two types of behavioural tests: A novel environment test, conducted in a standard observation room (analogous to an open field test)(Walsh & Cummins 1976) was followed by two tests of the reaction to different novel objects conducted in the home cage (chapter 2). The combination of the novel environment score (further referred to as exploration) and the novel object test score (further referred to as boldness) is referred to as early exploratory behaviour (EEB). EEB was used as the selection criterion in the bi-directional selection experiment of chapter 2. The exploration test was carried out between 30 and 35 days after hatching, the boldness tests 10 and 12 days later.

For the exploration test, five tree-like models (further referred to as trees) were placed in an observation room of 4.0x2.4x2.3 m (Dingemanse *et al.* 2002 chapter 2). The time a bird needed to visit the fourth tree was converted linearly to a scale of 0-10. Birds who reached the fourth tree within one minute were given a score of 10, birds that reached the fourth tree within two minutes were given a score of 9 etc. Birds that did not reach the fourth tree within 10 minutes, received a score of zero. The result of each boldness test was converted linearly to a 0-5 scale, with a score of five when a bird pecked the object and a score of zero when the bird did not reach the perch on which the object was placed within 120 seconds. The scores for the two novel objects were summed giving a total score of 0-10. The sum of the exploration and the boldness test scores gives the EEB score (for more details on the tests see chapter 2; Verbeek *et al.* 1994).

## Scaling

To study the relative levels of variation, it is necessary to know whether any differences are simply a consequence of scale (e.g. Houle 1992). To be secure a scale should be chosen, where the variance is independent of the mean, in which case significant differences in observed levels of variance between samples (i.e. groups) must be attributable to other factors than differences in the mean (Falconer & Mackay 1996; Lynch & Walsh 1998). Since the variance increased with the mean in the exploration test and decreased with the mean in the boldness test, the data had to be rescaled. A simple log-transformation, as often used in behavioural characters (Falconer & Mackay 1996; Stirling *et al.* 2002a), is therefore insufficient.

For optimal scaling we used the procedure CATREG, version 1.0 by DTSS, which is available in the statistical package SPSS 10.1 for Windows. CATREG uses categorical regression with optimal scaling, which quantifies categorical data by assigning numerical values to the categories (i.e. scores), resulting in an optimal linear regression equation for the transformed variables. The procedure treats quantified categorical variables in the same way as numerical variables. Using non-linear transformations allow variables to be analysed at a variety of levels to find the best-fitting model. CATREG was applied only on the scores of the original lines. Applying it on all groups would artificially lower all variance components other than additive

variance. The original scores of all birds (original lines and crosses and backcrosses) were then replaced by the computed scores. Although the analysis with the scaled data changed the exact values of the model parameters, the overall conclusions would have been the same when the analysis would have been done on the original data.

## Analysis of group means

The observed group means were analysed following the methods of Mather and Jinks (1971). The observed exploration, boldness and EEB means of the groups were used to estimate parameters, errors, and $\chi^2$ values of an initial model, using weighted least-squares methods (Mather & Jinks 1971; Kearsey & Pooni 1996; for details see Starmer *et al.* 1998; or Gilchrist & Partridge 1999). This initial model consisted of an overall mean *m* and additive [a] and dominance [d] genetic effects (Mather & Jinks 1971; following the notation of Kearsey & Pooni 1996). The estimated parameters were then used to calculate expected group means. For each group, the difference between the observed and the expected group means together with the weight values of the group means, was used to calculate a contribution-value for the $\chi^2$. All contribution values added up to the $\chi^2$ value, with the number of group means minus the number of estimated parameters, as the number of degrees of freedom. A significant $\chi^2$ indicates that the expected group means, generated through the model significantly deviate from the observed group means. This implies that the present model insufficiently describes the observed means.

Adding the parameters of interest to the initial model produces an extended model. We calculated parameter estimates (with standard error), and expected group means as described above. To test whether added parameters in the extended model increased the fit of the model significantly compared to the initial model, we used a likelihood-ratio test (Lynch & Walsh 1998). A t-test was used to test the significance of the parameter estimates, with the degrees of freedom being the total number of offspring used in the model minus one (Zar 1999). The significance of the added parameters indicated which parameters could be omitted to simplify the model, where we started with the least significant parameter. When parameters were omitted the goodness of fit was recalculated. We repeated omitting parameters until the goodness of fit decreased significantly by omitting one more parameter. The model that results from this is referred to as the minimal adequate model.

Three sets of two different models were made. In the first model (model A) we used the original lines and the reciprocal $F_1$, where the male and female $F_1$ offspring were treated as two groups, to calculate whether or not the expression of EEB, exploration and boldness is sex-dependent. In the second model we used the original parental lines, the reciprocal $F_1$ and the reciprocal backcrosses to test whether an additive maternal effect ($[a]_m$} and a dominance maternal effect ($[d]_m$) are involved in the inheritance of EEB, exploration and boldness. Each model (A and B) was made for exploration, boldness and EEB separately, giving a total of six models. The parameter coefficients that we used for the two models are given in Table 2.

**Table 2.** The parameter coefficients used in model A and B. n = number of individuals per group, m = group mean, [a] = additive genetic component, [d] = genetic dominance component, $[a]_m$ = additive maternal component, $[d]_m$ = dominant maternal component (used in Model B only) and [sde] = sex-dependent component (used in Model A only).

| *Group* | n | m | [a] | [d] | $[a]_m$ | $[d]_m$ | [sde] |
|---|---|---|---|---|---|---|---|
| $P_1$ | 31 | 1 | 1 | 0 | 1 | 0 | 0 |
| $P_2$ | 35 | 1 | -1 | 0 | -1 | 0 | 0 |
| $F_1$ | 44 | 1 | 0 | 1 | 1 | 0 | |
| $F_1$m | 22 | 1 | 0 | 1 | 1 | | 1 |
| $F_1$f | 15 | 1 | 0 | 1 | 1 | | -1 |
| $F_1$R | 12 | 1 | 0 | 1 | -1 | 0 | |
| $F_1$Rm | 4 | 1 | 0 | 1 | -1 | | 1 |
| $F_1$Rf | 4 | 1 | 0 | 1 | -1 | | -1 |
| $B_1$ | 0 | 1 | 0.5 | 0.5 | 1 | 0 | |
| $B_1$R | 6 | 1 | 0.5 | 0.5 | 0 | 1 | |
| $B_2$ | 7 | 1 | -0.5 | 0.5 | -1 | 0 | |
| $B_2$R | 16 | 1 | -0.5 | 0.5 | 0 | 1 | |

**Table 3.** Mean scaled test scores for the parental, cross and backcross groups, with their SEM and with N = number of individuals for EEB, exploration and boldness.

| | EEB | | | EXPLORATION | | | BOLDNESS | | |
|---|---|---|---|---|---|---|---|---|---|
| *Group* | m | s.e.m. | n | m | s.e.m. | n | m | s.e.m. | n |
| $P_1$ | 1.851 | 0.139 | 31 | 0.991 | 0.050 | 31 | 0.86 | 0.116 | 31 |
| $P_2$ | -1.639 | 0.136 | 35 | -0.878 | 0.073 | 35 | -0.762 | 0.092 | 35 |
| $F_1$f | -0.807 | 0.321 | 13 | -0.243 | 0.294 | 14 | -0.463 | 0.164 | 14 |
| $F_1$m | -1.029 | 0.226 | 20 | -0.332 | 0.186 | 22 | -0.678 | 0.101 | 20 |
| $F_1$ | -0.916 | 0.181 | 34 | -0.251 | 0.155 | 40 | -0.601 | 0.088 | 35 |
| $F_1$Rf | -1.416 | 0.449 | 4 | -0.485 | 0.467 | 4 | -0.931 | 0.057 | 4 |
| $F_1$Rm | -1.294 | 0.49 | 4 | -0.534 | 0.481 | 4 | -0.76 | 0.093 | 4 |
| $F_1$R | -1.272 | 0.281 | 10 | -0.341 | 0.268 | 12 | -0.874 | 0.051 | 10 |
| $B_1$ | | | 0 | | | 0 | | | 0 |
| $B_1$R | -0.139 | 0.605 | 6 | 0.301 | 0.368 | 6 | -0.44 | 0.29 | 6 |
| $B_2$ | -1.279 | 0.305 | 7 | -0.684 | 0.268 | 7 | -0.595 | 0.254 | 7 |
| $B_2$R | -0.657 | 0.362 | 16 | -0.105 | 0.252 | 16 | -0.551 | 0.18 | 16 |

To test whether parameter estimates differed significantly from each other, t-tests were used (Zar 1999 page 124). We performed several t-tests, so a Bonferroni correction would be appropriate as the chance of a significant result increases with the number of tests. Since we did not formally test hypotheses, but relations we did not perform a Bonferroni correction and present original P-values.

## RESULTS

The observed group means (scaled) used in both models are shown in Table 3. For all traits (exploration, boldness, EEB) separately, expected group means were calculated from a simple genetic model containing a grand mean (m) and an additive component ([a]) only (maximum-likelihood additive model). The observed group means and the regression lines on the expected group means derived from this maximum-likelihood additive model for all traits are plotted in Figure 1. In no case did this model describe the observed means adequately (minimum $\chi^2=8.20$; $P<0.05$).

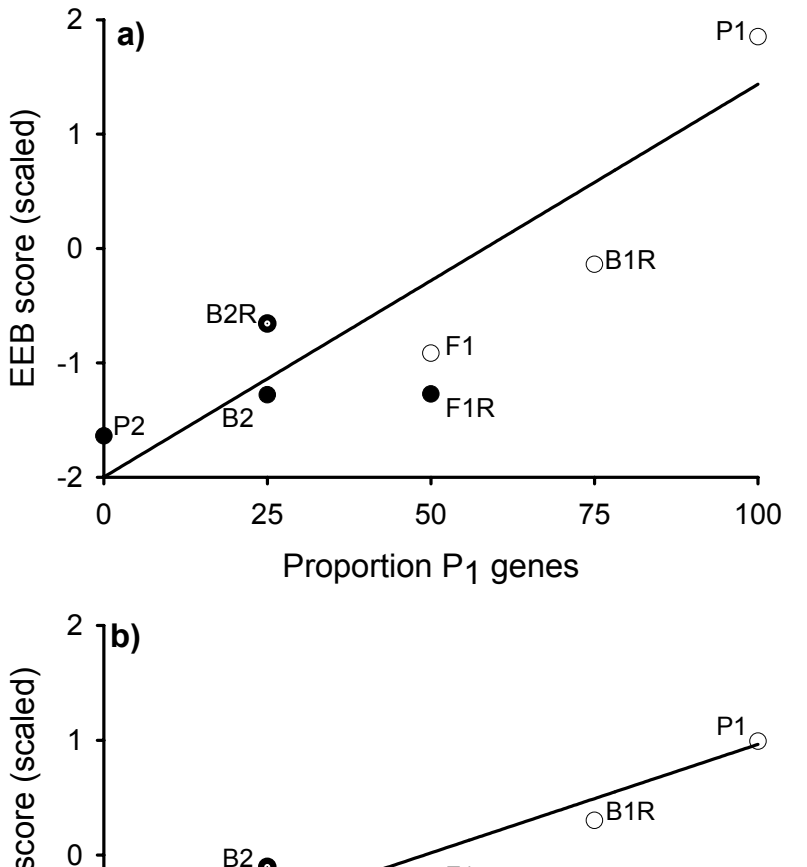
**Table 4.** Estimates of composite genetic effects underlying difference in EEB, exploration and boldness. Where: m = group mean, [a] = additive genetic component, [d] = genetic dominance component, $[a]_m$ = additive maternal component, $[d]_m$ = dominant maternal component and [sde] = sex-dependent component.

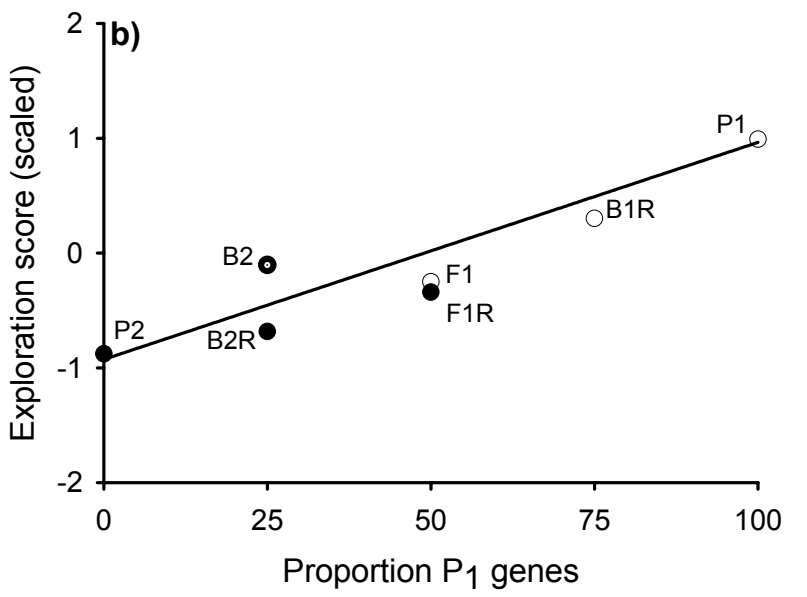
| | MODEL A | | | MODEL B | | |
|---|---|---|---|---|---|---|
| | **EEB** | **exploration** | **boldness** | **EEB** | **exploration** | **boldness** |
| **m** | 0.11 ± 0.10 | 0.06 ± 0.04 | 0.05 ± 0.07 | 0.17 ± 0.10 | 0.06 ± 0.04 | 0.05 ± 0.07 |
| **[a]** | 1.75 ± 0.10*** | 0.94 ± 0.04*** | 0.70 ± 0.09*** | 1.66 ± 0.10*** | 0.93 ± 0.04*** | 0.64 ± 0.09*** |
| **[d]** | -1.16 ± 0.19*** | -0.40 ± 0.15** | -0.84 ± 0.10*** | -1.02 ± 0.18*** | -0.29 ± 0.14* | -0.78 ± 0.09*** |
| **$[a]_m$** | 0.20 ± 0.19 | 0.10 ± 0.19 | 0.13 ± 0.05** | 0.23 ± 0.15 | 0.10 ± 0.14 | 0.14 ± 0.05** |
| **$[d]_m$** | | | | -0.33 ± 0.33 | -0.32 ± 0.22 | -0.16 ± 0.20 |
| **[sde]** | -0.11 ± 0.33 | -0.05 ± 0.30 | 0.08 ± 0.09 | | | |
| **df** | 3 | 3 | 2 | 4 | 4 | 3 |
| **$\chi^2$** | 1.494 | 0.369 | 3.704 | 7.651 | 3.586 | 4.267 |

* Significant at the 0.05 level
** Significant at the 0.01 level
*** Significant at the 0.001 level

In a first model, means were calculated for the separate sexes of the reciprocal $F_1$. An initial model was made in the form TRAIT = m + [a] + [d] (model A). To test whether the expression of exploration, boldness and EEB is sex dependent, we used group means and their standard errors of the original 'fast' and 'slow' lines and the reciprocal $F_1$. This model described the observed means adequately in the cases of exploration and EEB, but not boldness (Table 4, model A). Both [a] and [d] contributed significantly in all models. As m was scaled around 0, the grand mean is expected to be, and was, equal to zero in all cases. To test sex-dependence, this parameter was added to the model. In all cases this was done together with a maternal additive parameter ($[a]_m$), since the observed mean of the $F_1$ differed from the mean of the $F_1R$ cross, in boldness ($t_{42.6}$=2.674, p<0.05). The means did not differ from each other in exploration and EEB respectively ($t_{50}$=0.281, p=0.78; $t_{42}$=0.971, p=0.34). As expected, the parameter estimate for $[a]_m$ was not significant in either exploration and EEB when running the models in the form TRAIT = m + [a] + [d] + $[a]_m$ + [sde], but it was significant in boldness (Table 4, model A). So to test sex-dependent expression, $[a]_m$ was included in the model of boldness, but not in the models of exploration and EEB. In neither of the tests sex-dependent expression was significant, and the fit of the models did not increase significantly when sex-dependent expression was added (boldness: $\chi_1^2$=0.66; P=0.42; exploration: $\chi_1^2$=0.02; P=0.88; EEB: $\chi_1^2$=0.04; P=0.85). None of the minimal adequate models were significantly different from the observed means.

In a second model (model B), the observed means of all available groups were used to estimate $[a]_m$ and $[d]_m$. A new base model was made, in the form TRAIT = m + [a] + [d], with the means of all available lines and crosses ($P_1$, $P_2$, $F_1$, $F_1R$, $B_1$, $B_2$, $B_2R$). Since sex-dependent expression was not significant in model A, all groups were combined for sexes. Again the initial model adequately described the observed means in the cases of exploration and EEB, but this was not the case for boldness (Table 4, model B). The additive and the genetic dominance parameter were significant in all models. To these models the maternal parameters were added, so a model was formed, in the form TEST = m + [a] + [d] + $[a_m]$ + $[d_m]$. In all cases $d_m$ was the least significant parameter and the fit did not increase in comparison to the same models without $d_m$ (boldness: $\chi_1^2$=0.55, P=0.46; exploration: $\chi_1^2$=0.19, P=0.66; EEB: $\chi_1^2$=0.13, P=0.72). After removing $d_m$ all other parameters were significant in the boldness model. Removing $a_m$ from this model would significantly decrease the fit of the model ($\chi_1^2$=9.91, P<0.005). This was not the case for both exploration and EEB models (exploration: $\chi_1^2$=0.27, P=0.60; EEB: $\chi_1^2$=0.40, P=0.53). This does not automatically mean that the results for the two tests and the combined test are different. The additive maternal parameter of boldness does not differ significantly from either that of exploration ($t_{137}$=0.87, p=0.39) or of EEB ($t_{137}$=0.78, p=0.44). Removing any of the other (all significant) parameters would decrease the fit significantly in these models. All expected means generated through the minimal adequate models were not significantly different from the observed means (Table 4, model B).



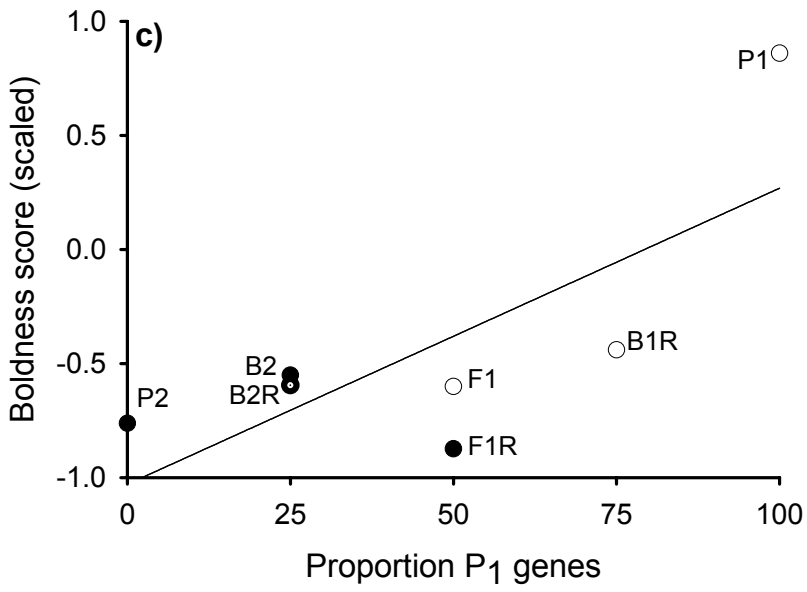




**Figure 1.** Mean observed test score of the lines and crosses, for (a) EEB, (b) exploration and (c) boldness. Cytoplasm origin of the lines are indicated with: ○ = $P_1$ cytoplasm; ● = $P_2$ cytoplasm; ⊙ = group is mix of individuals with $P_1$ and individuals with $P_2$ cytoplasm. For reasons of clarity, mean values are plotted without standard errors. The lines are the regression lines based on the expected means.

## DISCUSSION

Our results demonstrate that substantial additive and dominance effects are present in both the exploration test and the boldness test, and in the combination of the test scores. Traditionally it has been assumed that that there is little or no additive genetic variation in traits that influence fitness, as they are supposed to be under strong directional selection (e.g. Jones 1987). In the last decades however this assumption has been under discussion (Frank & Slatkin 1992). Controversy has arisen about implicit assumptions and interpretations of Fisher's 'Fundamental Theorem of Natural Selection' (Fisher 1930). Price & Schluter (1991) and Houle (1992) showed that low heritabilities in fitness related traits are not automatically caused by low amounts of additive genetic variation, but rather by a high residual variance. Estimates of the additive genetic component turned out not to be different from those in morphological traits, when expressed as a fraction of the mean value. Moreover, it is perhaps unreasonable that selection will constantly act in one direction in variable environments (Roff 1997). The net selection pressure over a longer time might therefore be low. This is expressed in the balancing selection view, which states that existing genetic variation available for adaptation is protected from selection by fluctuating selection pressures. Examples of this are antagonistic pleiotropy or frequency-dependent selection. But alternative theories like selection-mutation equilibrium, may also be plausible causes for the maintenance of additive genetic variation in avian personalities (Mousseau & Roff 1987; see Roff 1997).

Personality traits are most likely influenced by many loci, with each locus having a small effect on the trait. Therefore it may be important to not consider only additive but also non-additive sources of phenotypic variation (Falconer & Mackay 1996; Lynch & Walsh 1998). Merilä and Sheldon (1999) also pointed out that dominance variance is an important variance component in selection studies. In contrast to additive genetic variation, quantitative genetic theory predicts that the relative amount of genetic dominance variation should increase under selection (Mousseau & Roff 1987; Roff 1997; Merilä & Sheldon 1999; Mousseau & Roff 1987). This is confirmed in the study of Crnokrak & Roff (1995), who found that levels of dominance variance in life-history traits were higher than those in morphological traits.

A substantial dominance effect is more likely to occur in traits where variation is due to a relatively low number of variable loci. However, our results have to be seen in the right context. The lines used for the crosses have been selected for four generations with a small population size, and our results are therefore dependent on the animals chosen for the selection experiment. This implies that extrapolations from these results could be unreliable (Hill 1977).

Significant heritabilities of personalities in great tits are found both in the laboratory (Chapter 2) and in natural populations (Dingemanse *et al.* 2002). Since $h^2$ represents the ratio of additive genetic variance to total phenotypic variance, and environmental variance is smaller in the laboratory than in field populations, laboratory estimates of heritabilities are possibly overestimating natural heritabilities (Riska *et al.* 1989). Studies show however that laboratory estimates provide reasonable

estimations of magnitude and significance of heritabilities in the wild (Riska *et al.* 1989; Weigensberg & Roff 1996), but any difference may depend on the maternal and dominance effects (Blanckenhorn 2002). The fact that the laboratory estimate of the realised heritability derived from a regression on the cumulative response to selection as a function of the cumulative selection differential ($h^2 = 0.54$; chapter 2) is about twice the estimate of heritability derived from the parent-offspring regressions in natural populations (h2 = 0.34 Dingemanse *et al.* 2002) could possibly be caused by the large dominance effect found in this study (Blanckenhorn 2002). The correspondence however between both heritabilities was fairly high and the difference was not significant.

We found no evidence for sex-dependent expression of either of the traits, which is a remarkable finding since sex dependent expression is reported in extraversion in humans (Costa Jr *et al.* 2001). Extraversion is considered as boldness in non-human animals (Budaev 1999). Moreover, both in humans and in other animals sex differences in personality traits are reported (Buirski *et al.* 1978; Budaev 1999; Benus 2001). Other personality traits like aggression in mice clearly are differently expressed in both sexes (Sluyter 1994; Benus 2001). In humans, sex differences are found in the main personality axes Agreeableness, Neuroticism and Extraversion. Both biological and social psychological theories try to explain the existence of gender differences in personalities. Some biological theories predict that sex-dependent expression in personality traits arise from innate temperamental differences between the sexes, evolved by natural selection (Costa Jr *et al.* 2001). Evolutionary psychology predicts that sexes will differ in domains in which they have faced different adaptive problems throughout evolutionary history (Buss 1995). These are confirmed by some other biological theories, which point to hormonal differences and their effects on personality. Studies on human personalities confirm that the sex differences in androgens during development, cause differences in interests, activities and aggression (Berenbaum & Resnick 1997; Berenbaum 1999).

The presence of an additive maternal component in boldness and not in exploration was the only difference we found in the analysed traits. The calculated estimated additive maternal parameters for the different traits are however not significantly different from each other in a t-test, which indicates an effect of the small sample size and we recognise that with including the additive maternal parameter, we have reached the limits of the detection ability. The significant maternal component in boldness suggests however, that maternal effects are likely to play a role in both exploration and boldness. Since we collect the eggs just after laying, the most plausible maternal influence would be through the deposition of substances (e.g. maternal hormones) in the egg. Studies on maternal hormones (Schwabl *et al.* 1997; Eising *et al.* 2001), on the relation between maternal environment and antibodies (Heeb *et al.* 1998) and on the influence of females on their offspring sex ratio (Komdeur *et al.* 1997; Sheldon *et al.* 1999) show that female birds may control a surprisingly wide range of characteristics of their offspring. Recent theoretical, laboratory work and work in natural populations (McAdam *et al.* 2002), has suggested that heritable maternal effects can have important influences on the potential of

evolution (Wolf *et al.* 1998). Our results show that phenotypic expression is likely to be influenced by maternal hormones, but that this is independent of the offspring sex.

Although there is no evidence yet for a direct relationship between fitness and personalities, evidence is accumulating that personality traits affect reproduction, survival and dispersal (Armitage 1986; Réale & Festa-Bianchet 2002; Dingemanse *et al.* 2003). Our study on the genetic structure of avian personality traits, show that these traits have a substantial amount of additive genetic variance, a considerable dominance variance and sex-dependent expression is absent.

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CHAPTER 4

# Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities

**Kees van Oers[1,2], Pieter J. Drent[1], Piet de Goede[1] and Arie J. van Noordwijk[1]**

1. Department of Population Biology of Animals, Netherlands Institute of Ecology, PO Box 40, 6666 ZG, Heteren, The Netherlands
2. Evolutionary Population Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

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## ABSTRACT

Personalities are general properties of humans and other animals. Different personality traits are correlated and heritabilities of personality traits have been reported in humans and various animals. In great tits consistent, heritable differences have been found for exploration, which is correlated with various other personality traits. In this paper we validate if risk taking behaviour falls into the concept of these avian personalities. We found that risk-taking behaviour is repeatable and correlated with exploratory behaviour in wild-caught, hand-reared birds, and lines of a bi-directional selection experiment on 'fast' and 'slow' early exploratory behaviour differed for risk-taking behaviour. In addition to this, we found that within nest variation of risk-taking behaviour was smaller than the between-nest variation. To show that risk-taking behaviour has a genetic component in a natural bird population, we bred great tits in the laboratory and artificially selected on 'high' and 'low' risk-taking behaviour for two generations. Here we report a realized heritability of 19.3 ± 3.3 % for risk-taking behaviour. With these results we show in several ways that risk-taking behaviour is part of the avian personality construct. Moreover we prove that there is heritable variation for more than one correlated personality traits in a natural population, which demonstrates potential for correlated evolution.


## INTRODUCTION

Consistent individual differences in behaviour have been found in many animal species (Wilson *et al.* 1994; Gosling & John 1999). These differences in a range of behavioural traits have been labelled as temperament, coping strategies, styles or syndromes (Wechsler 1995; Boissy 1995; Koolhaas *et al.* 1999), comparable with human personalities (Zuckerman 1991). Evidence is accumulating, that the personality construct not only exists in humans, but also in other animals (Wilson *et al.* 1994; Clarke & Boinski 1995; Gosling 2001; Gosling & Vazire 2002). Two conditions have to be fulfilled for separate personality traits to represent a syndrome. First, the behavioural traits must be repeatable and heritable. Secondly, the behavioural traits have to be correlated with each other, within a single context.

In describing these behavioural syndromes, several domains are distinguished. Two broad personality dimensions are approach and avoidance motivation (Budaev & Zhuikov 1998; Elliot & Thrash 2002). Approach motivation is defined as behaviour that is directed by a positive event, while in avoidance motivation the behaviour is directed by negative events (Elliot & Covington 2001). An important field of study in behavioural ecology is that on the trade-off between approach and avoidance in the form of a cost-benefit trade-off between foraging and avoiding the risk for predation (Lima & Dill 1990; Lima 1998). Foraging activity may lead to an increase in predation risk (Godin & Smith 1988), but postponed foraging may have effects on the nutritional state of an animal (Van der Veen & Sivars 2000), and could thereby increase starvation probability (Sih 1997). Therefore, hungry animals are willing to take more risk, simply

as the costs of hiding and the benefits of risk taking increase with increasing hunger levels (Damsgard & Dill 1998). On the other hand, the absolute (Martin & Lopez 1999) and relative predation risk, and the predictability of predation risk (Sih 1992) may alter the balance between foraging and risk avoidance. Other factors influencing the trade-off between predation risk and feeding are food availability (Dill & Fraser 1997; Martin *et al.* 2003) and food properties (Cooper 2000), the quality of hiding places (Martin & Lopez 2000) and the distance to a possible hiding place or shelter (Walther & Gosler 2001). Culshaw and Broom (1980) showed, that when chicks were startled while foraging, the type of behaviour and the duration of the behavioural bout prior to the startle influenced the response to a startle.

Apart from the environmental factors mentioned above, individual characteristics such as e.g. age, size, sex, reproductive state, parasite prevalence or dominance status (Koivula *et al.* 1994; Candolin 1998; Abrahams & Cartar 2000; Kavaliers & Choleris 2001; Lange & Leimar 2001) can be responsible for differences in risk taking behaviour. Predation risk itself has on the other hand effects on scale of life-history decisions (for refs see Kavaliers & Choleris 2001). Also the phenotypic and genetic relation with other personality traits could directly or indirectly influence the trade-off between risk taking and foraging. Consistent individual differences in risk taking have already been reported in e.g. guppies (Godin & Dugatkin 1996) and pumpkinseed sunfish (Coleman & Wilson 1998). In humans it is suggested that approach-avoidance motivation even represents the foundation of several personality dimensions (Elliot & Thrash 2002). Covariation between risk-taking behaviour and individual differences in boldness and aggression has been found in several species, such as mice (Blaszczyk *et al.* 2000) and cichlid fish (Brick & Jakobsson 2002), but the genetic basis of risk taking behaviour and its relation to other personality traits is unknown in wild animal populations.

In great tits differences in exploration are phenotypically correlated with those in boldness (Verbeek *et al.* 1994), aggression (Verbeek *et al.* 1996; Drent & Marchetti 1999), feeding behaviour (Drent & Marchetti 1999; Marchetti & Drent 2000) and the reaction to physiological stress (Carere *et al.* 2001). In a four-generation bi-directional selection experiment on the combination of exploration and boldness (further referred to as early exploratory behaviour), in chapter 2 we showed that early exploratory behaviour has a genetic basis. In a study on wild great tits that were tested for exploration in the laboratory individuals also differed consistently in exploration behaviour and a comparable heritability was found through parent-offspring regression (Dingemanse *et al.* 2002). These consistent, heritable and co-varying reactions towards novel challenges can be seen as proof for the concept of avian personalities, comparable to human personalities (Gosling & Vazire 2002). To study personalities with a non-human animal as model species, one would preferably take a multidimensional personality approach (Budaev 1997), especially when studying personalities from an adaptive point of view. Natural selection influences different characters at the same time, and phenotypic correlations between personality traits have been shown in many studies Therefore we appreciate the need for the study of multiple behavioural traits in an integrative approach. Hereby we will be able to

incorporate ecological reality and evolutionary explanations of the underlying genetic structure of personalities.

To investigate whether and how risk-taking behaviour falls into the concept of avian personalities, we tested whether (I) risk-taking behaviour is repeatable and whether exploration and risk-taking behaviour are correlated. We therefore on one hand, investigated (II) whether this correlation exists in hand-reared great tits collected from a natural population and on the other hand, if (III) lines bi-directionally selected for 'fast' and 'slow' exploration differ for risk-taking behaviour. Furthermore, we assessed two estimations of heritability: we (IV) tested whether similarity is greater within broods than across-broods, which gives a grove heritability estimate. Moreover we (V) assessed the realized heritability of risk-taking behaviour by selecting for 'low' and 'high' risk-taking behaviour for two generations.

## MATERIAL AND METHODS

### Subjects

For purposes (I), (II), (IV) and (V) (see above) of this study we collected 94 great tits (*Parus major*) nestlings of 15 nests from two wild populations in 1998. Their biological parents raised these birds until an age of 10 days after hatching. At this point we took the birds from their nests, brought them to the laboratory and hand-reared them under standard conditions in the laboratory until independence (for details see chapter 2). After this period the juveniles were housed individually, tarsus was measured and they were tested for exploration as described below. At an age of 10 weeks, a blood sample was taken for sex determination. Birds were sexed according to the method of Griffiths (1998). Further more we used 73 birds of the fourth generation of the line bi-directionally selected for 'fast' (FE; n = 38) and 'slow' (SE; n = 35) early explorative behaviour (chapter 2), for purpose (III).

### Behavioural tests

To measure the early exploratory behaviour score (exploration and boldness) we performed two types of behavioural tests: A novel environment test (analogous to an open field test; Walsh & Cummins 1976), was followed by two tests of the reaction to different novel objects. The combination of the score of the novel environment test (further referred to as exploration) and the novel object test score (further referred to as boldness) is referred to as early exploratory behaviour. Early exploratory behaviour was used as the selection criterion in the bi-directional selection experiment of Drent *et al.* (chapter 2). The exploration test was carried out between 30 and 35 days after hatching, the boldness tests 10 and 12 days later (for details on the tests see chapter 2; Verbeek *et al.* 1994).

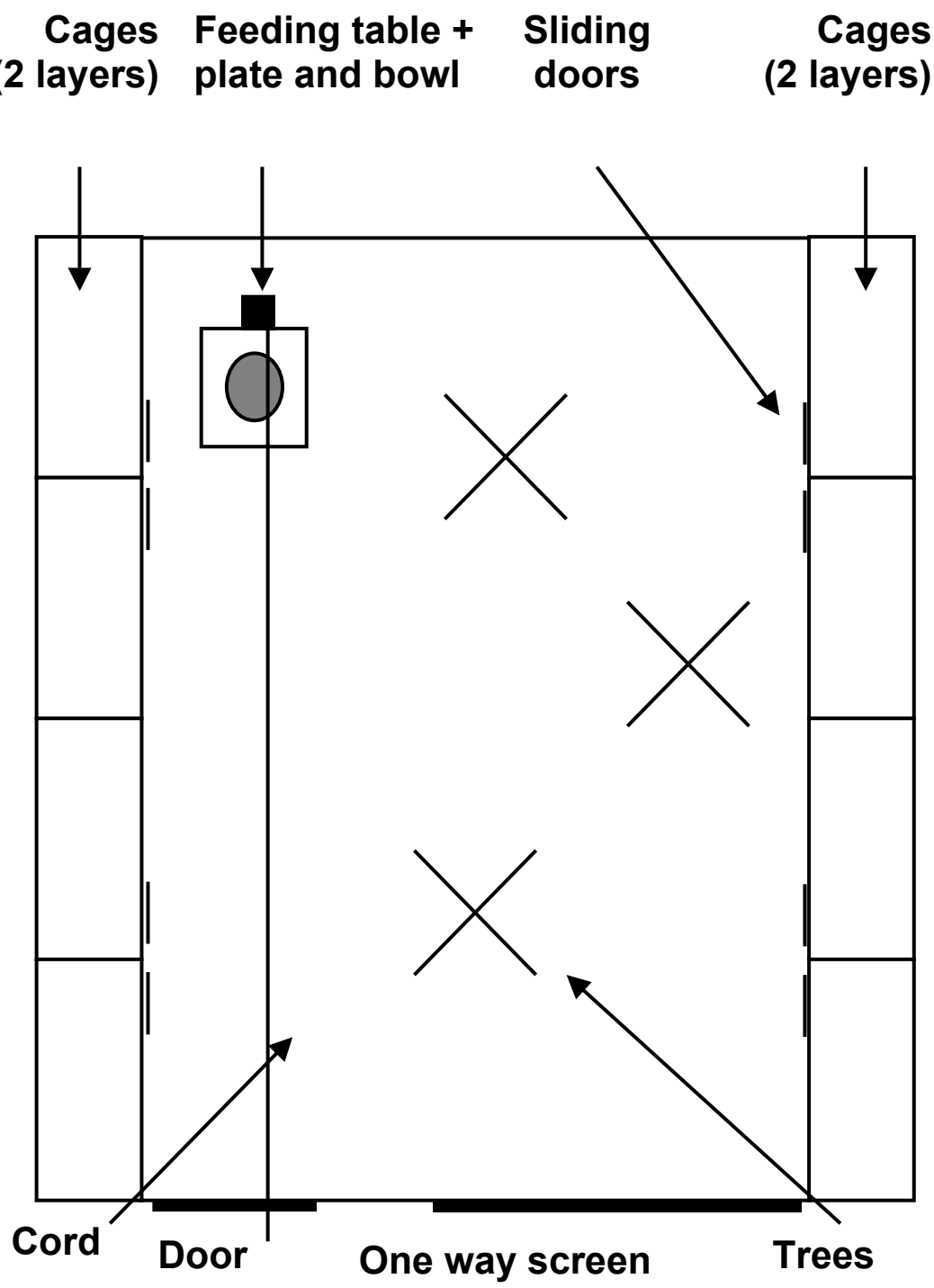


**FIGURE 1.** Plan of the observation room in which we tested risk-taking behaviour. Along each side-wall are eight sliding doors (in two rows of four above each other), which connect the cages to the room. The front wall had a 0.9x2.0 m door at the left side and a 1.1x0.16 m one-way screen for observation. The room contained three artificial trees and a feeding table (1.30 m high; platter 30 x 30 cm). The trees were made of wood with a trunk of 4x4 cm and a height of 1.5 m. Each tree had four cylindrical branches of 20 cm length. The upper two branches (5 cm below the top) were placed on opposite sides of the trunk, perpendicular to the lower branches (25 cm below the top). Birds entered the room through one of the sliding doors.

## Startle latency test

Risk-taking behaviour was tested in a test on the latency to return after a mild startle in a food context. This startle latency test took place 6-8 weeks after the boldness tests. For this test, we placed three trees and a feeding table in the observation room (Figure 1). The feeding table was equipped with a spring loaded, hinged steel plate of 7 x 7 cm, which was attached to the back of the tabletop. On the centre of the table we placed a bowl (diameter, 15 cm), of which the bottom was covered with mealworms. A cord attached to the plate, which was controlled from outside the observation room, made it possible to startle a bird: releasing the pressure on the cord at once caused the plate to spring up in front of the bird. After pulling the cord the plate got back into its initial position, invisible for the bird.

The test was build up into three phases. After entering the room, birds landed on a tree after flying around for a short time (1-10 seconds). We measured the time from the moment birds landed on a tree until the moment they took the first worm from the feeding bowl (first worm latency). In all cases the birds ate the worm in an artificial tree. Since the experimental set-up in combination with the observation room was new to the birds, we expect that the first latency to reflect a novelty effect. This first phase of the experiment, makes the birds familiar with the situation, to reduce this effect. In the second phase, we measured the time from the moment birds had eaten the worm until birds got back to the feeding table again and tried to get a second worm. Before the bird was able to actually take a worm, just as it landed on the feeding bowl, we startled the bird. We refer to this as 'second worm latency'. In the third phase, after the startle, we measured the time it took the bird to return to the table and actually take the second worm, the startle latency (also referred to as risk-taking behaviour).

If birds did not return to the table to take a worm within 20 minutes after the startle, we stopped the test (only 1 case). The time the birds took to handle and eat a worm was not included in any measure.

## Housing

After hand rearing, birds were kept individually in cages of 0.9x0.4x0.5 m with solid bottom, top, side and rear walls, a wire-mesh front and three perches. After the exploration and boldness tests (see below) the birds were housed in semi-open outdoor aviaries (2.0x4.0x2.5 m) in unisex flocks of 6-8 individuals, for a period of 6-8 weeks. After which they were placed back into their home-cage for the startle test. During all stages, we provided the birds with *ad libitum* water, commercial seed mixture and calcium. This was supplemented daily with mealworms and a mixture containing sour milk, ground beef heart, a multivitamin- and calcium solution and commercial egg mixture. Birds were kept under natural light conditions, with visual and vocal contact with other birds. Birds had no access to food two hours prior to the tests, and were deprived of mealworms two days, to increase their tendency to take mealworms during the test.

Boldness tests were conducted in the home cages. The exploration and the startle tests took place in an observation room of 2.4x4x2.3 m with sixteen individual cages connected to the room via sliding doors of 20x20 cm (chapter 2). Birds were let into the observation room without handling by manipulating the light conditions in the observation room and the adjacent cages before testing.

## Bi-directional artificial selection for risk-taking behaviour

For the parental generation, we selected those juveniles, which had the longest startle latency and the shortest startle latency. However, in order to get a labelling consistent with but distinguishable from former work, we will use 'high risk-taking' for birds with a short latency and 'low risk-taking' for birds with a long latency. Both 'high risk' and 'low risk' lines were started with 9 pairs. For the second generations we formed pairs from the first generation offspring by selecting the individuals with the shortest startle latency for the "high risk" line and longest startle latency for the "low risk" line, avoiding full sib and first cousin mating. The second generation of the 'high risk' and 'low risk' line were based on 8 and 5 pairs respectively. Pairs were kept in aviaries of 2.0 by 4.0 by 2.5 m from December onwards. All aviaries contained 4 nest boxes, so birds were able to choose between nest boxes to breed or roost in. Our aviary pairs lays eggs synchronous with birds of natural populations. In spring, aviaries were checked weekly when no nesting activity was observed (no material in nest boxes nor on feeding table). This frequency was increased to ones a day, when birds started nesting. Eggs were collected and exchanged with dummy eggs. Clutches of eight eggs from the same pair were brought to the field and incubated by foster females. Nestlings were collected at an age of 10 days and then hand-reared in the lab (for details on hand rearing see chapter 2).

The parental generation was sampled from two field populations in 1998. Because of sample problems, we needed three years (1999, 2000 and 2001) to obtain enough first-generation individuals to be able to produce enough second-generation pairs. The second generation was born in 2002.

**Table 1.** Mean log transformed first-worm, hunger and startle latencies with their standard errors. (Abbreviations: year, year of birth and first measurement; n, number of birds)

| year | n | first-worm | hunger | startle |
|---|---|---|---|---|
| 1998 | 109 | 1.90 ± 0.04 | 1.69 ± 0.03 | 1.82 ± 0.05 |
| 1999 | 43 | 1.60 ± 0.08 | 1.70 ± 0.03 | 1.91 ± 0.06 |
| 2000 | 37 | 1.30 ± 0.09 | 1.71 ± 0.06 | 1.85 ± 0.07 |
| 2001 | 39 | 1.15 ± 0.07 | 1.71 ± 0.04 | 1.65 ± 0.05 |
| 2002 | 22 | 1.15 ± 0.04 | 1.75 ± 0.03 | 1.62 ± 0.06 |

## Statistical analyses

The untransformed data were used in a Wilcoxon signed-ranks test to compare within-individual latencies and in a Spearman's rank correlations to compare the correlations between latencies. Because the variance in startle latency time increased with the mean value, this variable was log transformed (Zar 1999) for all analyses where normal distributions are assumed. Part of the phenotypic change from one generation to the next, might result from environmental variation between successive years or generations. To control for this between-year variation in the selection experiment, we used 250 birds raised during the same years as these generations as a control population (see table 1). The mean logarithmically transformed first-worm, second-worm and startle latencies for these birds per year (Table 1) were subtracted from the individual values of the animals involved in the selection experiment (Walsh & Lynch 2000). This same procedure was used for repeatability analyses, to separate sequence from year effects.

We calculated repeatability of risk-taking behaviour for all individuals for which we obtained multiple measurements (52 individuals 2 times; 40 individuals 3 times; 9 individuals 4 times). Repeatability, the proportion of the phenotypic variance explained by the individual (Falconer & Mackay 1996), was calculated following (Lessells & Boag 1987) and its standard errors following (Becker 1984). To test whether variation in risk-taking behaviour is related to sex, time of the day (in seconds after sunrise), age (juvenile or older) and size (tarsus at age of independence) we used a General Linear Model (GLM) and type III sums of squares to evaluate the influence of these factors and covariates. We constructed a model with all explanatory variables and all two-way interactions, for all first tests of an individual. We performed Pearson correlations to investigate relations between early exploratory behaviour, exploration and boldness, and first-worm latency, second-worm latency and risk taking behaviour. We used t-tests, assuming equal variation, to test whether lines bi-directionally selected for 'fast' and 'slow' early exploration behaviour differed in their time to take the first worm, second-worm latency and risk-taking behaviour.

One method to measure the heritability ($h^2$) of a trait is estimating the within-nest variance in relation to the between-nest variance by using a One-way ANOVA with nest as a grouping variable. Heritability was calculated as twice the intra-class correlation coefficient (Falconer & Mackay 1996). The intra class coefficient (repeatability) is calculated as the between-nest variance divided by the sum of the between-nest and the within-nest variance. This is however a rough estimate of $h^2$, and sets just sets an upper limit to the heritability (but see Dohm 2002), as it is most likely inflated due to common environment and genetic dominance effects (Falconer & Mackay 1996).

Realized heritabilities for each generation separately, were calculated by dividing the cumulative selection response by the cumulative selection intensity (Falconer & Mackay 1996; Lynch & Walsh 1998). The narrow sense heritability ($h^2$) measures the proportion of the total variance that is attributed to the effect of genes. This is defined as the ratio of the additive genetic variance ($V_A$) to total phenotypic variance ($V_P$), with $h^2 = V_A/V_P$ (Falconer & Mackay 1996). Realized heritability of the selection experiment

was measured as the unweighted linear regression of the cumulative selection differential and the cumulative response to selection (Walsh & Lynch 2000). All statistical tests are two-tailed, and p values $< 0.05$ are considered as being significant. We used SPSS version 10.1 for Windows for all analyses.

## RESULTS

### Test results

In our startle test we measured the length of the latencies in three phases. In each of the latencies there was considerable individual variation. Since part of the phenotypic variation may be due to factors with non-permanent effects, and we are mainly interested in phase three, we tested whether variation in risk-taking behaviour (phase 3) was related to several explanatory variables (time of the day, sex, age, size). Risk-taking behaviour was not related to either of these variables (GLM; time of the day, $F_{1,257} = 2.40$, $p = 0.12$; sex, $F_{1,328} = 2.22$, $p = 0.14$; age (juvenile or older), $F_{1,333} = 0.74$, $p = 0.39$; size (tarsus), $F_{1,209} = 0.29$, $p = 0.59$) , nor any of the interactions (GLM; all $p > 0.20$).

The latency to return to the feeding table after having eaten the first worm (second-worm, $\xi = 69.28 \pm 10.16$ sec) was significantly shorter (Wilcoxon signed-ranks test; $n = 94$, $z = -6.05$ and $p < 0.0001$) than the latency to take the first worm (first worm latency, $\xi = 142.31 \pm 18.13$ sec); birds almost immediately returned to the feeding table to try to get another mealworm. The mean startle latency (startle latency, $\xi = 144.30 \pm 19.00$ sec) was again significantly larger than the second-worm latency (Wilcoxon signed-ranks test; $n = 94$, $z = -4.59$ and $p < 0.0001$), which shows that the startle had an effect on the behaviour of the birds. The first worm latency (novelty effect) was positively correlated with the second-worm latency ($r_p = 0.58$, $n = 15$ and $p = 0.02$) and there was a tendency for a correlation with the startle latency ($r_p = 0.51$, $n = 15$ and $p = 0.05$). The second-worm latency was not correlated with startle latency ($r_p = 0.20$, $n = 15$ and $p = 0.49$).

### Repeatability

Due to learning effects, repeatability's are difficult to measure over a relative short period (Dingemanse *et al.* 2002). When we measured repeatability of risk-taking behaviour (phase 3) with between test intervals of 1 year, we found a repeatability of $0.26 \pm 0.07$. Neither the first-worm latency ($r = 0.06 \pm 0.07$) nor the second-worm latency ($r = 0.11 \pm 0.07$), were repeatable between tests, and no within-individual variables (time of the day, sex, age and size) or any interactions for first-worm latency (all $P > 0.34$) and second-worm latency (all $p > 0.35$) were good predictors for the phenotypic variation.

To see whether a learning effect exists in the different phases of the test, we compared only the first two tests of each individual. We found a sequence effect for the first-worm latency (GLM; $F_{1,201} = 8.36$, $p = 0.005$), but not for the second-worm

latency (GLM; $F_{1,201}$ = 0.71, p = 0.40) or for the risk-taking behaviour (GLM; $F_{1,201}$ = 0.25, p = 0.62).

These measurements are based on individual test scores. As siblings are expected to be more alike than non-relatives, these tests contain a certain amount of pseudo-replication. This implies that F-values may be overestimated in tested hypotheses, but this does not influence our result of finding no relations.

## Risk taking behaviour and early exploratory behaviour in hand-reared nestlings

In the sample, which was collected as nestlings from the wild, no significant correlation could be detected between early exploratory behaviour and either first-worm latency ($r_p$ = 0.42, n = 15 and p = 0.12) or second-worm latency ($r_p$ = 0.14, n = 15 and p = 0.62). In figure 2 risk taking behaviour is plotted against early exploratory behaviour (EEB). Although no significant relation was found ($r_p$ = 0.45, n = 15 and p = 0.09), there is a tendency that fast explorers come back to the feeding table after a startle sooner than slow explorers do.

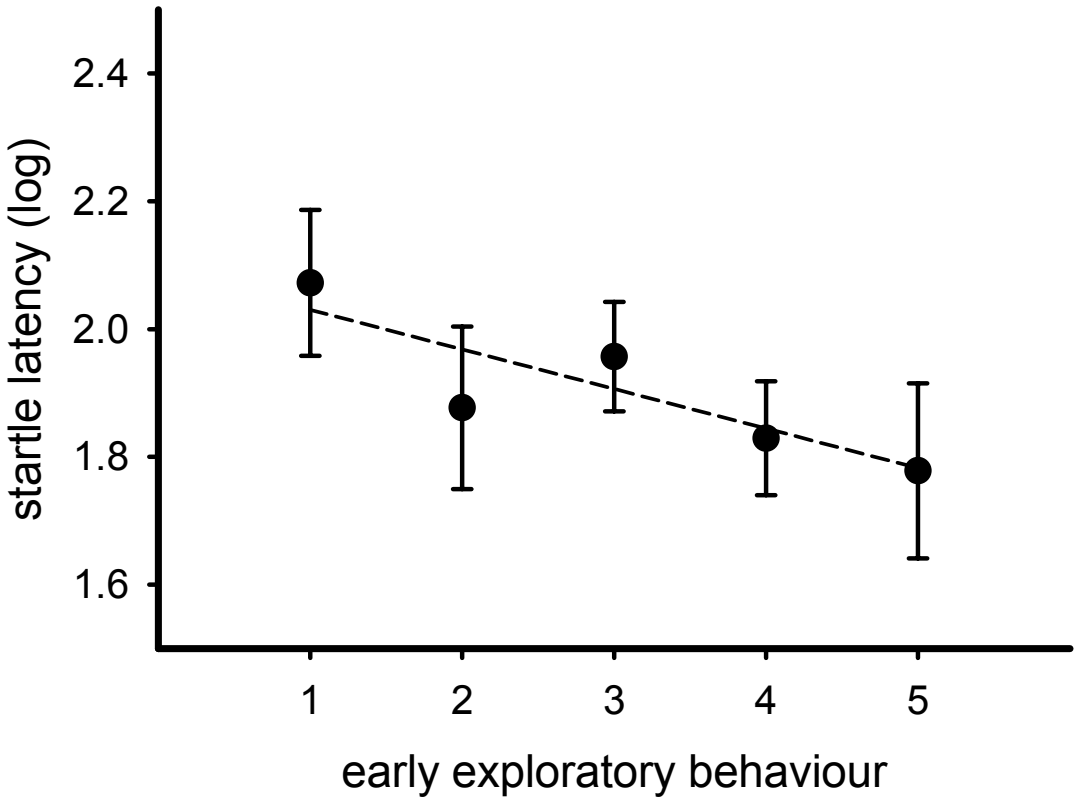


**FIGURE 2.** Mean startle latency with se. Individuals used are unselected birds collected from natural populations as juveniles. For reasons of clarity, early exploratory scores are presented as into 5 groups (1-4, 5-8, 9-12, 13-16, 17-20). Statistical testing has been done on original values. Original scores have been used, as all tests have been done during one year.

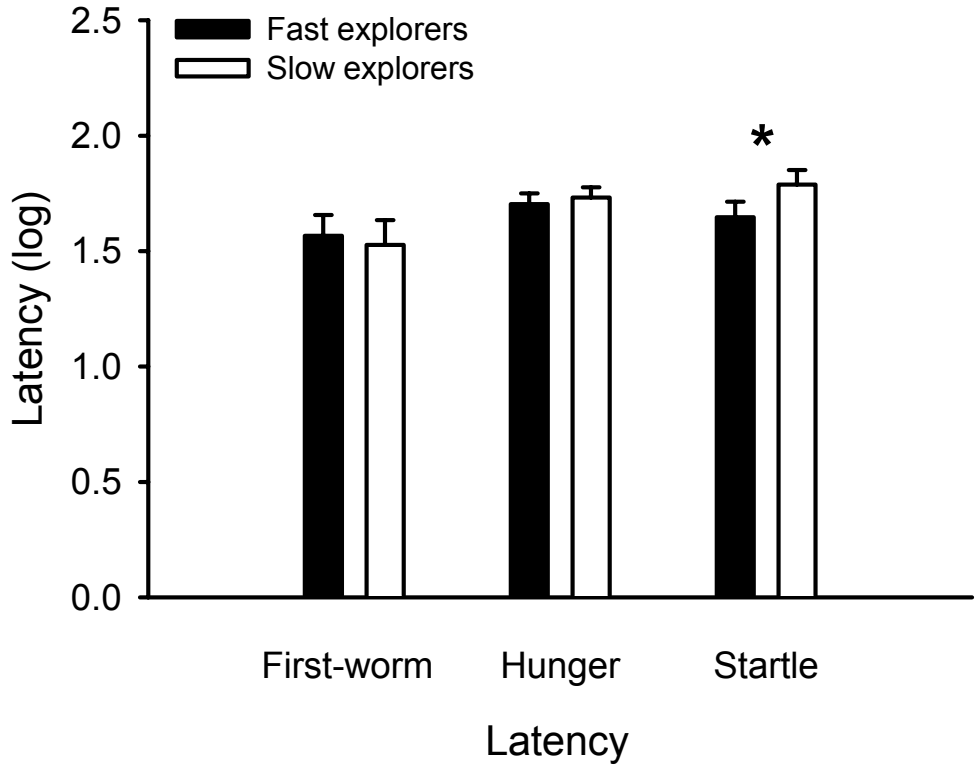


**FIGURE 3.** The time to take the first worm, and the latencies to return to the feeding table after having eaten the first worm and the startle plotted for the lines selected for "fast" and "slow" early exploratory behaviour. * $p < 0.05$. Latencies represent means and SE for the different part of the startle test.

When looking at the separate components of early exploratory behaviour (exploration and boldness) we see that the first-worm latency is correlated with exploration ($r_p = 0.84$, $n = 15$ and $p < 0.0001$), but not with boldness ($r_p = 0.21$, $n = 15$ and $p = 0.46$), which also counts for the second-worm latency (exploration: $r_p = 0.62$, $n = 15$ and $p = 0.02$; boldness: $r_p = 0.31$, $n = 15$ and $p = 0.26$). Risk-taking behaviour was correlated with exploration ($r_p = 0.37$, $n = 15$ and $p = 0.02$) but not with boldness ($r_p = 0.17$, $n = 15$ and $p = 0.55$). All phenotypic correlations are calculated as being positive, as risk-taking behaviour, exploration and boldness have all originally been measured in seconds. Exploration and boldness are however converted to inverted scores, which would have caused phenotypic correlations to be negative.

### Risk taking behaviour in lines selected for early exploratory behaviour

The results in the hand-reared juveniles were confirmed when looking at the lines bi-directionally selected for 'fast' and 'slow' early exploratory behaviour (figure 3). The lines neither differed in the first worm latency (t-test; $t_{71} = -0.91$ and $p = 0.37$) nor in the second-worm latency (t-test; $t_{71} = -1.08$ and $p = 0.29$). In contrast to the first two phases, the latency to come back to the feeding table after the startle differed for the two lines: fast explorers came back sooner to the feeding table than slow explorers did (t-test; $t_{71} = -2.15$ and $p = 0.04$).

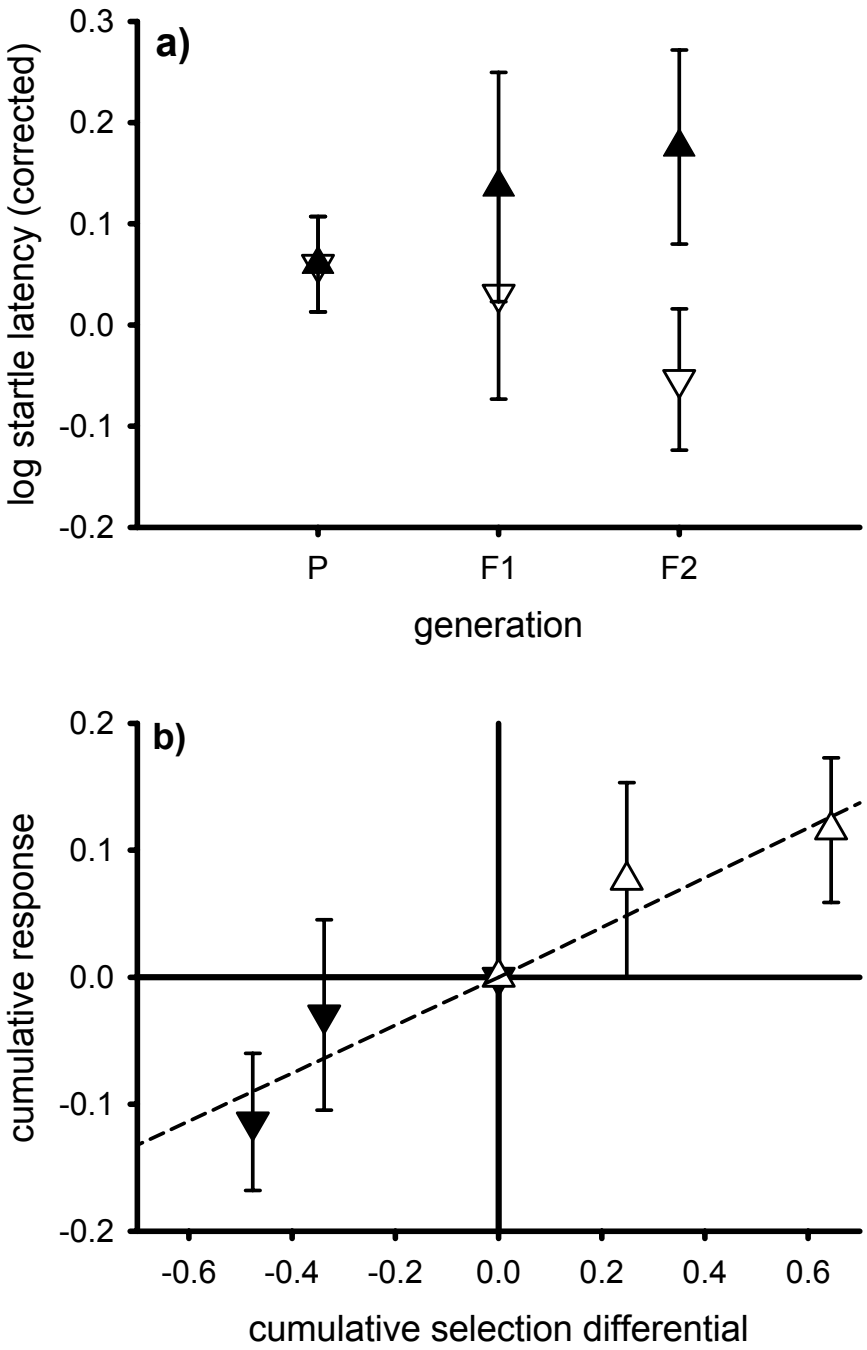


**FIGURE 4.** Response to artificial selection per generation (a) and relative to the cumulative selection differential (b) for both up-selection (filled triangle) and down-selection (inverted open triangle) with standard error of mean. Lines represent regression lines for up- and down-selection. The slopes for up- and down-selection separately are 0.19 (linear regression; $r^2 = 0.96$, $F_{1,2} = 21.14$ and $p = 0.136$) and 0.20 (linear regression; $r^2 = 0.88$, $F_{1,2} = 6.97$ and $p = 0.230$), respectively.

## Heritability of risk-taking behaviour

The mean brood size of the collected nests was 6.27 ± 0.87 (range 3-10). The within-nest variance was smaller than the among-nest variance (GLM; $F_{14,79} = 2.21$, $p = 0.014$). This demonstrates that young from the same brood show more resemblance to each other than to offspring of other broods. The heritability derived from this full-

sib analysis was 0.32 ± 0.20, but sample size is to small to make this significantly different from zero (t-test; $t_{14} = 1.88$, $p = 0.13$).

In table 2 the population measures of risk-taking behaviour are given for the parental generation, first and second generation of the bi-directional selection on 'high' and 'low' risk taking behaviour. Although the response to selection fluctuated during selection, we found a significant difference between the two lines (t-test; $t_{48} = -1.982$ and $p = 0.05$) after two generations of selection (figure 4a), with birds of the 'high' risk line returning 73 seconds earlier to the feeding table than birds of the 'low' risk line. In figure 4b the cumulative response to selection (response compared to the starting population) has been plotted against the cumulative selection differential (the deviation of mean of the individuals used as parents from the mean value in their generation). The realized heritability in the base population is the total of the observed phenotypic variance that can be attributed to genetic factors, which in this case is calculated from the regression coefficient of the cumulative response to selection over the cumulative selection differential, through the origin: 0.19 ± 0.03 (linear regression; $r^2 = 0.93$, $F_{1,4} = 37.85$ and $p < 0.01$). The heritabilities calculated refer to log latency, which implies that changes are proportional rather than additive.

**Table 2.** Population measures of risk-taking behaviour for the juvenile populations of the P, first and second generation of selection, corrected for between-year differences.
(Abbreviations: $S_{cum}$, the cumulative selection differential; n, total number of offspring tested; m, mean; s.e.m., standard error of mean; $h^2$, heritability; $V_P$, phenotypic variance; $V_A$, additive genetic variance.)

| *type* | *generation* | $S_{cum}$ | *n* | *m* | *s.e.m.* | $h^2$ | $V_P$ | $V_A$ |
|---|---|---|---|---|---|---|---|---|
| down-selection | P | | 94 | 0.060 | 0.047 | | 0.209 | |
| | 1 | -0.338 | 10 | 0.030 | 0.104 | 0.09 | 0.108 | 0.010 |
| | 2 | -0.476 | 28 | -0.054 | 0.070 | 0.24 | 0.137 | 0.033 |
| Up-selection | 1 | 0.249 | 23 | 0.136 | 0.113 | 0.31 | 0.295 | 0.091 |
| | 2 | 0.644 | 22 | 0.176 | 0.096 | 0.18 | 0.202 | 0.036 |

## DISCUSSION

We showed that individual great tits collected from two populations and hand reared in the laboratory, consistently differ in risk-taking behaviour, independent of sex, size or time of the day at which the test was carried out. There is a tendency that risk-taking behaviour is correlated with early exploratory behaviour in these birds. Lines selected for 'fast' and 'slow' exploration differ in risk-taking behaviour. Fast explorers responded less to a startle, and thereby returned sooner to a feeding table than slow explorers do. We also demonstrate that within-brood variation in risk-taking behaviour is smaller than the among-nest variation. In a full-sib analysis, we found a

heritability of 32 %. Moreover our results of a bi-directional selection experiment on 'high' and 'low' risk taking behaviour proved that variation in risk taking behaviour in a wild bird population is heritable. We found a realized heritability of 19 %, based on the selection over two generations.

Risk-taking behaviour as measured in our test in the laboratory can be seen as a standardised measure of the individual outcome of the trade-off between finding food and avoiding risk of predation. Although no predator was present in the vicinity of the birds, this test still reflects situations occurring in the wild. The behaviour of birds startled by predators is similar to when they are disturbed by an unknown cause (Van der Veen 2000). When attacked by a predator, birds fly away from the place they are foraging, fly around and land in a tree or seek shelter ( see e.g. Ficken & Witkin 1977). We recognise that the time to restart foraging also depends on social interaction with flock mates; a next step would therefore be to see whether the presence of other birds, and their behaviour would influence these decisions.

The time it took birds to take the first worm was correlated with exploration, but not with boldness and early exploratory behaviour. So, as expected novelty effects were found the moment birds entered the room, but birds only experienced the room or the experimental set-up, and not the attributes themselves as novel. This can be explained by the experience the birds gained before testing. The observation room was not new anymore, since they experienced it in the exploration test, six to eight weeks earlier. After the tests for boldness and exploration, birds were submitted to group living in aviaries, where a feeding table and artificial trees were present. Therefore, the attributes were not novel to them at the time of testing, and no fear of novelty was present. The particular set-up of the trees and table in the room were new when the test for risk-taking behaviour was conducted for the first time, but not when this test was conducted for the second time, a year later. The decrease in latency between the years is therefore comparable with the difference between the first-worm latency and the second-worm latency within a test. This learning effect is also present in the exploration test itself, which has been shown on wild great tits (Dingemanse *et al.* 2002). The shorter the time between the separate tests, the faster the birds do explore the observation room. Also the correlation between the first-worm latency and the startle latency would suggest that both are measures of a common motivational state, elucidated by different challenges: a novel experimental set-up at the beginning of the test, and an unexpected startle in a food context later on.

The fact that the first-worm latency is correlated with the second-worm latency indicates that on hand, the novelty effects present in the first-worm latency have not completely vanished in the second-worm latency. This is confirmed by the correlations with exploration, which are present for both latencies, but the correlation between exploration and second-worm latency is smaller that the one with the first-worm latency, indicating an eroding effect. On the other hand, if the second-worm latency resembles a hunger state, this is also likely to be also present in both measurements. Risk-taking behaviour tends to be correlated with the first-worm latency, this points to a common motivational background for these two latencies. As it is however not

correlated with the second-worm latency, the hunger state seems relatively less important.

The heritability found for risk-taking behaviour in this study is lower than the heritability found for early exploratory behaviour in the four-generation bi-directional selection experiment on 'fast' and 'slow' exploration ($h^2$ = 54%; chapter 2). This confirms comparable findings in human personalities using a Three-dimensional Personality Questionnaire (TPQ). Here heritabilities for harm avoidance (i.e. risk taking behaviour) are typically lower than those for e.g. novelty seeking (i.e. exploration and novelty; Ebstein *et al.* 2000). We can find two reasons for this difference. First, the test on risk-taking behaviour is taken later in life than the exploration and boldness test. This leaves more scope for learning effects. Secondly, when the difference is a more adaptive one, it could be that there is more selection on risk-taking behaviour in the natural populations of which we derived the birds. Due to stronger selection additive genetic variation could decrease (e.g. Jones 1987; but see e.g. Frank & Slatkin 1992).

Laboratory estimates of heritabilities may not be good predictors of heritabilities in natural populations, owing to a reduction in environmental variability in the laboratory (Riska *et al.* 1989; but see chapter 2 and Dingemanse *et al.* 2002). Results of several comparative studies however showed that laboratory estimates are somewhat higher, but not different (Weigensberg & Roff 1996; Bryant & Meffert 1998; Blanckenhorn 2002).

We cannot completely exclude environmental maternal effects on the estimation of $h^2$ for risk-taking behaviour. Females can alter the concentration of maternal hormones deposited in the eggs (Schwabl 1993). Individual differences in female behaviour are known to cause between-nest differences in egg hormone concentrations (Whittingham & Schwabl 2002). The female is thereby able to indirectly influence the behaviour of her young (Schwabl 1993; Eising *et al.* 2001), despite the fact that our young were raised by foster parents until 10 days after hatching, and hand reared from day 10 until independence. Also heritable maternal effects may have influences on heritabilities (McAdam *et al.* 2002). In earlier findings on the influence of maternal effects on the phenotypic variation in exploration however, we revealed that although maternal effects are present, they are relatively small compared to additive and dominant genetic effects (Chapter 3). Hormones are however known to play a role in risk-taking behaviour (Boissy 1995; Koolhaas *et al.* 1999; King 2002).

Our selection results are clear after correction for year effects. The correction for these kinds of effects through the use of a control population is a standard technique in selection experiments (Walsh & Lynch 2000). At the same time between-year variation in risk-taking behaviour cannot be explained by variation in age, sex or size in our sample. This indicates that environmental factors, in the sense of experiences early in life, are important in the expression of risk-taking behaviour. These effects, however seem to shift the distribution between years, rather than interact with year.

We find that risk-taking behaviour is correlated to other aspects of avian personalities. Novelty, exploration and risk-taking behaviour seem to be part of one personality concept, which is in line with the results of other studies on personalities (Mather & Anderson 1993; Budaev & Zhuikov 1998; Weiss *et al.* 2000), and coping

styles (Benus *et al.* 1991) in domesticated animals. Iguchi *et al.* (2001) showed in a study on two groups of cloned siblings of red-spotted cherry salmon, that there were heritable consistent between-clone differences in three principal components derived from several behavioural measurements. They labelled these PC's boldness, activity and carefulness (comparable to boldness, novelty and risk-taking in our study), showing an integration of these genetically governed components. Whether these behaviours are real independent behaviours is still unclear and studies on the functional architecture of personality traits in natural populations are needed.

Risk taking behaviour is known to influence life-history decisions (Grand 1999), and evidence is also accumulating that other personality traits affect reproduction, survival and dispersal (Armitage 1986; Eaves *et al.* 1990; Réale *et al.* 2000; Fraser *et al.* 2001; Dingemanse *et al.* 2003). Our study shows that personality traits are correlated and have a substantial amount of additive genetic variance, and therefore gives scope for co-selection for different traits or dimensions. This implies that natural selection on a trait in one context could have consequences on evolution of another trait (Price & Langen 1992). To study the co-existence of adaptive individual strategies in natural populations, these genetic correlations between different personality traits need more study.

Budaev (1998) already showed in his study on Guppies, the importance of the use of more than one dimension in animal personality studies. With the results of two artificial selection experiments, we now have proof for the genetic basis of at least two personality dimensions in great tits. These traits are correlated and consistent within context. With this we have a powerful tool to investigate the interactions between multiple personality dimensions, but moreover we will be able to get a better grip on the genetic architecture of personalities in animals from a wild population.

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CHAPTER 5

# Context dependence of avian personalities: risk-taking behaviour in a social and a non-social situation

**Kees van Oers[1,2], Margreet Klunder[1], and Pieter J. Drent[1]**

1. Department of Population Biology of Animals, Netherlands Institute of Ecology, PO Box 40, 6666 ZG, Heteren, The Netherlands
2. Evolutionary Population Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

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## ABSTRACT

We studied context dependence of avian personalities in the great tit (*Parus major*). Individual birds were tested for their latency to come back to a feeding table after a gentle startle (startle latency) in a social and a non-social context. In the social context an accompanying bird was foraging in a parallel section of the same room, separated by a transparent foil. We found a context dependent reaction that differed for both sexes. Females became slower in general in the social test, while male reaction to context change was dependent on behavioural type. Slow males had shorter startle latency in the presence of a companion that decreased with increasing activity of the companying bird, while the latency of fast male explorers was independent on the presence and behaviour of an accompanying bird. These results show that individual differences in behaviour are context dependent and that sexes react differently to the social context. These results are discussed in the perspective of domain specificity and domain generality of personalities.

## INTRODUCTION

Consistent individual differences in behaviour have been found in many animal species (Wilson *et al.* 1994; Gosling & John 1999). These differences in a range of behavioural traits have been labelled as temperament, coping strategies, styles or syndromes (Wechsler 1995; Boissy 1995; Koolhaas *et al.* 1999), comparable with human personalities (Zuckerman 1991; Bouchard & Loehlin 2001). Evidence is accumulating, that personalities not only exists in humans, but also in other animals (Wilson *et al.* 1994; Clarke & Boinski 1995; Gosling 2001; Gosling & Vazire 2002). Two conditions have to be fulfilled for separate personality traits to represent a syndrome. First, the behavioural traits must be repeatable and heritable. Secondly, several behavioural traits have to be correlated with each other, within a single context. Although in many animals, personalities are considered as general characteristics, with an underlying physiological mechanism (Koolhaas *et al.* 1999) that are expressed across many situations, it is suggested that individual differences may well be context dependent (Wilson *et al.* 1994).

Two broad dimensions in behaviour are approach and avoidance motivation (Elliot & Covington 2001). Approach motivation is defined as behaviour that is directed by a positive event, while in avoidance motivation the behaviour is directed by negative events (Elliot & Covington 2001). An important field of study in behavioural ecology is that on the trade-off between approach and avoidance in the form of a cost-benefit trade-off between foraging and avoiding the risk for predation (Lima & Dill 1990; Lima 1998). Foraging activity may lead to an increase in predation risk (Godin & Smith 1988), but postponed foraging may have effects on the nutritional state of an animal (Van der Veen & Sivars 2000), and could thereby increase starvation probability (Sih 1997). Therefore, hungry animals should be willing to take more risk, simply because the costs of hiding and the benefits of risk taking increase with increasing hunger

levels (Damsgard & Dill 1998). On the other hand, the absolute (Martin & Lopez 1999) and relative predation risk, and the predictability of predation risk (Sih 1992) may alter the balance between foraging and risk avoidance. Other factors influencing the trade-off between predation risk and feeding are food availability (Dill & Fraser 1997; Martin *et al.* 2003) and food properties (Cooper 2000), the quality of hiding places (Martin & Lopez 2000) and the distance to a possible hiding place or shelter (Walther & Gosler 2001). Culshaw and Broom (1980) showed, that when chicks were startled while foraging, the type of behaviour and the duration of the behavioural bout prior to the startle also influenced the response to a startle.

It is generally known that the presence and number of conspecifics also plays an important role in this decision conflict (for a review see Lima & Dill 1990). Individuals within groups may be more risk-prone than solitary individuals, since living in groups decreases the individual predation risk (Elgar 1989). As group-living also increases competition for food (Goss-Custard 1980), an alternative hypothesis is that certain individuals might be more willing to take risks than others, to get their share of the available resource (Grand & Dill 1999). The composition of a group may therefore influence individual risk-taking behaviour. Most studies hereby focus on differences in e.g. age, size, sex, reproductive state, parasite prevalence or dominance status (Koivula *et al.* 1994; Lange & Leimar 2001).(Candolin 1998; Abrahams & Cartar 2000; Kavaliers & Choleris 2001) A third hypothesis states that individuals may benefit from subtracting information or from copying behaviour from other individuals within the group. This information will on one hand depend on the behaviour if the tutor. On the other hand, individuals differ in the information they subtract from other individuals (see Marchetti & Drent 2000) and individual differences in risk-taking behaviour are related to other behaviours (e.g. Blaszczyk *et al.* 2000). Approach and avoidance motivation have been identified as an important factor in studies on consistent individual differences in behavioural traits (Budaev & Zhuikov 1998; chapter 4; Elliot & Thrash 2002). Covariation with other consistent individual differences may therefore directly or indirectly influence the trade-off between risk taking and foraging. Although the necessity of taking a more integrative approach to behaviour is increasingly appreciated, this aspect has had little attention (Brick & Jakobsson 2002).

The great tit is a small hole nesting passerine that inhabits almost all Eurasian wooded areas. Males are actively defending territories during breeding and early spring, but great tits are highly social outside these periods. Verbeek *et al.* (1994) showed consistent individual differences in great tits, based on early exploratory behaviour (EEB). Exploratory behaviour of great tits is heritable (Dingemanse *et al.* 2002; chapter 2) and correlated with aggressive behaviour in pair-wise confrontations (Verbeek *et al.* 1996), recovery time and behaviour after a contest (Verbeek *et al.* 1999), reaction to stress (Carere *et al.* 2001) and foraging behaviour (Drent & Marchetti 1999; Marchetti & Drent 2000). Another behavioural trait, which is related to exploration, is risk-taking behaviour. In chapter 4 we showed that fast explorers return quicker to a feeding table with mealworms after being startled than slow explorers do. In this startle test birds were tested in a non-social context. However, great tits live an important part of the year in social groups (Drent 1984) and several

studies have shown that this social life can affect the behaviour of the animal (for a review: see Galef & Giraldeau 2001).

In this study we investigate whether the relation between exploratory behaviour and risk-taking is dependent on social circumstances. We thereby concentrate on difference in risk-taking behaviour between a test with and a test without a companion. Our aim is to assess (i) whether risk-taking behaviour in great tits depends on the presence of a companion bird and (ii) the behaviour of this companion. For both issues we examined whether birds of different exploratory types (Verbeek *et al.* 1994; chapter 2) reacted in a different way to this change in context.

## METHODS

### Subjects and housing

Ten days after hatching we took juvenile great tits from their nests in natural populations and hand-reared them under standard conditions in the laboratory until independence (for details see chapter 2). After this period the juveniles were housed individually in cages of 0.9x0.4x0.5 m, solid bottom, top, side and rear walls a wire-mesh front and three perches. When birds were about 10 weeks old, a blood sample was taken for sex determination. We measured exploration behaviour when the juveniles were five weeks old. After the exploration test (see below) the birds were housed in semi-open outdoor aviaries (2.0x4.0x2.5 m) in unisex flocks of 6-8 individuals, for a period of 6-8 weeks. After which they were placed back into their home-cage for the startle test (see below). During all stages, we provided the birds with *ad libitum* water, commercial seed mixture and calcium. This was supplemented daily with mealworms and a mixture containing sour milk, ground beef heart, a multivitamin- and calcium solution and commercial egg mixture. Birds were kept under natural light conditions, with visual and vocal contact with other birds. Birds had no access to food two hours prior to the tests, and were deprived of mealworms two days before testing, to increase their tendency to take mealworms during the test.

We used 49 juveniles (16 FE males, 13 SE males, 9 FE females and 11 SE females) and 16 adult males (8 being FE and 8 SE). These adults were birds from lines selected for 'fast' and 'slow' early exploratory behaviour (chapter 2). These adult birds will further be referred to as companions. Before the experiment started, we weighed all birds and measured their tarsus.

### Behavioural tests

*exploration*

As a measure of exploration we used a novel environment test, in which the way birds explore an experimental room of 2.4 x 4 x 2.3 m with five artificial trees was recorded. We used the time it took a bird to visit four out of five trees to classify birds in either Fast Explorers (FE) or Slow Explorers (SE) (for details on the tests see chapter

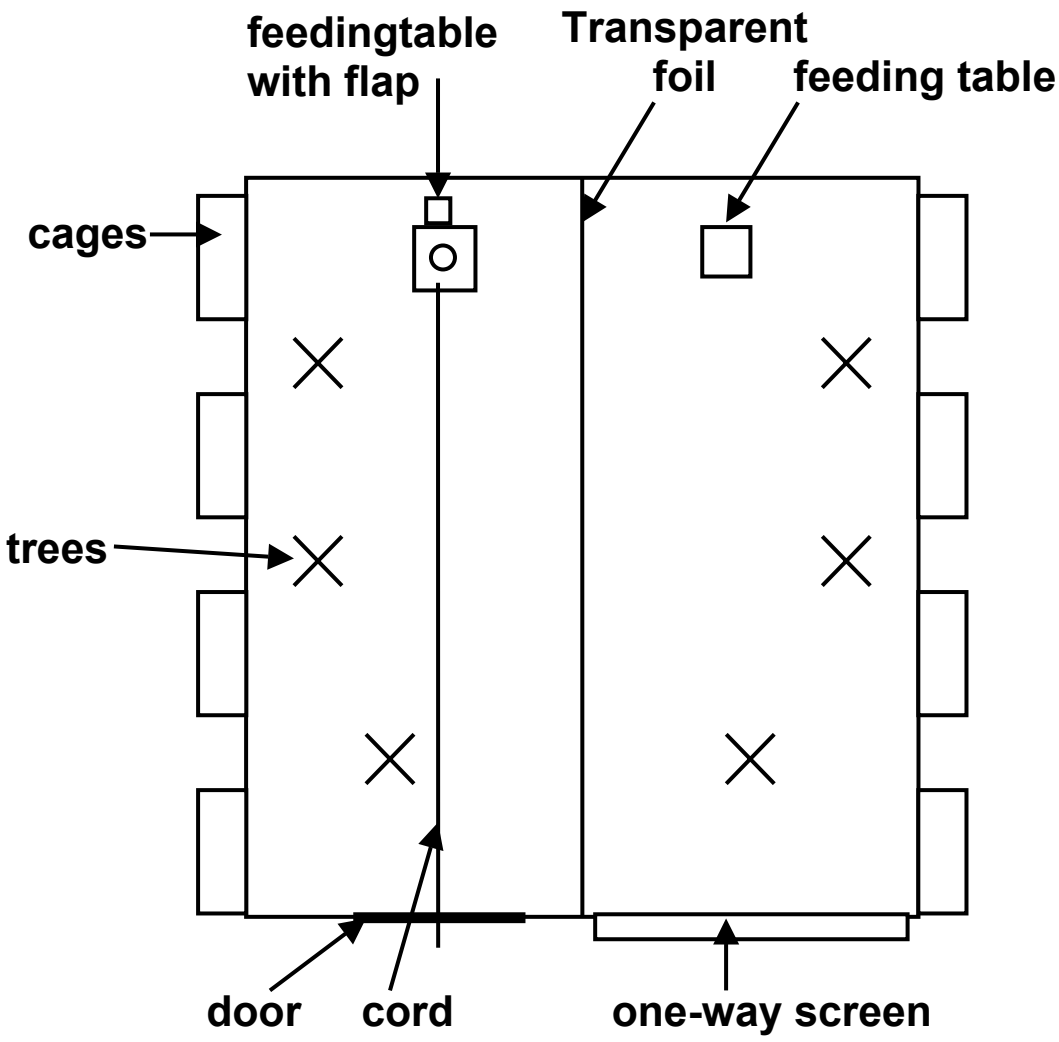


**Figure 1.** A top view of the observation room. Only eight out of 16 cages are shown. Focal animals are housed in the 8 left cages, companions in the right 8 (only 4 visible).

2; Verbeek *et al.* 1994). The exploration test was carried out between 30 and 35 days after hatching.

*Startle test*

The startle tests took place in two experimental rooms of 2.4x4x2.3 m each, which were divided in two equal parts by a transparent foil (figure 1). Sixteen individual cages were connected with the room via sliding doors of 20x20 cm (only one of two layers is visible in the figure). Three artificial trees and a feeding table with a dish with mealworms were present in both parts of the observation rooms. The feeding table on the left side was equipped with a hinged steel plate. A cord attached to this made it possible to startle a bird: decreasing the pressure on the cord caused the plate to spring back. After pulling the cord the plate got back into its initial position, invisible for the bird.

*Non-social startle test*

All birds were first tested in a non-social context. This test took place in the left part of the observation room. Apart from the division of the experimental rooms, the test is similar to the test described in chapter 4. To let birds enter the room without handling, we darkened their cages with a towel. The test was build up into three phases. After entering the room, birds landed on a tree after flying around for a short time (1-10 seconds). We measured the time from the moment birds landed on a tree until the moment they took the first worm from the feeding bowl (first worm latency). In all cases the birds took the worm to and ate it in an artificial tree. Since the experimental set-up in combination with the observation room was new to the birds, we expect that the first latency to reflect a novelty effect. This first phase of the experiment, makes the birds familiar with the situation, to reduce this effect. In the second phase, we measured the time from the moment birds had eaten the first worm until birds got back to the feeding table again and tried to get a second worm. Before the bird was able to actually take a worm, just as it landed on the feeding bowl, we startled the bird. We refer to this as 'second worm latency'. In the third phase, after the startle, we measured the time it took the bird to return to the table and actually take the second worm, the startle latency (referred to as risk-taking behaviour). When a bird didn't take the first worm within 30 minutes, we stopped the test (1 case). This was also done when the second-worm latency exceeded 10 minutes (1 case), or the startle latency exceeded 15 minutes (5 cases).

*Social startle test*

In the social startle test the 28 FE and 26 SE juveniles were randomly paired with one of the 8 FE and 8 SE male companions. Each companion was used for four times at a maximum. At the beginning of the test we let a companion enter in the right part of the room in the same way as the juveniles. In this phase of the test the companions were alone, to be able to accustom to the observation room and the feeding table, and to evade a possible novelty effect in the companions behaviour. After a companion had eaten a worm or when the companion did not eat a worm within five minutes (4 cases), we switched off the light and let the juvenile test bird enter the left part of the observation room. In the social startle test the same measurements were taken as in the non-social test, in addition we counted the number of table visits by the companion. As a measure of the activity of the companions, we used the mean number of table visits per minute (companion activity rate). In eight cases birds did not go back to the feeding table within 15 minutes. These birds were assigned a latency of 15 minutes.

## STATISTICAL ANALYSES

We log-transformed all latencies, as they show an increased variability with increasing values (Zar 1999). The residuals of the regression of weight against tarsus were used as a measure of the physical condition of the bird (Horak *et al.* 1998). In 5 cases we

missed either an individual's value for the non-social or for the social startle test. These individuals were omitted, leaving 49 cases for the analysis.

In the startle tests we could find no evidence of observer (one-way ANOVA: $F_{1,49}$=0.04, $P$=NS), condition (linear regression: $R^2$=0.006, $F_{1,48}$=0.31, $P$=NS) nor time of the day (linear regression: $R^2$=0.002, $F_{1,48}$=0.10, $P$=NS) effects, nor any of the interactions between these variables.

Adult properties in relation to behavioural type were analysed using t-tests. We used Generalised Linear Modelling (GLM) to test the influence of exploration type, sex and the interaction between these factors on the time to the first worm, hunger motivation and startle latency. A repeated measures GLM was used to analyse the changes in these variables from a non-social to a social context. Repeated-measures ANOVA's thereby use the relative change in latency from the non-social startle test to the social startle test as a dummy variable (context). In this model we included behavioural type (type; FE or SE) and sex as factors and companions' activity rate (companion) as a covariate. All analysis on context dependence are therefore using context as main factor and the interactions of context with the variables of interest. General context dependence was tested with only context in the model. All other variables were then added to the model (full model) and with a backward procedure removed when least significant, until only significant variables were present in the model (minimal adequate model). Variables were tested in this minimal adequate model (final model). We used SPSS 10.05 software to analyse all data.

## RESULTS

### Non-social startle test

The non-social startle test was always carried out before the test with the companion. The startle test can be divided into three phases. In the first phase of the test, the birds explored the room and the set-up. SE and FE birds differed in their time to take the first worm (first-worm latency; GLM: $F_{1,47}$=7.48, P=0.009); like what was expected from this earlier paper, FE took less time than SE birds did. In the second part of the test, the birds tried to take a second worm, after which they got startled. This interval between the first worm and the startle, the second-worm latency did not differ for the exploration types (GLM: $F_{1,47}$=0.14, P=0.71). In the last phase after the startle was given, we again found a significant difference in startle latency between FE and SE birds (GLM: $F_{1,47}$=9.88, P=0.003). FE birds returned faster to the feeding table after the startle than SE birds did. Again no significant sex differences or interactions between sex and exploration type were found for any of the latencies (all P>0.35).

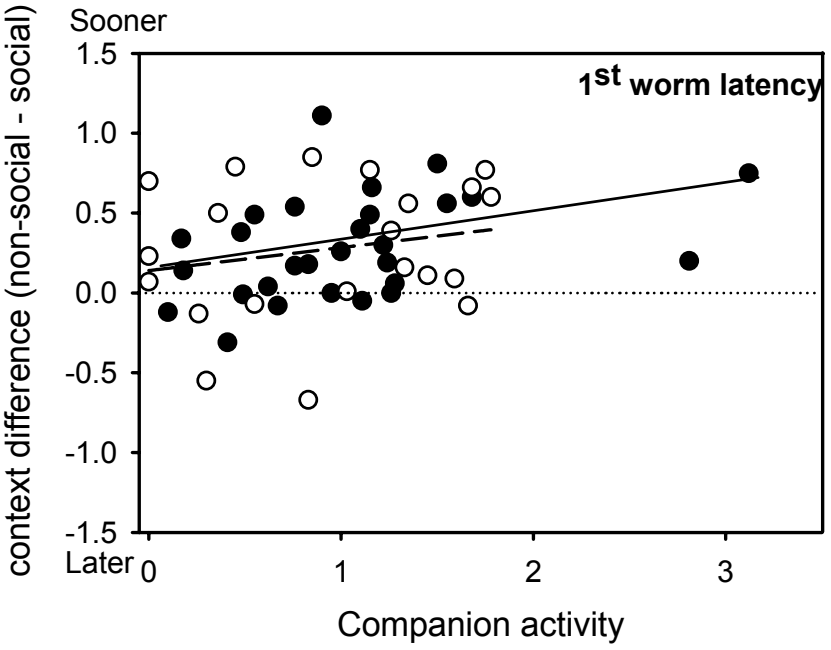


**Figure 2.** Relation between the companion activity rate and the difference in first worm latency between a non-social and social context (context difference). Open circles and solid line: Fast explorers; closed circles and dashed line: slow explorers. Lines are regression lines.

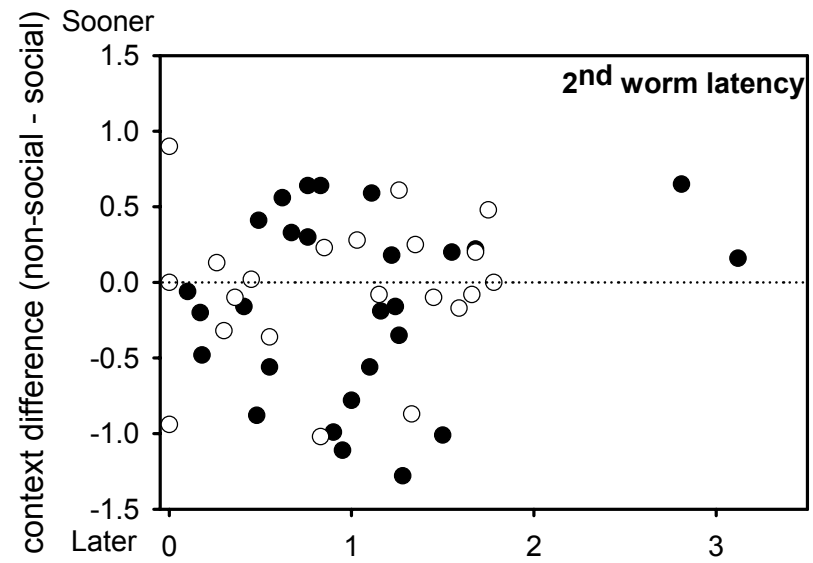


**Figure 3.** Relation between the companion activity rate and the difference in second worm latency startle latency between a non-social and social context (context difference). Hunger motivation is measured as the time between the first worm eaten and the attempt to take the second worm. Open circles = Fast explorers; closed circles = slow explorers.

## Non-social versus social context

In the habituation phase of the adults (without focal birds), FE birds took less time to take the first worm from the feeding table than SE birds did (t-test; $t_{47}=2.74$ and $P=0.009$). In the actual experiment, we used the companion's activity rate as a measure of its behaviour. The companion's activity rate reflects their phenotype and we expect the focal birds to react to the behaviour of the companions rather then to their genotype. The analysis of the companions activity rate showed that FE birds visited the feeding table more often per minute than SE birds did (GLM: $F_{1,47}=7.48$, $P=0.009$).

To test whether any of the latencies increased or decreased in a social context compared to a non-social context, we used the two tests of one individual as two samples of the same bird in a repeated measures analysis. We first analysed whether first-worm latency is context dependent and which role a companion plays on this (figure 2). In general, focal birds took less time to take their first worm when a companion was present (repeated measures GLM: context; $F_{1,48}=28.27$, $P<0.0001$). Moreover, this was dependent on the companion's activity rate: the higher the companion's activity, the bigger this difference was (repeated measures GLM: context*companion; $F_{1,47}=4.37$, $P=0.04$), independent of the exploration type (repeated measures GLM: context*type; $F_{1,46}=0.01$, $P=0.97$) or sex (repeated measures GLM: context*sex; $F_{1,46}=0.21$, $P=0.65$) of the focal bird.

In the second part of the experiment, we analysed the change in second-worm latency from the non-social to the social situation, and whether this change depends on the companion activity. Figure 3 shows that neither companion presence (repeated measures GLM: context; $F_{1,48}=2.68$, $P=0.11$) nor his activity rate (repeated measures GLM: context*companion; $F_{1,47}=0.39$, $P=0.53$) had an effect on the change second worm latency. The change in second-worm latency (non-social to social) was the same for sexes and types.

The third part and most important part of the experiment is whether risk-taking behaviour is context dependent. The change in startle latency from a non-social to a social context differed for the sexes (repeated measures GLM: context*sex; $F_{1,47}=7.13$, $P=0.01$), but no sex differences were found for the startle latency in the non-social startle test (GLM: sex; $F_{1,47}=0.21$, $P=0.65$). We therefore analysed the context dependence of the startle latency for females and males separately. In contrast to all other context effects, females had longer startle latencies in the presence of a companion, than in absence of a companion (repeated measures GLM: context; $F_{1,19}=4.93$, $P=0.04$; figure 4). This was independent of exploration type (repeated measures GLM: context*type; $F_{1,18}=0.08$, $P=0.78$) or companion's activity (repeated measures GLM: context*companion; $F_{1,18}=0.58$, $P=0.46$). In contrast to females, males tended to have shorter startle latencies in the presence of a companion, than in the absence of a companion (repeated measures GLM: context; $F_{1,28}=3.54$, $P=0.07$; figure 4). Males focal birds from the two exploration types reacted different to the activity of a companion (repeated measures GLM: context*type*companion; $F_{1,25}=5.99$, $P=0.02$;

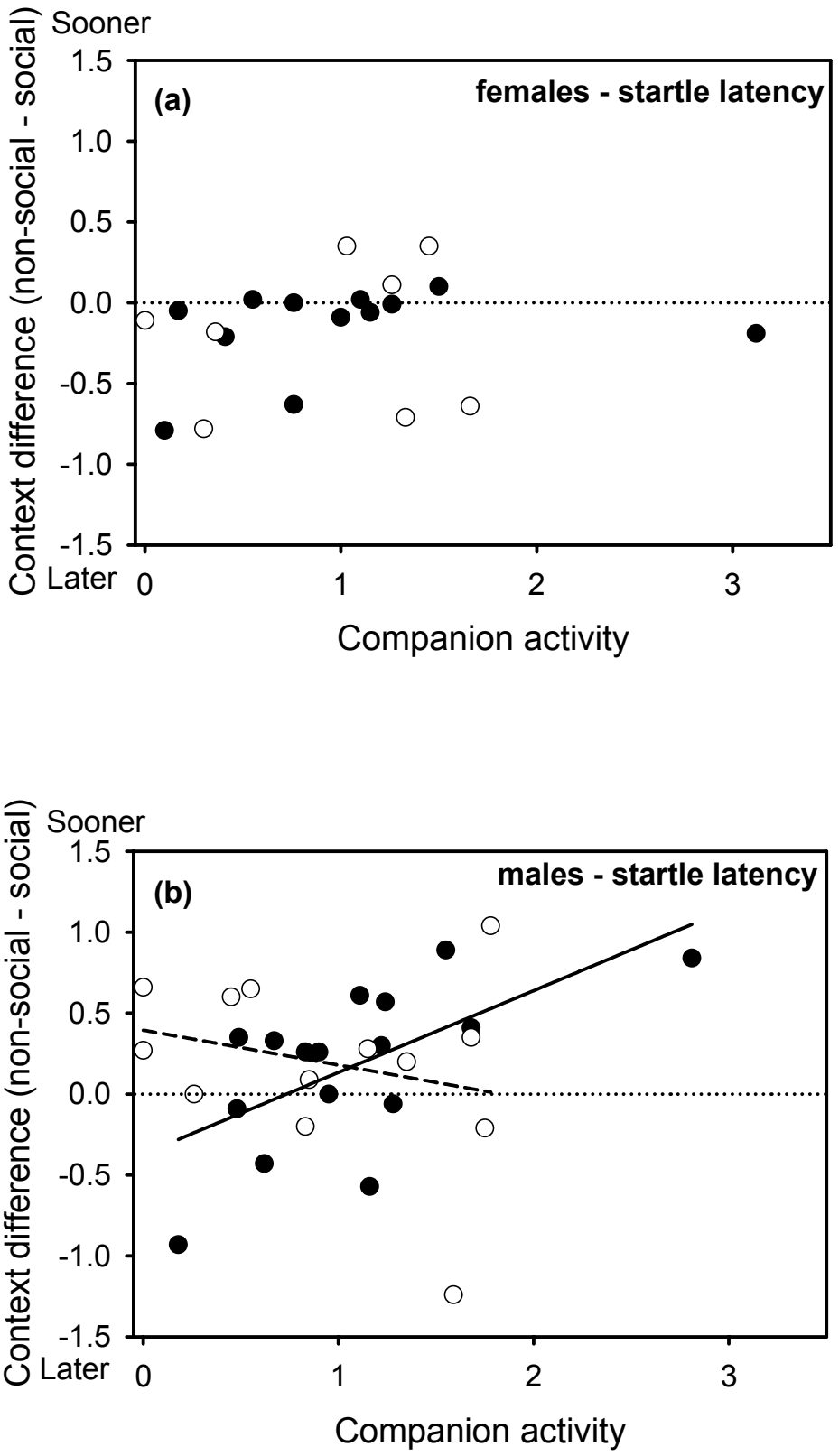


**Figure 4.** Relation between the companion activity rate and the difference in latency startle latency for females **(a)** and males **(b)**. Positive values indicate that birds return faster to the feeding table in the presence than in the absence of a companion. Open circles and solid line = Fast explorers; closed circles and dashed line = slow explorers. Lines are regression lines.

figure 4). SE males had lower startle latencies in the presence of a companion, compared to startle latencies without the companion (repeated measures GLM: context*companion; $F_{1,14}$=8.79, $P$=0.01), while no relation was detected between context difference and companion activity for FE males (repeated measures GLM: context*companion; $F_{1,10}$=0.77, $P$=0.40).

## DISCUSSION

Although we used only half of the test room for the non-social startle test our results in the non-social context are identical to the results of our earlier study, in which the same test was conducted using the whole room (chapter 4). FE birds had lower first-worm and startle latencies than SE birds did and there was no difference in second-worm latency between the behavioural types. This is also in agreement with the findings of other studies (e.g. Benus *et al.* 1987; Verbeek *et al.* 1999) that show that bold individuals are more risk prone than cautious ones are.

When comparing the non-social with the social startle test, we should remember that all birds experienced the non-social context before the social one. Therefore absolute differences between the two tests (context differences) could be due to either the companion's presence or learning effects due to the repetition of the test. However, it is unlikely that interactions between context and sex or behavioural type are due to habituation effects. Our discussion will therefore focus on these aspects.

Our study confirms that a bird's behaviour is influenced by the presence of a conspecific in the same room. The presence and feeding activity of the companion enhanced the time to take the first-worm. The first-worm latency can be seen as a measure of exploration, rather than a measure of boldness (chapter 4) and habituation effects, which fade away differences in exploration due to repeated testing are known to exist for great tits (Dingemanse *et al.* 2002). The approach towards the feeding table was additionally enhanced by an increase in activity of the male companion, which cannot be ascribed to habituation effects alone. The high feeding activities of the adult males are likely to reduce the focal birds' fear of the feeding table (Cadieu & Cadieu 2002) thereby changing the motivational state of the bird. Additionally, focal birds could have been copying the behaviour of the companion. In Florida scrub-jays (Midford *et al.* 2000) learning of a novel foraging patch from group members was more effective than individual learning. This so called social or observational learning is also known to have an effect on the use of novel food sources in great tits (Fisher & Hinde 1949; Sasvári 1979; Marchetti & Drent 2000 and refs in there). According to the findings of Marchetti and Drent (2000) however, we would have expected an interaction of context difference and behavioural type, when social learning would have played a role. They found that male fast explorers copied the behaviour of a tutor, while slow explorers didn't. We therefore conclude that habituation effects and fear reduction due to the companion, rather than social learning mainly affect context differences in exploration.

From our previous study we know that the second phase of the test, the second-worm latency, reflects the hunger state of the animal. The hunger state was affected neither by the presence nor by the activity rate of the companion. We found no type or sex effects. This indicates that the approach motivation for feeding is strong relative to novelty effects that are possibly still present in this phase. Moreover, birds are influenced neither by the presence nor the behaviour of a conspecific, in this phase.

In the last phase of the test males and females reacted differently to a change in context. All females were less prone to take risks while in males context dependence was dependent of behavioural type. Since no sex-differences were observed in the non-social test this gender difference can only be due to differences between the sexes in the social context.

Three causes for the gender difference in context dependence can be identified. One possibility is that physiological reactions to stressful situations are different for females and males. Sex differences have been found for a solitary test set-up in rats. Heinsbroek *et al.* (1991) showed that shortly after exposure to inescapable electric shocks male rats were more severely affected than female rats. Males performed less subsequent shock-escaping behaviour than females did. In our case however the sex difference only occurred in a social context. Therefore the difference is most likely caused by the presence of the companions, and the fact that all companions were males. Females could then be more vigilant in the social context either because they are attracted to the males or because males dominate females (Drent 1983). First, females could have waited longer with returning to the feeding table, when they recognise the companion as a possible partner. In this case they could have lost time in social interaction. Second, the females could also have associated the startle with an aggressive act of the neighbouring bird. They would than behave subordinate towards the other bird which could have increased their vigilance regarding foraging by waiting to decrease the chance of behavioural interference while foraging.

We found that SE males returned faster when the activity rate of the neighbouring bird increased, while FE males didn't behave differently with the presence of their companions. Habituation effects and fear reduction due to the companion's foraging activity can explain the reaction of SE males. Increasing activity of the companion strengthens this safety effect. The result that FE males didn't react to the foraging activity of his neighbour, confirms earlier studies of individual differences (Benus *et al.* 1990; Verbeek *et al.* 1994) where aggressive, fast explorers are more routine-like and behave more autonomous than non-aggressive slow explorers.

As was proposed by Wilson *et al.* (1994), and confirmed in some other studies (Coleman & Wilson 1998; Réale *et al.* 2000), our study shows the context dependence of a personality trait. Domain specificity, or context dependence is most often linked to the absence of phenotypic correlations of one trait measured in multiple contexts (Réale *et al.* 2000; Van der Kooij *et al.* 2002; Sih *et al.* 2003). This should however not be confused with flexibility of personalities. As was shown in several studies, different avian personality traits are phenotypically (Verbeek *et al.* 1996; chapter 4) and genetically (chapter 6) positively correlated within the same context (Buirski *et al.* 1978). As the behavioural traits studied are measurements of reactions to an external

stimulus, context dependent variation in behaviour can be caused by several factors. Besides the effect of the context itself, learning effects, variation in the stimulus or measurement variation can be responsible for the lack of phenotypic correlation between traits in multiple contexts.

Our results support the hypothesis that personalities not match the traditional concept of a one-dimensional behavioural continuum. This idea was already established in studies on animal personalities that make use of factorial analysis like in human personality studies (for a review see Gosling & John 1999). So, personalities consist of multiple traits, but phenotypic correlations between these traits over contexts can be low. This shows the importance of estimates of genetic correlations. As most of the above mentioned studies are studies on animals used as models for human personalities, the traits of interest do not automatically match traits that are of ecological and evolutionary interest.

In conclusion, it is clear that the presence of conspecifics indeed influences the way great tits cope with negative experiences. Moreover, the foraging activity of a companion has an important effect on foraging behaviour in novel environments and risk-taking behaviour. This context dependence differs between sexes and behavioural type. The present study is carried out with two individuals only, while in nature great tits live in larger flocks during winter. Earlier studies of individual differences show that members of a group behave differently from pairs, caused by the more complex nature of interactions in groups (Verbeek *et al.* 1999). Research on individual differences in risk-taking behaviour in more complex group situations is therefore required. Our study shows that phenotypic correlations between personality traits are not consistent between contexts. Therefore fitness differences between personalities are also expected to be context dependent. To get a better knowledge on the structure of personalities, genetic correlations of ecological important traits that are known to be part of the personality concept are needed.

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CHAPTER 6

# Genetic correlations of avian personality traits: Correlated response to artificial selection

**Kees van Oers[1,2], Gerdien de Jong[1,2], Pieter J. Drent[1] and Arie J. van Noordwijk[1]**

1. Department of Population Biology of Animals, Netherlands Institute of Ecology, PO Box 40, 6666 ZG, Heteren, The Netherlands
2. Evolutionary Population Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

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## ABSTRACT

Personalities are general properties of humans and other animals, where individuals differ over a range of correlated heritable behavioral traits. In great tits consistent differences have been found for several personality traits. These traits have a genetic basis and are phenotypically correlated. Estimates of genetic correlations are however fundamental to understand the evolution of consistent individual differences in behaviour. Genetic correlations can be due to pleiotropic effects or to linkage disequilibrium. In this study we used two selection experiments of two independent avian personality traits, early exploratory behaviour and risk-taking behaviour. These selection lines were both started using wild great tits (*Parus* major) from two natural populations. Genetic correlations were calculated using the response and the correlated response to artificial selection. We found genetic correlations ranging from 0.51 to 0.66 based on individual values and from 0.84 to 1.00 based on nest means. The different behavioural traits might therefore have a common genetic basis, possibly constraining independent evolution of personality traits in natural populations. These results are discussed in the relation with domain generality and domain specificity of personalities.

## INTRODUCTION

Consistent individual differences in behaviour have been found in many animal species (Wilson *et al.* 1994; Gosling & John 1999). These differences in a range of behavioural traits have been labelled as temperament, coping strategies, styles or syndromes (Wechsler 1995; Boissy 1995; Koolhaas *et al.* 1999), comparable with human personalities (Eysenck & Eysenck 1985; John 1990; Zuckerman 1991). Evidence is accumulating, that the personality concept not only exists in humans, but also in other animal species (Wilson *et al.* 1994; Clarke & Boinski 1995; Gosling 2001; Gosling & Vazire 2002). Two conditions have to be fulfilled for separate personality traits to represent a syndrome. First, the behavioural traits must be repeatable and heritable. Secondly, the behavioural traits have to be correlated with each other, within a single context. Phenotypic correlations between behavioural traits have been reported for several domesticated and wild species (for refs see Sih *et al.* 2003). In contrast, other studies found low phenotypic correlations between personality traits and subscribed this to context specificity (Coleman & Wilson 1998; Réale *et al.* 2000). The evolution of quantitative characters however depends on the genetic variation and genetic correlations. A genetic background for personality traits is generally accepted, but studies on the genetic structure of personality traits in natural populations are missing. The genetic structure however both determines the course of selection and is itself determined by selection on trait combinations.

Genetic correlations between traits might constrain evolutionary change, as during selection genetic correlations influence the selection response. In a non-selected

population, genetic correlations might provide information on past selection for combinations of particular traits (Lande & Arnold 1983; Maynard Smith *et al.* 1985). Two possible mechanisms may be responsible for genetic correlations. First, in the case of pleiotropy, individual genes have effects on several traits. Second, traits can be affected by different sets of genes, but a selective force, which generates and preserves particular combinations of alleles at a particular locus, causes linkage disequilibrium (Price & Langen 1992; Falconer & Mackay 1996; Lynch & Walsh 1998).

In great tits, exploration is phenotypically correlated with boldness (Verbeek *et al.* 1994), aggression (Verbeek *et al.* 1996), foraging behaviour (Drent & Marchetti 1999; Marchetti & Drent 2000), the physiological reaction to stress (Carere *et al.* 2001) and with risk-taking behaviour (chapter 4). An early exploratory behaviour score was developed on the combination of two tests. First a test was conducted on the reaction to a novel environment (exploration) and second a test on the reaction to a novel object (boldness). In a four-generation bi-directional selection experiment on early exploratory behaviour, in chapter 2 we showed that early exploratory behaviour has a genetic basis. In chapter 4 we (chapter 4, figure 1) repeated this selection experiment on risk-taking behaviour. In a study on wild great tits that were tested for exploration in the laboratory individuals also differed consistently in exploration behaviour and a comparable heritability was found through parent-offspring regression (Dingemanse *et al.* 2002). These consistent, heritable and co-varying reactions towards novel challenges can be seen as proof for the concept of avian personalities.

The existence of several avian personality traits that are phenotypically correlated and have an additive genetic component, give scope for the co-evolution of different traits. Genetic correlations may serve as an adequate measure for this in the study on the co-existence of individual differences in behaviour in natural populations. Genetic correlations are however difficult to estimate in natural populations, as they require large datasets on individuals with known pedigrees (Cheverud 1988). In this study we therefore used a traditional quantitative genetic method to calculate genetic correlations, based on data of two bi-directional selection experiments. The responses and correlated responses to selection are used to calculate genetic correlations between separate avian personality traits. These artificial selection experiments were conducted on early exploratory behaviour (combination of boldness and exploration) and on risk-taking behaviour. These two behaviours are assumed to be independent personality traits (chapter 4), comparable with Novelty seeking and Harm avoidance in the Three-dimensional Personality Questionnaire (TPQ), used in human personality research (Eysenck & Eysenck 1985).

Here, we (1) investigate whether there are significant genetic correlations between risk-taking behaviour and early exploratory behaviour (EEB). Furthermore we consider whether the two components of EEB, exploration and boldness, have different genetic correlations with risk-taking and discuss potential implications. Furthermore, we (2) use these results to consider existing ideas on the functional structure of personalities and the domain specificity of personality traits. We compare our genetic correlations with previous found phenotypic correlations, to see if phenotypic correlations between these traits are similar to estimates of genetic correlations. Finally we (3) place our

results in a broader framework, thereby discussing the evolution of adaptive behavioural traits in natural populations.

## METHODS

### Early exploratory behaviour

To measure the early exploratory behaviour score (exploration and boldness) we performed two types of behavioural tests: A novel environment test (analogous to an open field test; Walsh & Cummins 1976), was followed by two tests of the reaction to different novel objects. The combination of the score of the novel environment test (further referred to as exploration) and the novel object test score (further referred to as boldness) is referred to as early exploratory behaviour. The exploration test was carried out between 30 and 35 days after hatching, the boldness tests 10 and 12 days later (for details on the tests see chapter 2; chapter 6; Verbeek *et al.* 1994).

### Startle latency test

To measure the latency to restart foraging after a startle, we placed three artificial trees and a feeding table with a bowl with mealworms in the observation room. The feeding table was equipped with a spring loaded, hinged steel plate to startle a bird: releasing the pressure on the cord at once caused the plate to spring up in front of the bird. After birds entered the room, the birds took a worm. Birds returned to the feeding table to take a second worm, but we startled them just before they were able to take the second worm. After the startle we measured the time to actually take the second worm: the startle latency (for more details on the startle test see chapter 4). This startle latency test took place 6-8 weeks after the boldness tests (90-100 days after hatching).

### Bi-directional artificial selection experiments

Early exploratory behaviour was used as the selection criterion in the bi-directional selection experiment of chapter 2. In total 79 nestlings of 11 nests were used for the analyses on the parental generation. In the fourth generation of selection, we had 14 nests with 38 individuals of the high line and 9 nests with 35 birds of the low line available for analysis.

The startle latency was used as selection criterion for the bi-directional selection on risk-taking behaviour (chapter 4). In the second generation data was available for 7 nests of the high line (38 individuals) and 8 nests of the low line (35 individuals).

Both selection procedures were similar, and details of pair/formation, incubation, cross fostering and hand rearing are described in chapter 2.

## Genetic correlations

The genetic correlations were calculated using the response and the correlated response to artificial selection using the formula (Falconer & Mackay 1996: pages 316–318):

$$r_A = \sqrt{[(CR_A / R_A) \times (CR_B / R_B)]} \quad (1)$$

where $CR_A$ is the correlated response of behaviour A to the artificial selection on behaviour B, $CR_B$ is the correlated response of behaviour B to the artificial selection on behaviour A. $R_A$ and $R_B$ are the responses to artificial selection of behaviour A and behaviour B respectively. Genetic correlations were calculated for risk-taking behaviour and early exploratory behaviour as well as for risk-taking behaviour and boldness, and for risk-taking behaviour and exploration separately.

To obtain a measure of confidence, two different types of estimates (with in total 5 estimates) of each of the three genetic correlations were calculated. For the first type of estimate, the response and correlated response were calculated as the difference between the high and low lines of the selection line experiments (1 estimate). For the second type of estimate, the responses of each of the selection line experiments were split into two parts: The difference between the mean of the high and the mean of the original parental generation (high) and the difference between the mean of the low lines and the mean of the parental generation (low). This creates four possible combinations to calculate genetic correlations (high-high, high-low, low-high, low-low = 4 estimates). These estimates are not independent measures of the genetic correlations and are therefore not used to assess the variation in genetic correlations. Genetic correlations were calculated using both individual values and nest means.

## Statistical analyses

Because the variance in startle latency time increased with the mean value, this variable was log transformed (Zar 1999) for all analyses where normal distributions are assumed. To control for between-year variation we used 250 birds raised during the selection experiment as a control population by subtracting the mean value of the control group from the individual values. None of these control birds were part of either selection experiment. All tests were based on nest means, unless stated otherwise. We used t-tests to compare line means. The estimation of the power and the required minimal sample size for a power of 80% of these tests was done with a power test (Zar 1999: Pp 134–136). All statistical tests are two-tailed, and p values $<0.05$ are considered as being significant. We used SPSS version 10.1 for Windows for all analyses.

## RESULTS

The responses and correlated responses to selection, corrected for the number of generations, are given in Figure 2. Based on the analysis of nest means, the lines selected for early exploratory behaviour, significantly differed in their correlated response of risk-taking behaviour (Table 1). The lines selected for risk-taking behaviour significantly differed in exploration, but not in boldness and early exploratory behaviour (Table 1). The absence of significance for boldness and EEB is most likely caused by a lack of power due to a small sample size, since the lines differed in their correlated responses in the same manner for all traits. This is confirmed in a power analysis and when the minimal adequate sample sizes were calculated (Table 1). Therefore we may conclude that 'fast' explorers have shorter startle latencies than 'slow' explorers and 'high' risk-takers explore a novel room more quickly and are bolder towards a novel object than 'low' risk takers.

**Table 1.** Correlated responses for the selection experiments on risk-taking behaviour (RTB) and early exploratory behaviour (EEB). Correlated responses (± standard error) are given for the high and low lines separately. N = number of nests, Power = power of test given the sample size, M.A.S. = minimal adequate sample size for reaching a power of 80%.

| | CORRELATED RESPONSE | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **trait** | **high line** | **n** | **low line** | **n** | **t** | **p** | **power** | **m.a.s.** |
| RTB | -0.259 ± 0.081 | 14 | 0.064 ± 0.092 | 9 | -2.570 | 0.02 | 74.8% | 10 |
| EEB | -0.749 ± 1.162 | 8 | 1.584 ± 1.150 | 7 | -1.427 | 0.18 | 29.7% | 21 |
| Exploration | -1.112 ± 0.553 | 7 | 1.281 ± 0.896 | 7 | -2.273 | 0.04 | 62.3% | 8 |
| Boldness | -0.428 ± 0.605 | 7 | 0.375 ± 0.780 | 7 | -0.825 | 0.42 | 12.9% | 69 |

**Table 2.** Genetic correlations and their standard errors between risk-taking behaviour (RTB) and early exploratory behaviour (EEB), exploration and boldness. Values are calculated using individual test scores.

| Traits | $r_A$ | t | p |
|---|---|---|---|
| RTB and EEB | 0.58 ± 0.25 | 2.30 | 0.08 |
| RTB and exploration | 0.51 ± 0.15 | 3.46 | 0.03 |
| RTB and boldness | 0.66 ± 0.34 | 1.96 | 0.12 |

**Table 3.** Genetic correlations and their standard errors between risk-taking behaviour (RTB) and early exploratory behaviour (EEB), exploration and boldness. Values are calculated using nest means.

| Traits | $r_A$ | t | p |
|---|---|---|---|
| RTB and EEB | 0.84 ± 0.27 | 3.11 | 0.04 |
| RTB and exploration | 1.00 ± 0.32 | 3.15 | 0.03 |
| RTB and boldness | 0.94 ± 0.29 | 3.26 | 0.03 |

Genetic correlations calculated on individual scores range from 0.51 to 0.66 (Table 2). Genetic correlations calculated from nest means range from 0.84 to 1.00 (Table 3). The correlation between RTB and EEB (t-test; $t_5 = -1.53$ and $p = 0.20$) and between RTB and boldness (t-test; $t_5 = -2.01$ and $p = 0.12$), calculated using individual scores did not differ from the correlation calculated from nest means. The correlations between RTB and exploration did differ (t-test; $t_5 = -2.89$ and $p = 0.045$) between the two methods.

## Discussion

We demonstrate that avian personality traits are genetically correlated and present genetic correlations ranging from 0.53 to 0.67 for individual test values and from 0.84 to 1.00 based on nest means. These results indicate that natural selection on one trait has consequences for the correlational selection on other traits. Either shared genes or strong correlated selection can be the cause of these high genetic correlations.

The five different estimates of the genetic correlations are not independent measures of the genetic correlation between the avian personality traits. We are aware that this is not ideal, but this is the best method available to get a measure of confidence.

The genetic correlation between risk-taking and novelty seeking was close to one, and higher than the correlation between risk-taking and exploration. This suggests that the same genes are involved in the reaction towards a novel object and the response to a possible risk (i.e. a predator). This is an interesting finding as boldness is often measured as the propensity to take risks in animal research (e.g. Coleman & Wilson 1998), but risk-taking behaviour is seen as a separate domain in human personality research (Eysenck & Eysenck 1985). The high correlation between risk-taking and exploration indicates that the two traits have many genes in common but are not completely identical. It could therefore well be that the traits are not independent traits, but different measurements of the expression of the same group of genes. Differences between the genetic correlations are then a result of sampling errors. Genetic correlations have high standard errors, which become evident in repeated selection experiments, since each sample from a natural population contains just a sample of the polymorphic pleiotropic loci that cause the genetic correlation (Gromko 1995). In each sample some of the genes may be lacking variation just by chance. A second reason for sampling errors can be the sample size. In a review, Cheverud (1988) studied the difference in correlation level between phenotypic and genetic correlations. He showed that the discrepancy between the two estimates decreases with increasing effective sample size. Since we are aware of the relatively low effective sample sizes in our study, this has to be taken into account.

## Domain specificity vs. Domain generality

In the study on animal personalities two different approaches can be discriminated. One approach makes use of factorial analysis as used in human personality studies and describes the same main dimensions, with in addition two extra dimensions (for a review see Gosling & John 1999). The other part of the research on consistent individual differences in animals is based on studies of single traits (Armitage 1986; Benus *et al.* 1987; Wilson *et al.* 1993; Sluyter *et al.* 1996b; Réale *et al.* 2000; Benus 2001). In studies on coping styles or strategies, mostly on domesticated animals, these behavioural traits are linked to physiological parameters (Koolhaas *et al.* 1999). These different approaches both find genetic and environmental influences on the traits but a general discussion within each of these approaches is whether these traits are domain general or domain specific (Wilson *et al.* 1994). Domain specificity, or context dependence is most often linked to the presence of phenotypic correlations of one trait measured in multiple contexts (Réale *et al.* 2000; Van der Kooij *et al.* 2002; Sih *et al.* 2003; chapter 4). Since the behavioural traits studied are measurements of reactions to an external stimulus, variation in behaviour between contexts can be dependent on several factors. In addition to the effect of the context itself, learning effects, variation in the stimulus or measurement variation can be responsible for the lack of phenotypic correlation between traits in multiple contexts.

Our genetic correlations are higher than the phenotypic correlations calculated in a previous study (ranging from 0.17 –0.45), which is in line with other studies (Roff 1996; Réale & Festa-Bianchet 2000). We show that phenotypic correlations of avian personality traits are not good predictors of genetic correlations. Several causes can be responsible for this discrepancy between phenotypic correlations and genetic correlations. First, phenotypic correlations are a combination of genetic and environmental factors (Falconer & Mackay 1996). Heritabilities of personality traits typically do not reach one, which gives scope for environmental effects influencing phenotypic correlations. Phenotypic correlations could therefore well be undervalued due to environmental effects (Roff 1995). Environmental variance can be either general ($V_{eg}$) or special ($V_{es}$). Sampling effects typically characterize $V_{es}$. General environmental variance can have various origins. One example is the variation in the amount of maternal hormones females deposit with the laying order of the eggs. This has been shown for several bird species (Eising *et al.* 2001; Groothuis & Schwabl 2002) and indications for a relation of androgens with laying order have been found in great tits (Carere 2003). Learning effects are a second example of causes for general environmental effects, and they are known to influence personality traits. Thereby the expression of a trait may change when measuring one and the same trait in different environmental contexts at different stages in life.

In our results, genetic correlations are higher when we control for within nest variance. Since genetic correlations are influenced by special environmental effects only, we can expect that general environmental effects are not responsible for this difference between the genetic and phenotypic correlations. This is confirmed by our earlier studies, where repeatability estimates of EEB and RTB were not higher than our heritability estimates (Dingemanse *et al.* 2002; chapter 2; chapter 4).

## Pleiotropy vs. linkage disequilibrium

The functional architecture of personality traits has been debated in various approaches to personality research, but empirical evidence was lacking (Bouchard & Loehlin 2001). All approaches on consistent individual differences in behavioural traits have in common that they report an underlying genetic structure that causes the coherence of these traits, either due to pleiotropic effects (multiple effects of individual genes) or to linkage disequilibrium (non-random association between alleles at different loci). Personality traits are influenced by several loci each in its way responsible for a single metabolic or developmental pathway. Genetic variance in a specific personality trait may then be a product of the variance in these separate loci and the variance in the functional architecture of the observed trait (Houle 1991). A general set of pleiotropic genes could be the basis for differences in personalities, but some exclusive genes additionally influence each separate personality trait.

Elliot and Thrash (2002) stated that approach-avoidance motivation might be seen as such a unifying thread of personality, since it forms the foundation of several of the main traits as used in human psychology. Approach-avoidance can thereby describe variation in many personality traits, but certainly not all. In the studies of coping styles in rodents where a more physiological approach is used to study consistent individual differences, personalities are believed to be rooted in a genetically based differential use of physiological mechanisms (Koolhaas *et al.* 1999). The combination of a genetic system and epigenetic factors influencing the physiological mechanism rather early in life, are believed to be responsible for consistent phenotypic characteristics. Alternatively, the assumed independent personality traits may solely reflect differences in expression of the same set of genes.

Consistent individual differences are generally accepted to be adaptive (Wilson 1998; Buss & Greiling 1999): evolution is responsible for numerous solutions to problems, rather than one adaptive mean surrounded by non-adaptive variation. The coherence between different personality traits could therefore also be a product of natural selection, instead of a product of a static underlying genetic and/or physiological architecture. Even if the separate traits inherit due to independent sets of loci, but interactively affect fitness, linkage disequilibria may build up through correlated selection (Lande & Arnold 1983). This is also referred to as fitness epistasis (Whitlock *et al.* 1995). Selection against particular combinations of traits, cause other combinations to be more frequent. In a study on in humans, Eaves *et al.* (1990) linked the number of biological children to personality traits. They showed that individuals with certain trait-value combinations had a higher fitness than other combinations, when looking at the fitness surface of a combination of two personality domains. This sets the possibility for the selection of particular allele combinations. One limitation in human personality research is however, that cultural effects are difficult to be separated from genetic effects (Eaves *et al.* 1990; Gosling 2001). Nevertheless, these results imply a potential basis for correlated selection. Correlated selection has been proved to be responsible for the genetic correlations in the side-blotched lizard polymorphism (Sinervo & Svensson 2002). However, unless correlational selection is

strong and chronic (Sinervo & Svensson 2002), linkage disequilibria build up by correlational selection are expected to weaken rapidly (Falconer & Mackay 1996).

Purely from our results we are not able to distinguish whether our genetic correlations originate from pleiotropy or from linkage disequilibrium due to correlated selection or a mixture of the two (Conner 2002). Nevertheless, we can develop expectations combining our findings with those in the literature.

Comparable studies on the genetic structure of personality traits in humans are rare, but Eley (1997) found that the correlation between Anxiety and Depression was mainly determined through a genetic factor (80%). This gives proof for pleiotropic effects in these two emotionality dimensions. In a study using bivariate analyses to calculate genetic correlations between TPQ traits and alcoholism, Czerwinsko *et al.* (1999b) found large pleiotropic effects for novelty seeking and alcoholism. Also when looking to our great tit system, it is likely that different personality traits share a greater part of their genes. The large genetic correlations of this study are one indication for this. A second indication was found by Dingemanse (2003), who showed differences in selection pressures for males and females and different selection pressures over three different years, in a study on exploration in a natural population. Consider this together with the prerequisites of correlational selection, genetic correlations found in our study will be built up and maintained by correlated selection alone if variation in natural selection on one trait covaries with selection on another trait. As we expect this to be unlikely, we do not expect that our results can be explained by correlational selection only.

## CONCLUSION

The genetic correlations found in this study are high, most likely caused by pleiotropic effects, but linkage disequilibrium due to correlated selection cannot be ruled out completely. The discrepancy between the genetic correlations of this study and the phenotypic correlations of earlier studies is caused by special environmental effects. Our results do not support suggestions from other studies, that correlated behaviours can be split up into two or more distinct axes, which are supposed to be under independent control, through e.g. separate sets of genes (Wilson *et al.* 1994; Budaev & Zhuikov 1998; Elliot & Thrash 2002). More likely selection on several correlated personality traits shape the form of any of the traits. Studying single behaviours will therefore only make sense when the results are considered in the context of the whole phenotype (Price & Langen 1992).

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CHAPTER 7

# Summarising Discussion

**Kees van Oers[1,2] , Arie J. van Noordwijk[1] and Pieter J. Drent[1],**

1. Department of Population Biology of Animals, Netherlands Institute of Ecology, PO Box 40, 6666 ZG, Heteren, The Netherlands
2. Evolutionary Population Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

The need for evolutionary studies on quantitative traits that integrate genetics, development and fitness consequences is increasing. Due to the complexity, coherence and variability of behavioural traits, evolutionary biologists are therefore more and more attracted to the study of behaviour. The use of the model system of consistent individual differences in personality traits in the great tit provides a good foundation to do controlled experiments on the mechanisms underlying the variation in complex behavioural traits, and to make the step to functionality and evolution. The study presented in this thesis is part of a NWO program on the heritability, ontogeny and fitness consequences of personalities. The genetic background and the structure of the genetic mechanism that underlies the inheritance of these personality traits were investigated in this study.

## Behavioural individuality

The long-held assumption that individuals within populations are identical changed with the observation of a mix of consistent behavioural phenotypes within single populations. These individual differences are most apparent, and therefore best described in male mating systems (for a review see Taborsky 1994; Gross 1996) and trophic polymorphisms (for a review see Robinson & Wilson 1994). Only in the late 1980s variation between individuals in a third category of behaviour is regarded as more than a deviation from an adaptive mean, in the reaction to environmental challenges (e.g. Armitage 1986; Clark & Ehlinger 1987; Benus *et al.* 1987; Greenberg 1988). These individual differences in e.g. aggression, neophobia, exploration and boldness in social and non-social conditions have since been investigated in behavioural, physiological, psychological, ecological and agricultural studies and have been demonstrated in many domesticated and wild animal species (Wilson *et al.* 1994; Koolhaas *et al.* 1999; for reviews see Gosling & John 1999). Although widely discussed, the sources that cause this between individual variation within natural populations have rarely been investigated (Chitty 1967; Wilson 1998).

## Heritability of consistent individual behaviour

Most behavioural traits are expected to be the target of selection, and are therefore likely to play a role in evolution. A requirement for a particular trait to be evolvable is that the phenotypic variation is partly caused by a heritable component. Therefore, the identification of this heritable influence is the essential starting point for all evolutionary research and all other genetic analyses (Boake *et al.* 2002).

In our study on personality traits in the great tit we have been able to breed animals experimentally and to artificially select for our traits of interest. We show with two independent artificial selection experiments that a significant part of the phenotypic variation in early explorative behaviour (chapter 2) and in risk-taking behaviour (chapter 4) can be ascribed to variation in the genetic make-up of these traits.

In Chapter 2, we also show that exploratory behaviour as we measure it on hand-reared juveniles corresponds to the outcome of the same test on their wild parents. An offspring-parent regression showed that 30% of the variation between nests could be

attributed to their parents. This is an important link with the field studies of our project (see Dingemanse 2003), since our tests could be condition dependent, and therefore could have reflected another behavioural response in hand-reared than in wild individuals. These findings were also confirmed in the field study, where an analysis on known family relationships revealed heritability estimates of about 30 % in exploratory behaviour (Dingemanse *et al.* 2002). We found a difference in the realized heritability between the two traits (54% for early exploratory behaviour and 19% for risk-taking). Complex traits are influenced by genetic and environmental effects, and by the interaction between genotype and environment (chapter 1). Since risk-taking behaviour is measured in a later stadium of a bird's life this difference could therefore be seen as an indication for the existence of learning effects in personality traits. Nevertheless it should be emphasised that heritability measures should be interpreted with caution, since they are not simple reflections of the amount of genetic variation in a trait, but a statistic which integrates effects of selection, structure and the interrelationship between variance components (Stirling *et al.* 2002a). Above everything else, heritability estimates reflect the amount of environmental variation, including measurement error.

It should well be noted that we did not unravel the direct mechanical relation between variation in particular genes and variation in the observed behaviour, despite demonstrating substantial amounts of genetic variation. Studies that have looked at the direct causes of individual variation in comparable behavioural traits found several possible origins. Lipp and co-workers for example found a relation between genetic variation in the infrapyramidal mossy fibre projection in the hippocampus and several behaviours, in laboratory rat and mice strains (for an overview and references see Lipp & Wolfer 1999). In humans a polymorphism of the Dopamine-4 receptor accounted for about 10 % of the variation in novelty seeking (Benjamin *et al.* 1996; Cloninger *et al.* 1996; but see Ebstein *et al.* 2000; Reif & Lesch 2003). Another finding was the relation between a functional polymorphism in a regulatory sequence for the serotonin transporter-gene and anxiety (for references see Eley & Plomin 1997; Reif & Lesch 2003). Whether these gene polymorphisms are present in wild animals, and also show associations with either explorative behaviour or risk-taking behaviour in great tits are certainly interesting questions for further study.

Many studies however, find it sufficient to provide a heritability measure for their trait of interest in order to draw conclusions about the genetic contribution to the phenotypic response to selection and the consequences for changes in the genetic composition of the population. Since heritability is only a measure of that fraction of the phenotypic variance that can be ascribed to additive genetic variance, interactions between genes (epistatic effects), alleles (e.g. genetic dominance), or interactions between genotype and environment (GEI) are not included. Therefore it is necessary to identify the genetic variance and covariance components that cause phenotypic variation.

## Genetic structure

With different methods we found different heritability estimates in our study. A possible cause for this variation in heritability estimates would be the existence of nonadditive (Falconer & Mackay 1996) or indirect genetic effects (IGEs Wolf *et al.* 1998) in the inheritance of these traits. The analyses of line crosses (i.e. using the original lines, F1 crosses and back-crosses) enabled us to separate the components of variation (Mather & Jinks 1971). With the use of these quantitative genetic methods, we were able to estimate the proportion of phenotypic variation contributed by additive genetic effects, genetic dominance effects, genetic maternal effects (additive and dominant) and sex-dependent expression.

The analysis (chapter 3) shows that besides a considerable amount of additive genetic variation also genetic dominance plays an important role in the structure of inheritance. Dominance variance was often generally considered as relatively unimportant since it does not predict the response to selection (Fisher 1930; Crnokrak & Roff 1995). Dominance variance ($V_d$) can however influence the heritability of traits when during bottleneck events nonadditive genetic variance can be 'converted' into or affect additive genetic variance (Crnokrak & Roff 1995). Life history traits are closely related to fitness, and are therefore expected to have high relative levels of $V_d$ compared to e.g. morphological characters. Behavioural traits like physiological traits are expected to be intermediate. A measure for this relative $V_d$ is:

$$D_\alpha = \frac{V_d}{V_d + V_a} \qquad (1)$$

where $V_d$ = the dominance variance and $V_a$ is the additive variance. In a compilation of studies, Cnokrak and Roff (1995) present the mean $D_\alpha$ for life-history (0.59), behavioural (0.28), physiological (0.53) and morphological (0.10) traits in natural populations). They show that traits closely related to fitness have high relative contributions of $V_d$ compared to $V_a$. In our study $D_\alpha$ = 0.44, which is high for a behavioural trait. This indicates that our behavioural traits have a possible strong link to life-history and physiological traits. Moreover, dominance variance can play a role in the maintenance of additive genetic variation in personality traits.

In one of the founding studies of individual differences in behaviour, clear sex-dependent expression was detected (Sluyter 1994). Since we did not detect sex-dependent expression in our study, we can assume that differences between sexes that have been found are due to differences in selection pressures (Dingemanse 2003) or interactions with the social environment (chapter 5), rather then a difference in expression of the same genes.

Other effects that could influence the response to natural and artificial selection are indirect genetic effects (IGEs). The main candidates in our study on personality are maternal effects and group- selection effects. Maternal effects arise when the phenotype of a mother or the environment she experiences has a phenotypic effect on her offspring (Mousseau & Fox 1998). Our analyses in chapter 4 showed that the part of the phenotypic variation that could be explained by heritable additive maternal effects was relatively low (7 %). Since our intention was to draw conclusions on the

genetic structure of personality traits, we minimised the social (parental and group) influence by collecting eggs before incubation, and using foster parents and hand rearing to raise the chicks. In natural situations the inheritance of IGEs may possibly be important sources of phenotypic variation (Wolf *et al.* 1998; Wolf 2003), which we were not able to investigate. Further quantitative genetic research on personality traits in natural populations is therefore needed starting with the knowledge provided by this thesis.

Traits that influence fitness often show high absolute levels of additive genetic variation, despite having a low heritability. A reasonable explanation for this is that they capture genetic variation and accumulate mutations from many loci (Merilä & Sheldon 1999). This hypothesis is only valid when the variation in these traits is really influenced by many loci and selection pressures are stable. It is commonly assumed, but hardly proven, that many loci with small effects are responsible for the variation in polygenic traits. A substantial genetic dominance effect is however more likely to occur in traits where the major part of the variation is due to variation in a relatively low number of loci. This in combination with the high amounts of additive genetic variance suggests that personality traits as we studied them, are closely related to fitness, but additive genetic variance is not eroded by directional selection. This is in line with the findings of Dingemanse (2003) who showed that exploratory behaviour in wild great tits has fitness consequences, but selection pressures vary between years. Consequently, the net selection pressure over a long time might be low. This idea is expressed in the balancing selection view: existing genetic variation available for adaptation is protected from selection by fluctuating selection pressures. Therefore theories like antagonistic pleiotropy, frequency-dependent selection or selection-mutation equilibrium may be plausible causes for the maintenance of additive genetic variation in avian personality traits (Roff 1997).

## Personalities

One of the biggest problems of the study of individual differences in behaviour is the nomenclature. Many different fields in several disciplines use different terminology for the same or extremely similar phenomena. One clear distinction can be made. Studies on e.g. boldness, neophobia, risk-taking, tenseness, fearfulness or exploration are studies on single behavioural traits, while studies on personalities, attitudes, temperament, individuality, emotion, coping styles, behavioural syndromes or strategies are studies on suites of traits. The different names used tell us more about the field of study than about the fundamental differences between them. What all studies have in common is the intention to study the mechanisms and causes of behavioural variation, and use these mechanisms to explain variation in other behaviours, well-being, behavioural disorders, illnesses or life-history and dispersal, survival and reproductive success (i.e. fitness).

Behavioural syndromes or personalities are defined as aggregated summary trends in behaviour, capturing an individual's characteristic patterns of behaviour that is persistent across time and situation (Gosling 1998). Human research studies thereby state that personality traits are expressed across many situations (Kagan *et al.* 1988).

In the ecology of individual differences, the question whether these differences are domain general or dependent on the context they are measured in (Coleman & Wilson 1998) is stated as one of the major problems to be solved.

To be able to study context dependence of traits that are heavily influenced by developmental and learning effects one has first to study these traits in a constant environment. In great tits, explorative behaviour showed to be phenotypically correlated with many other traits within the same context (see Chapter 1 for references). Moreover, we measured two presumably independent traits (exploratory and risk-taking behaviour) and found that besides the phenotypic correlation, these traits were strongly genetically correlated (chapter 6). In Chapter 2 we also showed that phenotypic plasticity in avian personality traits was relatively small compared to genetic factors during the first 10 days of their life. This would indicate that avian personality traits are relative inflexible stable characteristics as is proposed in human personality research (Kagan *et al.* 1988). This seems in contrast to theories and findings of Wilson and co-workers (Wilson *et al.* 1994; Wilson 1998; Coleman & Wilson 1998). They state every important situation that influences survival and reproduction potentially requires a different adaptive response, at least if these traits are adaptive. It might be therefore reasonable to expect a lack of phenotypic correlations between measures of the same individuals in different contexts. In our experiment of chapter 5, we showed that although consistent individual differences in risk-taking behaviour are repeatable and heritable characteristics (chapter 4), test results were not reproducible when the context of the test was changed (chapter 5). This is in line with the findings of Wilson and co-workers, but seems contradictionary to our own results of chapter 4. This apparent contradiction in our data, and between our data and Wilson and Kagan, might be a consequence of genetic variation in the way of reacting to the environment, and be explained as follows.

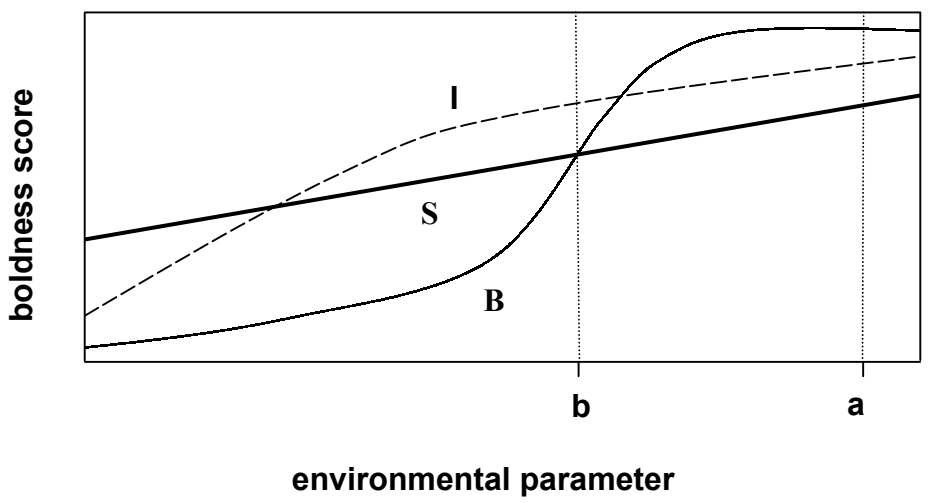


**Figure 1.** The reaction norms of three boldness genotypes (B = bold, I = intermediate, S = shy) for every value of an environmental parameter (after Van Noordwijk & Gebhardt 1987).

Behavioural (phenotype) measured in a certain environment is influenced by several factors. First there is the interaction of the genotype of the animal and the present environment in which the measurement is taken. This environment could be the social environment as well as the non-social environment, or both. When we assume to have three distinct genotypes, representing the three boldness groups presented by Coleman and Wilson (1998): **B**old, **I**ntermediate and **S**hy. We can plot the reaction norms of these genotypes against one dominant environmental parameter (Figure 1). In reality we are not able to measure the phenotype on a continuous environmental scale, so we do this at two points: a and b. When we classify our animals into three boldness groups when measuring at point a: B individuals get the highest score, I intermediate and S the lowest. Measuring the same genotypes in environment b according to the changes in reproducible reaction norms, the relative order of these genotypes has changed. Now no distinction is possible between B and S individuals, and I individuals get a higher score than B and S. This 'context-dependence' does not imply any flexibility of the behavioural reaction norms, but merely a difference in reaction at a certain point on an environmental axis.

This simple representation assumes that the developmental environment of these genotypes is the same. The genotypes measured in different contexts can also show variation in phenotype due to experience gained earlier in life. If the ontogeny of the behavioural trait, that means that there is an interaction between genes and the environment during the development of the reaction norm. Therefore, to reliably test the domain generality or context dependence of behavioural traits it requires a third axes in the analysis. Alternatively, one could first standardise the developmental conditions and then experimentally changes one at a time, like was done in the studies of Carere (Carere 2003).

A third point is that Coleman and Wilson measured the individuals while being in a group situation. Pumpkinseed sunfish, just like great tits, are social animals that live in groups during an important part of their life. Thereby, the conspecifics themselves can be seen as a very important environmental condition (Wolf *et al.* 1999). The decisions they make are therefore dependent on the decisions of and interaction with other group-members (Krams 1998; Verbeek *et al.* 1999; Carere *et al.* 2001). To explain the behaviour of individuals by their genotypic differences, often acquires knowledge of other social or non-social environmental components or interactions between them (chapter 5; Verbeek *et al.* 1999).

These points show that consistent individual differences in one context may produce predictable outcomes in other environments, provided that one knows the reaction norms and one has determined the changes in the environmental conditions. Context dependence of behavioural traits may be caused by more factors than the phenotypic expression on the environment these traits are measured in. It is therefore no proof for the adaptiveness of variation in behavioural traits over several environments. On the contrary, although every environmental condition could favour its own phenotype, changing environments in space and/or time, and therefore changing selection pressures could be a plausible mechanism for the existence of consistent behavioural variation in several traits. The reaction norms are not

determined by the adaptiveness of the variation in each context separately; but as a consequence of this constraint of varying selection, a compromise to optimise fitness over the whole range of contexts has evolved (De Jong 1999). It is therefore to be expected that not just one strategy, but several strategies may reach the same optimal solution for the combination of situations, in evolutionary terms.

## Relationship between avian personality traits and life-history traits

Although poorly investigated, personality traits seem to be related to life-history traits (Armitage 1986; Mealey & Segal 1993; Réale *et al.* 2000; Dingemanse 2003). One striking aspect in our study is the co-selection of timing of breeding in our two selection lines. Fast explorers and high risk-taking females started reproducing earlier in the season than slow explorers and low risk-takers did (van Oers, unp. data). This is in agreement with findings in a population of bighorn ewes (Réale *et al.* 2000). Individuals that were easily trappable and had a high struggling intensity reproduced earlier. Both in the study of the ewes and in great tits, early reproduction is correlated with high fitness (Verboven & Visser 1998; Visser & Verboven 1999; Réale *et al.* 2000).

## Evolution of personalities

In personalities, suites of traits are supposedly linked, either through genetic or environmental causes or a combination of these causes, like in e.g. physiological linkage. Why and how evolution plays a role in the coherence of these suites of traits is unspecified. Since many of these behavioural traits might share a common physiological background, most likely selection on one trait will shape the form of any of the other traits. In chapter 6 we showed that genetic correlations between two of those traits, exploration and risk-taking behaviour, is high. Most likely pleiotropic effects cause this high correlation, but linkage disequilibrium due to correlated selection cannot be ruled out completely. Our results therefore do not support the suggestions of other studies, which state that correlated behaviours can be split up into two or more axes that are supposed to be under independent control (e.g. Budaev & Zhuikov 1998). More likely the genetic variation in different behaviours is largely due to variation in the same genes and can be summarised in the one-dimensional axis of approach and avoidance motivation (Elliot & Covington 2001). This axis can be used 'a conceptual glue' between all different disciplines that study individual differences in the reaction to environmental stimuli. Moreover this result puts the methods used in human personality research into doubt; in human personality research principal component analyses (PCA) assert the complete independence of the major behavioural domains. This does not imply that the distinction between these traits is not valid, but that the independence of these traits or domains should not be assumed but investigated. A statistical technique to represent data as much as possible by independent axes, PCA, should not be confused with independence in mechanisms.

## Closing remarks

More and more studies realise that "in the absence of good genetic data, one simply cannot predict responses to selection or reconstruct the past forces of evolution" (Willis *et al.* 1991). In this thesis we have shown that (i) personality traits have a clear genetic basis, that (ii) the structure of inheritance is not simply additive and that (iii) personality traits do not inherit independently of each other, and that (iv) therefore the genetic structure has to be taken into account when looking at the expected response to natural selection and past evolutionary forces. This has brought us a large step further in understanding the inheritance of complex behavioural traits in natural populations and will help building more realistic models in studying the evolution of complex traits and syndromes of traits. Moreover, with this study we provide the starting point for future research on more detailed questions on several levels, however without ignoring the development in others.

From the genetic approach, a first possibility for further research is to study the genes that are involved in these traits. Both bottom-up and top-down methods can be used here. A good opportunity in using bottom-up methods is the 'candidate gene' approach. To test whether known polymorphisms with links to behaviour in humans (for a review see Reif & Lesch 2003), primates (Matsumoto *et al.* 1995) dogs (Niimi *et al.* 2001) and mice (Powell *et al.* 2003) can be found in the great tit, and whether the selection lines differ in these polymorphisms would be a great challenge. An example of a top-down method would be mapping the genome of our selection lines. With the use of neutral markers it will be possible to establish reference points on the chromosomes, and determine which flank the genes that account for the largest variation in behaviour. The most promising starting approach for the Great Tit would be to find an association between a set of neutral markers and our behavioural trait in the selection lines. With this technique it will be possible to identify single genes or QTLs. This approach becomes even more promising through the possibility to transport this knowledge on the genetic makeup to the field. By introducing F2 offspring into well-studied populations, phenotypic plasticity in response to growth conditions and social experiences early in life can be measured and the selection pressures on genetic variation in the field can be identified. The next step after this could be to develop a marker system for a natural population. All of these would enhance our understanding of the evolutionary forces that cause individual variation in personality traits to persist within populations and how they influence population dynamics.

Studies of behaviour have a long tradition of combining both ultimate and proximate questions. With our program on the heritability, ontogeny and fitness consequences of personalities in a wild bird species we present a complete study, in which we were able to answer some important nature-nurture questions in the function and evolution of personality traits and complex traits in general. What we should not forget when working in any field of science is that many studies only make sense in the context of a comprehensive study. The usual simplifications made by ecologists, developmental biologists and geneticists exclude the variables studied in the other disciplines (Van Noordwijk & Gebhardt 1987). However, only combing the efforts of these different disciplines enables us to draw conclusions that will bring us forward in unravelling the complex process of evolution.

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# NEDERLANDSE SAMENVATTING

## Adaptieve individuele verschillen.

In veel soorten is aangetoond dat individuen binnen natuurlijke populaties consistente verschillen vertonen in hun gedrag. Sommige van deze studies hebben laten zien dat deze verschillen een belangrijke rol spelen in life-history beslissingen van individuele dieren. Individuele verschillen in een scala van gedragseigenschappen worden temperament, coping strategieën of gedragsstrategieën genoemd, vergelijkbaar met humane persoonlijkheden. Ondanks dat verschillen in persoonlijkheid bij dieren voor de hand liggen, is er weinig onderzoek verricht aan dit thema met een evolutionaire grondslag. Dit was vooral te wijten aan de angst te worden beticht van antropomorfisme. Persoonlijkheden zijn in de tussentijd in de meeste vertebraten en enkele invertebraten aangetoond, en de gedachte dat het consequenties zal hebben voor vele huidige ecologische modellen heeft daarbij grond gevat.

## Persoonlijkheden en genetica

De meeste informatie over de overervingstructuur van persoonlijkheidskenmerken komen van studies aan mensen of knaagdieren. Al kunnen met tweelingstudies bij mensen vele vragen beantwoord worden, ze hebben enkele methodologische beperkingen aangezien experimenten bij mens studies geen volledige experimentele controle toelaten. Dier modellen zijn vaak een goed systeem gebleken om de aan kwantitatieve eigenschappen onderliggende genetische mechanismen te ontrafelen van zowel fysiologische als ook gedragseigenschappen. Daar de meeste van deze studies echter aan in gevangenschap gefokte populaties werden uitgevoerd geven zij geen inzicht in de evolutionaire processen die deze eigenschappen hebben gevormd.

In dit project bekijken we persoonlijkheden vanuit een evolutionair perspectief. Aangezien natuurlijke selectie alleen kan werken in de aanwezigheid van genetische verschillen, kan gedrag alleen in een voorspelbare wijze evolueren indien er gedragsalternatieven in de populatie voorhanden zijn en als dit fenotype overerfbaar is. In dit proefschrift beschrijven we de studie naar de genetische structuur die ten grondslag ligt aan de overerving van persoonlijkheden in de koolmees (*Parus major*). Ik probeer daarbij de volgende vragen te beantwoorden:

- Hebben polygene gedragseigenschappen een additieve genetische component, en zo ja, welk gedeelte van de fenotypische variatie kunnen we daarmee verklaren?
- Welke andere genetische effecten spelen een rol in de overerving van deze eigenschappen?
- Zijn deze eigenschappen deel van een functioneel syndroom, en zo ja, wordt dit syndroom veroorzaakt door genetische of omgevingseigenschappen.

## Modelsoort de koolmees

Dit proefschrift bevat de eerste genetische studie aan persoonlijkheidskenmerken aan een wilde diersoort, de mens uitgesloten. Om enkele redenen hebben we daarvoor de koolmees als modelsoort genomen. De koolmees is een zeer algemene monogame territoriale zangvogel, die zijn nesten bouwt in natuurlijke holtes, maar nestkasten indien aangeboden prefereert. Hij bewoond alle typen boomrijke omgevingen in heel Europa en gedeeltes van Azië en Noord Afrika. Mannetjes van de koolmees zijn territoriaal gedurende het hele jaar, mits de voedselcondities het toestaan. Vrouwtjes strijden onderling om de mannetjes met een territoriale status. Gedurende de herfst en winter wordt de plaatsgebonden intolerantie vaak vervangen door een hiërarchische intolerantie. Territorium eigenaren vormen samen met buren en niet-territoriale dieren groepen, met name als het voedselaanbod locaal onvoorspelbaar, schaars of slecht te vinden is. Laagrangige dieren dispergeren hierbij vaak van groep tot groep en daarbij tussen gebieden.

Koolmezen hebben legsels van 5-16 eieren die na 12-14 dagen broeden uitkomen. Als de vogels tussen de 16 en de 18 dagen oud zijn verlaten zij hun nest, maar worden door de ouders doorgevoerd tot volledige onafhankelijkheid. Na ongeveer 35 dagen zijn de jongen zelfstandig van hun ouders en vormen dan groepen met een sociale hiërarchie. In deze periode wisselen juvenielen geregeld van groep en gebied, eerst veroorzaakt door ervaringen van de ouders, later door voedselaanbod en dichtheid. Vanaf september van het jaar van uitvliegen worden jonge mannetjes locaal dominant tussen bestaande territoria, of in minder aantrekkelijke gedeeltes van door oudere vogels bezette territoria.

Naast de brede kennis die wij hebben van de ecologie en de '*levens-geschiedenis*' (life-history) van de koolmees is er nog een andere belangrijke reden om de koolmees als modelsoort te gebruiken: koolmezen kunnen onder laboratorium condities gehouden en gekweekt worden. Hun broedactiviteit kan worden gesynchroniseerd met natuurlijke populaties, wat noodzakelijk is voor cross-fostering. Nog belangrijker is echter dat jonge koolmezen met de hand onder gestandaardiseerde condities kunnen worden groot gebracht zonder de gedragsverschillen te beïnvloeden, beide voorwaarden voor een genetische studie aan persoonlijkheidskenmerken bij vogels.

## Persoonlijkheden in de koolmees

Sociale dominantie speelt een belangrijke rol in het leven van individuele koolmezen. Individuen hebben regelmatig te kampen met variatie in zowel hun sociale als niet-sociale omgeving in tijd en plaats. Monika Verbeek startte haar studie om te zien of individuele koolmezen consistent waren in vroeg agressief en exploratief gedrag met als doel dit te gebruiken als voorspeller voor latere dominantie verhoudingen en sociale structuur. Zij liet zien dat mannelijke koolmezen consistent verschillen in hoe zij een nieuwe omgeving exploreren en een onbekend voorwerp benaderen in hun eigen kooi. Deze verschillen in vroeg exploratief gedrag werken door in o.a. foerageergedrag en agressief gedrag.

## Overervingsgraad van consistent individueel gedrag

Van de meeste gedragseigenschappen wordt aangenomen dat zij aan selectie onderhevig zijn, wat betekent dat ze naar alle waarschijnlijkheid een rol in de evolutie spelen. Een voorwaarde dat evolutie grip op een bepaalde eigenschap kan krijgen is dat de fenotypische variatie gedeeltelijk wordt veroorzaakt door een overerfbare component. Daarom is de identificatie van deze overerfbare invloed een essentieel startpunt voor al het evolutionaire onderzoek en alle genetische analyses.

In onze studie aan persoonlijkheidskenmerken van koolmezen tonen we met twee onafhankelijke selectie experimenten aan dat een significant deel van de fenotypische variatie in vroeg exploratief gedrag (hoofdstuk 2) en in 'risico nemen' (hoofdstuk 4) kan worden toegeschreven aan variatie in de genetische make-up. In hoofdstuk 2 laten we ook zien dat vroeg exploratief gedrag, zoals we het meten aan met de hand opgefokte jonge vogels, overeenstemt met de uitkomst van dezelfde test aan hun wilde ouders. Een jong-ouder regressie liet verder zien dat 30% van de variatie tussen nesten kon worden toegeschreven aan hun ouders. Dit is een belangrijke link met het veldonderzoek van het programma. We vonden een verschil tussen de overervingsgraden van de twee geselecteerde eigenschappen: 54% voor vroeg exploratief gedrag en 19% voor 'risico nemen'. Complexe eigenschappen worden beïnvloed door zowel genetische en omgevingseffecten, en door de interactie van beiden. Aangezien 'risico nemen' in een later stadium van het leven gemeten word kan het verschil tussen de erfelijkheidsgraden worden gezien als een indicatie voor het bestaan van leereffecten in persoonlijkheidskenmerken. Desalniettemin moeten we hier benadrukken dat erfelijkheidsgraden met enige voorzichtigheid moeten worden geïnterpreteerd. Zij zijn niet de simpele reflectie van de hoeveelheid genetische variatie in een eigenschap, maar een statistische getal dat effecten van selectie, structuur en de verhoudingen tussen genetische componenten integreert. Boven alles reflecteren schattingen van overervingsgraden de hoeveelheid omgevingsvariatie, inclusief meetfouten.

## Genetische structuur

Erfelijkheidsgraden zeggen niets over interacties tussen genen (epistatische effecten), allelen (b.v.b. genetische dominantie) of interactie tussen genotype en omgeving. Daarom is het noodzakelijk de genetische variatie- en covariatie componenten te identificeren die verantwoordelijk zijn voor de fenotypische variatie. Met verschillende methoden hebben we verschillende schattingen van erfelijkheidsgraden gevonden. Een mogelijke oorzaak hiervoor is de aanwezigheid van non-additieve of indirecte genetische effecten bij de overerving van deze eigenschappen. Met de analyse van kruisingen tussen de selectie lijnen hebben we de proportie van de fenotypische variatie geschat die werd veroorzaakt door genetische dominantie, genetische moeder-effecten en sex-afhankelijke expressie. Hierbij hebben we gebruik gemaakt van de originele lijnen geselecteerd voor vroeg exploratief gedrag, de F1 kruisingen tussen de lijnen en de terugkruisingen. De analyse laat zien (hoofdstuk 3), dat naast een relatief groot additief effect ook genetische dominantie een rol speelt in de overervingsstructuur, genetsiche moedereffecten en sex-afhankelijke expressie spelen

geen of nauwelijks een rol. Dominantie effecten kunnen tijdens zogenaamde ‘botleneck events’ worden omgezet in additieve effecten, of kunnen deze beïnvloeden. Meer nog kunnen dominantie effecten een belangrijke rol spelen in behoud van genetische variatie in persoonlijkheidseigenschappen. De mate van genetische dominantie gevonden in onze studie is vergelijkbaar met die van life-history en physiologische eigenschappen, wat wijst op een sterk verband van vroeg exploratief gedrag met deze types eigenschappen.

## Context afhankelijkheid

In de ecologie van individuele verschillen in gedrag, wordt de vraag of deze verschillen wel of niet afhangen van de context waarin ze zijn gemeten, gezien als een van de grote raadsels die nog moet worden opgelost. Om de invloed van de context op de expressie van gedrag dat sterk wordt beïnvloed door ontwikkelings- en leereffecten te meten is het noodzakelijk om deze gedragingen eerst te meten in een constante omgeving. Eerdere studies aan koolmezen hadden al laten zien dat vroeg exploratief gedrag sterk fenotypisch gecorreleerd is met vele andere eigenschappen in dezelfde context. In hoofdstuk 6 laten we zien dat buiten deze fenotypische correlaties twee ogenschijnlijk onafhankelijke eigenschappen sterk genetisch gecorreleerd zijn. In hoofdstuk 2 laten we zien dat fenotypische plasticiteit relatief klein is ten opzichte van de genetische component. Dit alles duidt erop dat persoonlijkheidskenmerken van koolmezen relatief inflexibele eigenschappen zijn. In ons experiment van hoofdstuk 5 laten we zien dat, ook al zijn individuele verschillen in ‘risico nemen’ herhaalbaar en overerfbaar (hoofdstuk 4), test resultaten niet reproduceerbaar zijn als de context van de test wordt veranderd. Deze resultaten lijken in strijd met het vorige, maar kunnen duiden op genetische variatie in de manier waarop de strategieën reageren op veranderingen in de omgeving. Dat wil zeggen dat indien de reactienormen en de veranderingen in omgeving bekend zijn, de uitkomsten van een context verandering voorspelbaar kunnen zijn. De context afhankelijkheid van persoonlijkheden kunnen worden veroorzaakt door meer factoren dan enkel de fenotypische expressie en de omgeving waarin ze worden gemeten. Het is daarom geen bewijs voor de adaptiviteit van variatie in gedragseigenschappen gemeten in verschillende omgevingen. Het tegenovergestelde is waar: iedere omgeving kan een bepaald fenotype bevoordelen. Veranderende omgevingen in tijd en plaats en daarbij veranderende selectie drukken kunnen daarom een plausibel mechanisme zijn voor de aanwezigheid van consistente individuele verschillen in gedrag. Daarbij worden de reactienormen niet gevormd door de adaptiviteit van variatie in gedrag in iedere context apart, maar als een compromis fitness te optimaliseren over de hele range van contexten. Dit als consequentie van variërende selectiedrukken, waardoor fitness niet geoptimaliseerd kan worden voor iedere context apart.

## Evolutie van persoonlijkheden

In persoonlijkheden worden groepen van eigenschappen verondersteld te zijn gekoppeld. Dit kan veroorzaakt zijn door genetische- of omgevingsoorzaken, of door een combinatie hiervan, bijvoorbeeld door fysiologische koppeling. Hoe evolutie hierbij een rol speelt is onduidelijk. Omdat vele van deze gedragseigenschappen mogelijk een gemeenschappelijke fysiologische achtergrond bezitten, zal selectie op een eigenschap consequenties hebben voor vele andere eigenschappen. In hoofdstuk 6 laten we zien dat de genetische correlatie tussen vroeg exploratief gedrag en 'risico nemen' zeer hoog is. Het meest waarschijnlijke is dat dit wordt veroorzaakt door de gemeenschappelijke genen die betrokken zijn bij beide eigenschappen, 'linkage disequilibrium' kan echter niet worden uitgesloten. Onze studie laat zien dat persoonlijkheidseigenschappen niet duidelijk opgesplitst kunnen worden in onafhankelijke groepen van eigenschappen. Hoogstwaarschijnlijk is genetische variatie in verschillende gedragingen grotendeels veroorzaakt door variatie in dezelfde genen.

## Afsluitende opmerkingen

In dit proefschrift heb ik laten zien dat (i) persoonlijkheidskenmerken een duidelijke genetische achtergrond hebben, dat (ii) de overervingsstructuur niet alleen additief werkt, (iii) persoonlijkheidskenmerken niet onafhankelijk overerven en dat (iv) daarom de genetische structuur zeer belangrijk is in het onderzoek naar de verwachte respons op natuurlijke en evolutionaire krachten in het verleden. Deze wetenschap brengt ons verder in het begrijpen van de overervingsstructuur van complexe gedragseigenschappen in natuurlijke populaties en kan ons helpen realistischer modellen te maken over de evolutie van complexe eigenschappen en syndromen.

Gedragsstudies hebben een lange traditie in het combineren van zowel ultimate als proximate vragen. Met ons programma over de erfelijkheid, ontwikkeling en fitness consequenties van persoonlijkheden in en wilde vogelsoort, presenteren we een complete studie waarin we mogelijk waren om enige belangrijke vragen te beantwoorden over de functie en evolutie van complexe eigenschappen. Wat we hierbij niet mogen vergeten, is dat veel studies alleen nut hebben in de context van een allesomvattende studie. De vereenvoudigingen die meestal gemaakt worden door ecologen, ontwikkelingsbiologen en genetici, sluiten de variabelen uit die bestudeerd worden in de andere disciplines. Echter, alleen het combineren van de inspanningen van verschillende disciplines maakt het ons mogelijk om conclusies te trekken die ons verder brengen in het ontrafelen van de complexe evolutionaire processen.

# DEUTSCHE ZUSAMMENFASSUNG

## Adaptive individuelle Unterschiede

Bei vielen Arten konnte nachgewiesen werden, das Individuen innerhalb natürlicher Populationen konsistente Unterschiede im Verhalten zeigen. Einige Studien belegten, daß diese Unterschiede eine wesentliche Rolle für die sog. life-history Entscheidungen des einzelnen Individuums spielen. Die individuellen Unterschiede in einer Skala van Verhaltenseigenschaften werden Temperament, coping-Strategien oder Verhaltensstrategien genannt und sind vergleichbar mit menschlichen Persönlichkeiten. Obwohl diese Persönlichkeitsunterschiede auch bei Tieren auf der Hand liegen, wurde bis dato wenig Forschung zu diesem Thema verrichtet, das auf einer evolutionären Grundlage basiert. Dies ist in erster Linie der Angst zu verdanken, des Antropomorphismus verdächtigt zu werden. In der Zwischenzeit konnten bei den meisten Vertebraten und einigen Invertebraten Persönlichkeiten nachgewiesen werden. Dies legt den Gedanken nahe, daß Konsequenzen für die viele der heute bestehenden ökologischen Modelle nicht mehr ausgeschlossen werden können.

## Persönlichkeiten und Genetik

Die meisten Informationen zur Vererbungsstruktur von Persönlichkeitsmerkmalen stammen aus Untersuchungen an Menschen oder Nagetieren. Obwohl durch die humane Zwillingsforschung viele Fragen beantwortet werden konnten, unterliegen diese Untersuchungen doch gewissen methodischen Einschränkungen, da sie keine vollständige experimentelle Kontrolle erlauben. Tiermodelle haben sich bei dieser Problematik als ein gutes System erwiesen. Sie bieten die Möglichkeit, quantitativen Eigenschaften unterliegenden genetische Mechanismen von sowohl physiologischen als auch Verhalentseigenschaften zu scheiden. Die meisten dieser Studien werden jedoch an in Gefangenschaft gezüchteten Populationen ausgeführt und bieten dadurch keine Einsicht in die evolutionären Prozesse, die diese Eigenschaften hervorgebracht haben.

In dieser Studie werden Persönlichkeiten aus der evolutionären Perspektive untersucht. Da eine natürliche Selektion nur durch das Vorhandensein von genetischen Unterschieden funktionieren kann, kann sich auch das Verhalten nur in vorhersagbarer Weise evoluieren unter der Voraussetzung, daß innerhalb der Population Verhaltensalternativen vorhanden sind und dieser Phänotyp vererblich ist. In der vorliegenden Dissertation wird die Untersuchung der genetischen Struktur beschrieben, auf der die Vererbung von Persönlichkeiten bei der Kohlmeise (*Parus major*) basiert. Dabei sollen die folgenden Fragen beantwortet werden:

- Weisen polygene Verhaltenseigenschaften additive genetische Effekte auf, und, wenn ja, welcher Teil der phänotypischen Variation kann dadurch erklärt werden?
- Welche anderen genetischen Effekte spielen bei der Vererbung dieser Eigenschaften eine Rolle?
- Sind diese Eigenschaften Teil eines funktionellen Syndroms, und, wenn ja, wird dieses Syndrom durch genetische Eigenschaften oder die Umwelt beeinflußt?

## Modellart Kohlmeise

Mit dieser Dissertation wird erstmals die genetische Untersuchung von Persönlichkeitsmerkmalen bei einer wilden Tierart beschrieben, den Menschen ausgeschlossen. Aus mehreren Gründen wurde die Kohlmeise als Modellart ausgewählt. Die Kohlmeise ist ein weitverbreiteter, monogam und territorial lebender Singvogel, der seine Nester in natürlichen Höhlen baut, jedoch Nistkästen den Vorzug gibt, wenn sie ihm angeboten werden. Man findet ihn in allen baumreichen Umgebungen Europas, sowie in Asien und Nordafrika. Seine soziale und nicht-soziale Umwelt unterliegt sowohl zeitlich als auch räumlich großen Variationen. Männliche Kohlmeisen sind während des gesamten Jahres territorial, wenn die Nahrungsbedingungen es erlauben. Die weiblichen Kohlmeisen kämpfen miteinander um die Männchen, die in Besitz eines Territoriums sind. Im Herbst und Winter weicht die ortsgebundene Intoleranz häufig einer hierarchischen Intoleranz. Besitzer eines Territoriums schließen sich mit benachbarten Tieren und Tieren die kein Territorium besetzen, zu Gruppen zusammen. Dies geschieht in erster Linie, wenn das örtliche Nahrungsangebot schlecht vorhersehrbar, knapp oder schlecht zu finden ist. Tiere von niedrigem Rang wechseln dabei häufig in verschiedene Gruppen und damit verbunden auch in verschiedene Gebiete.

Kohlmeisen haben Gelege mit 5 bis 16 Eiern, aus denen nach einer Brutzeit von 12 bis 14 Tagen die Jungen schlüpfen. Im Alter von 16 bis 18 Tagen verlassen die Jungvögel das Nest. Auch nach dem Ausfliegen werden die Jungen bis zur vollständigen Unabhängigkeit noch von den Eltern gefüttert. Sind die Jungtiere selbstständig, bilden sie Gruppen, in denen eine soziale Hierarchie entsteht. In dieser Periode wechseln die juvenilen Tiere regelmäßig die Gruppe und das Gebiet. Dies wird erst durch Erfahrungen der Eltern verursacht, später durch das Nahrungangebot und Besiedlungsdichte. Etwa im September des Jahres in dem die Vögel ausgeflogen sind, werden die jungen Männchen örtlich dominant.

Außer der guten Kenntnis der Ökologie und der „Lebensgeschichte“ (life-history) von Kohlmeisen besteht noch ein weiterer wichtiger Grund, der die Kohlmeise als Modellart geeignet macht: Kohlmeisen können unter Laborbedingungen gehalten und gezüchtet werden. Ihre Brutaktivität kann mit der von natürlichen Populationen synchronisiert werden; eine unerläßliche Bedingung für cross-fostering. Noch wesentlicher ist aber der Aspekt, daß es möglich ist, junge Kohlmeisen per Handaufzucht unter Standardbedingungen heranzuziehen ohne daß Unterschiede im

Verhalten beeinflußt werden. Beides sind Kernvoraussetzungen für eine genetische Studie von Persönlichkeitsmerkmalen bei Vögeln.

## Persönlichkeiten bei der Kohlmeise

Die soziale Dominanz spielt eine wesentliche Rolle im Leben der einzelnen Kohlmeise. Jedes Individuum unterliegt regelmäßig Veränderungen, sowohl seiner sozialen als auch nicht-sozialen Umgebung in Zeit und Ort. Monika Verbeek begann ihre Studie, um zu untersuchen, ob individuelle Kohlmeisen in Bezug auf frühes aggressives und exploratives Verhalten konsistent waren. Ihre Zielsetzung bestand darin, die Ergebnisse als Vorhersage auf später entwickelte Dominanzverhältnisse und die soziale Struktur zu gebrauchen. Sie zeigte auf, daß männliche Kohlmeisen sich konsistent darin unterscheiden, wie sie eine neue Umgebung entdecken und auf einen ihnen unbekannten Gegenstand innerhalb ihres vertrauten Käfigs reagieren. Diese Unterschiede im frühen Explorationsverhalten setzen sich u.a. auch in der Nahrungssuche und im Aggressionsverhalten durch.

## Vererbungsgrad von konsitent individuellem Verhalten

Von den meisten Verhaltenseigenschaften nimmt man an, daß sie der Selektion ausgesetzt sind. Dies bedeutet, daß sie aller Wahrscheinlichkeit nach eine Rolle in der Evolution spielen. Eine Voraussetzung, daß Evolution sich auf eine bestimmte Eigenschaft auswirkt, besteht darin, daß die phänotypische Variation zum Teil durch eine vererbliche Komponente verursacht wird. Deshalb ist die Identifizierung dieses vererblichen Einflusses ein wesentlicher Ausgangspunkt für alle evolutionären Untersuchungen und alle genetischen Analysen.

In unserer Studie über die Persönlichkeitsmerkmale der Kohlmeisen, zeigen wir mittels zwei voneinander unabhängigen Selektionsexperimenten, daß ein signifikanter Anteil der phänotypischen Variation im frühen Explorationsverhalten (Kap. 2) und dem „Nehmen von Risiken“ (Kap. 4) einer Variation im genetischen Make-up zugeschrieben werden kann. In Kapitel 2 wird ebenfalls gezeigt, daß das frühe Explorationsverhalten, so wie es sich bei den mit der Hand aufgezogen Jungvögeln darstellte, mit dem der Eltern übereinstimmt, die dem selben Testverfahren unterzogen wurden. Die Regression von Jungen und Eltern läßt außerdem erkennen, daß 30% der Variation zwischen den einzelnen Nestern auf die Eltern zurück geht. Dies ist eine wichtige Verbindung zur Freilandstudie, die ebenfalls im Untersuchungsprogramm enthalten ist. Auch können wir einen Unterschied im Grad der Vererbung der beiden selektierten Eigenschaften aufweisen: 54% für das frühe Explorationsverhalten und 19% für die „Risikobereitschaft“. Komplexe Eigenschaften werden sowohl durch genetische als auch durch Umwelteffekte beeinflußt, aber auch durch die Interaktion beider Effekte. Da die Risikobereitschaft in einem späteren Lebensstadium der Tiere gemessen wurde, kann der Unterschied zwischen den Vererbungsgraden als Indikation für das Bestehen von Lehreffekten bei Persönlichkeitsmerkmalen betrachtet werden. Dennoch muß an dieser Stelle nachdrücklich darauf hingewiesen werden, daß der Grad der Vererbung mit einiger Vorsicht zu interpretieren ist. Er ist nicht die einfache Widerspiegelung der Menge an genetischer Variation in einer Eigenschaft, sondern eine statistische Größe,

die den Effekt von Selektion, Struktur und die Verhältnisse zwischen genetischen Komponenten integriert. Übergeordnet betrachtet, reflektiert die Schätzung von Vererbungsgraden die Menge der Umweltvariation, Messungsfehler eingeschlossen.

## Genetische Strukur

Der Erblichkeitsgrad sagt nichts über die Interaktion zwischen den Genen (epistatischer Effekt), Allelen (z.B. genetische Dominanz) oder der Interaktion zwischen Genotyp und Umwelt aus. Aus diesem Grund ist es unerläßlich, um die genetischen Variations- und Covariationskomponenten zu identifizieren, die sich verantwortlich zeichnen für die phänotypische Variation. Mit unterschiedlichen Methoden ergaben sich bei unserer Untersuchung dabei verschiedene Schätzungen von Erblichkeitsgraden. Eine mögliche Ursache hierfür könnte im Vorhandensein von nicht-additieven oder indirekten genetischen Effekten bei der Vererbung dieser Eigenschaften liegen. Durch die Analyse der Kreuzungen zwischen den Selektionslinien wurde das Ausmaß der phänotypischen Variation geschätzt, die durch genetische Dominanz, genetische Muttereffekte und geschlechtsabhängige Expression verursacht wird. Hierzu wurden die ursprünglich auf frühes Explorationsverhalten selektierten Linien, die F1-Kreuzungen zwischen den Linien und die Zurückkreuzungen herangezogen. Deren Analyse zeigt auf, daß neben einem relativ großen additiven Effekt auch die genetische Dominanz eine Rolle spielt, wohingegen genetische Muttereffekte und die geschlechtsabhängige Expression nicht oder kaum von Bedeutung sind. Dominanzeffekte können durch sogenannte „bottleneck events“ in additive Effekte umgewandelt werden oder Einfluß auf diese ausüben. Darüberhinaus können Dominanzeffekte eine wichtige Rolle beim Erhalt von genetischer Variation von Persönlichkeitseigenschaften einnehmen. Das Ausmaß der in der vorliegenden Studie gefundenen genetischen Dominanz ist vergleichbar mit denen von life-history- und physiologischen Eigenschaften, was auf einen engen Verband zwischen dem frühen Explorationsverhalten und diesen Eigenschaftstypen hinweist.

## Kontextabhängigkeit

In der Ökologie von individuellen Unterschieden im Verhalten wird die Frage, ob diese Unterschiede abhängig sind vom Kontext in dem sie gemessen wurden, als eines der großen Rätsel gesehen, die es noch zu lösen gilt. Um den Einfluß des Kontextes auf die Expression des Verhaltens bestimmen zu können, müssen diese Verhaltensweisen zunächst unter Konstantbedingungen gemessen werden. Dabei ist zu beachten, daß auch die Verhaltensexpression stark durch Entwicklungs- und Lerneffekte beeinflußt wird. Frühere Studien an Kohlmeisen ließen bereits erkennen, daß frühes Explorationsverhalten stark phänotypisch mit einer Vielzahl anderer Eigenschaften korrelliert ist, wenn sie im selben Kontext gemessen werden. In Kapitel 6 zeigen wir auf, daß neben den phänotypischen Korrelationen auch zwei anscheinend unabhängige Eigenschaften stark genetisch korreliert sind. In Kapitel 2 wird beschrieben, daß die phänotypische Plastizität gegenüber der der genetischen Komponente relativ klein ist. Dies alles weist darauf hin, daß die Persönlichkeitsmerkmale von Kohlmeisen verhältnismäßig unflexibele Eigenschaften

darstellen. Im Experiment, das in Kapitel 5 beschrieben wird, lassen wir sehen, daß Testergebnisse in verändertem Kontext nicht reproduzierbar sind, obwohl die individuellen Unterschiede in der Risikobereitschaft ansich wiederholbar und vererblich sind (siehe Kapitel 4). Diese Ergebnisse scheinen im Widerspruch miteinander zu stehen, aber deuten auf eine genetische Variation in der Art und Weise wie Strategien auf Veränderungen in ihrer Umwelt reagieren. Dies bedeutet, daß, insofern Reaktionsnormen und die Veränderungen der Umwelt bekannt sind, die Resultate einer Kontextveränderung vorhersagbar sein können. Die Kontextabhängigkeit von Persönlichheiten werden durch mehr Faktoren verursacht, als allein die phänotypische Expression und die Umgebung in der sie gemessen werden. Darum gibt es keinen Beweis für die Adaptivität in der Variation von Verhaltenseigenschaften, die in unterschiedlichen Umgebungen gemessen wurden. Im Gegenteil: jede Umgebung kann einen bestimmten Phänotyp bevorzugen. Deshalb kann eine sich in Zeit und Ort veränderende Umgebung und ein sich dadurch geänderte Selektiondruck ein plausibler Mechanismus für das Vorhandensein von konsistente individuellen Unterschieden des Verhaltens sein. Die Reaktionsnormen werden hierbei nicht in jedem Kontext einzeln durch die Adaption der Variation im Verhalten geformt, sondern als ein Kompromiss um die Fitness in der gesamten Spannweite der Kontexte zu optimalisieren. Dies ist die Konsequenz des variierenden Selektionsdrucks, weshalb die Fitness nicht in jedem einzelnen Kontext optimalisiert werden kann.

## Evolution von Persönlichkeiten

Man nimmt an, daß bei Persönlichkeiten Gruppen von Eigenschaften aneinander gekoppelt sind. Dies kann verursacht werden durch genetische Ursachen oder durch Umweltfaktoren oder durch eine Kombination von beiden, wie z.B. einer physiologischen Verbindung. Weil möglicherweise viele Verhaltenseigenschaften einen gemeinsamen physiologischen Hintergrund besitzen, wirkt sich die Selektion einer Eigenschaft ebenfalls auf viele andere Eigenschaften aus. In Kapitel 6 wird gezeigt, daß genetische Korrelation von frühem Explorationsverhalten und der Risikobereitschaft sehr hoch ist. Es erscheint am wahrscheinlichsten, daß die Ursache hierfür in gemeinsamen Genen zu sehen ist, die beiden Eigenschaften zu Grunde liegen. Allerdings kann auch „linkage disequilibrium“ nicht ausgeschlossen werden. Die Studie zeigt, daß Persönlichkeitsmerkmale nicht in voneinader unabhängige Gruppen von Eigenschaften unterteilt werden können. Höchstwahrscheinlich wird die genetische Variation unterschiedlichen Verhaltens zum größten Teil durch eine Variation ein- und derselben Gene verursacht.

## Abschließende Bemerkungen

In der vorliegenden Dissertation habe ich gezeigt, daß (i) Persönlichkeitsmerkmale einen deutlichen genetischen Hintergrund haben und daß (ii) die Vererbungsstruktur nicht allein additiv ist. Weiterhin werden (iii) Persönlichkeitsmerkmale nicht unabhängig vererbt und daß deshalb (iv) der genetischen Struktur eine bedeutende Rolle bei der Untersuchung der zu erwartenden Respons auf natürliche Selektion und

den evolutionären Kräften in der Vergangenheit zu kommt. Die Kenntnis hiervon liefert einen Beitrag beim Verständis der Vererbungsstrukturen von komplexen Verhaltenseigenschaften bei natürlichen Populationen und kann uns helfen realistischere Modelle von der Evolution von komplexen Eigenschaften und Syndromen zu entwerfen.

Verhaltensstudien stehen in einer langen Tradition bei der Kombination von der Fragen nach den „ultimate factors“ als auch den „proximate factors“. Mit unserem Untersuchungsprogramm zur Vererbung, Entwicklung und den Konsequenzen für die Fitness von Persönlichkeitsmerkmalen einer wilden Vogelart, sind wir in der Lage eine umfangreiche Studie vorzulegen, die Antwort auf einige der wichtigsten Fragen zur Funktion und Evolution von komplexen Eigenschaften liefert. Hierbei dürfen wir nicht aus dem Auge verlieren, daß viele Forschungsarbeiten erst im Zusammenhang mit einer allesumfassenden Studie wirklich Sinn machen. Eine Vereinfachung, wie sie häufig durch Ökologen, Entwicklungsbiologen und Genetiker vorgenommen wird, schließen Variabeln aus, die in anderen Disziplinen untersucht werden. Allerdings macht erst die Kombination der Resultate verschiedenster Disziplinen das Ziehen von Schlußfolgerungen möglich, die es zulassen, Licht in das Entwirren von komplexen evolutionären Prozessen zu bringen.

# NAWOORD

De ‘opleiding’ zit erop, het werk kan beginnen! Met dit proefschrift hoop ik dat te hebben bereikt wat altijd mijn droom is geweest: bioloog zijn! Hoe het leven verder loopt blijft gelukkig een vraagteken, maar dat ik een boel ervaringen heb opgedaan in de laatste jaren staat als een huis boven water. Natuurlijk is het onmogelijk het onderzoek zoals beschreven in dit proefschrift alleen te doen. Buiten dat is het zeker niet reëel te denken dat alleen de mensen die met hun handen hebben bijgedragen aan het tot stand komen van dit boekje belangrijk voor me waren. Natuurlijk hebben veel meer mensen dan die ik hier met name noem hun aandeel gehad in het tot stand komen van dit proefschrift. Mochten er dus nog personen zijn die niet in dit dankwoord staan en die toch gehoopt of verwacht hadden hier in dit stukje te staan, dan dank ik hen bij deze.

Als eerste wil ik Piet Drent bedanken. Al waren de officiële verhoudingen tussen ons niet altijd even duidelijk voor anderen, ik denk dat wij vanaf het begin precies wisten wat we aan elkaar hadden. Als dagelijkse begeleider, praatpaal, koppelaar (netwerken, Kees!!), mede oc-lid, ‘vader van het project’ en als collega behield je altijd het overzicht, was je altijd geïnteresseerd, betrokken en had jij al oog voor dingen voor dat ik er überhaupt bij stil stond. Dit gold niet alleen voor wetenschappelijke aangelegenheden, ook bij persoonlijke zaken had je aan een half woord genoeg. Bedankt daarom niet alleen voor het fungeren als goede leermeester, maar vooral voor het meer zijn dan dat, een goed en sociaal mens! Arie, als promotor, brein achter mijn project en leraar in de kwantitatieve genetica was je vaak iets meer op de achtergrond, maar je had altijd goed in de gaten hoe het project verliep. Jij wist ook manuscripten net dat stukje duidelijkheid te geven, zonder te hoog van de toren te blazen. Wat je me vooral geleerd hebt is net even anders over dingen na gaat denken, dat je bijvoorbeeld een doel soms beter via een kleine omweg kunt bereiken. Arie, je geeft me een boel extra kennis mee op mijn weg en ik ben trots je als leermeester te hebben gehad. Al was het contact niet altijd even intensief, ook zonder jouw Gerdien, was mijn proefschrift niet geworden wat hij nu is. Met name in de laatste periode heb je een grote bijdrage geleverd in de soms ongewone en gecompliceerde analyses. Vaak gaf je net dat beetje inzicht mee uit Utrecht waar ik weer weken mee vooruit kon. Verder wil ik mijn mede OIO’s, Niels en Claudio, uit het NWO programma bedanken. Vooral in het begin hebben we veel aan elkaar gehad en veel samengewerkt wat tot een grote meerwaarde door het hele project heeft geleid. Ik denk dat we alle drie kunnen zeggen dat het niet aan ons heeft gelegen als het geen vruchtbaar project is geworden (maar dat is het duidelijk wel geworden). Maar ook zonder de inbreng van de anderen uit het programma was ‘mijn deel’ een stuk minder interessant geweest. Al had ik in het begin het idee het ondergeschoven kindje te zijn van het programma, naar mate het project vorderde liet eenieder blijken dat onze kracht lag in het zijn van een groot team. Dit bleek vooral in het gezamenlijk optreden bij diverse internationale congressen. Ton Groothuis, Jaap Koolhaas, Christiaan Both en Joost Tinbergen,

bedankt voor de goede samenwerking, de discussies en de tips. Naast de vaste mensen in het programma wil ik de studenten waar ik direct mee gewerkt heb bedanken voor hun belangrijke inbreng: Esther van de Meer, Leontien Witjes, Margreet Klunder, Vera Ros en Marianne Heijkoop, bedankt voor jullie bijdrage, ik hoop dat jullie net zoveel van mij hebben geleerd als ik van jullie.

Een heel belangrijk deel van 'ons' onderzoek wordt gedaan en mogelijk gemaakt door de mensen die eigenlijk het meest worden ondergewaardeerd. Het wetenschappelijk ondersteunend personeel, zoals het zo mooi heet, was de grote basis voor het project. Piet de Goede jij hebt als multi-inzetbare, altijd goedgemutste en geduldige 'veld-assistent', in het veld maar ook in het lab bakken met werk verzet. Maar ook bij jouw zou ik toch willen benadrukken dat ik je niet slechts voor je grote inzet bewonder, maar meer voor je uitzonderlijk fijne persoonlijkheid. Het was altijd prettig om met je samen te werken. Ik hoop dat je uit de taak als paranimf de waardering kunt halen die ik voor je heb. Even belangrijk waren de dierverzorgers met Bart van IJmeren, Tanja Thomas, Mary-Lou Aaldering en Janneke Venhorst als vaste rotsen in de branding. Bedankt voor het voeren en vertroetelen van de mezen, voor het poepkrabben, weekend diensten en de flexibiliteit in de samenwerking. Meer nog wil ik jullie bedanken voor de gezelligheid, de negerzoenen, kopjes koffie en thee bij het testen en het begrip dat je toch moet hebben om met zulke eigenwijze wetenschappers samen te kunnen werken. Naast de mensen binnen het team, wil ik ook Christa bedanken voor de goede samenwerking en de zeer belangrijke eerste lessen in de moleculaire genetica. Ook speciale dank aan Willie Keultjes, zonder jouw interesse in 'alweer een nieuw apparaat of opstelling' waren de gegevens nooit geworden zoals ze nu zijn. Ik kan me geen enkel experiment voor de geest halen, waar geen 'Keultjes-uitvinding' bij te pas kwam.

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# Curriculum Vitae

Kees van Oers was born the 26th of Februari 1971 on Wieringen, The Netherlands. He attended the Katholic Highschool Etten-Leur (K.S.E.), where he finished his H.A.V.O exam in 1988 and his V.W.O. exam in 1990. In that same year he started his study Biology at Utrecht University. In 1994 he switched to Groningen University where he obtained his masters degree in 1996. In the practical part of his undergraduate period, Kees focused on different aspects of the life history of several bird species. He performed a M.Sc.-project on the functional and numerical response of Oystercatchers on their bivalve prey (Groningen University). Besides that he also took part in a conservation project on the Great Green (Buffon's) Macaw of Guayquil, in which he studied the basic ecology of this bird in South-West Ecuador, South America (University of Amsterdam). During a three-month project in de Ooypolder, the Netherlands, he looked at the breeding success of black terns (Alterra; IBN-DLO at that time). After his graduation he worked on different projects dealing with the effect of parasite infections on avian reproduction. On Schiermonnikoog he conducted an experiment on the effect of gut parasites on juvenile survival in the Oystercatcher (University of Groningen). On Cousin Island, The Seychelles he combined a field assistantship with a study on blood parasites in the Seychelles Warbler (University of Groningen/ University of Melbourne). This continued in laboratory research on blood parasites in several European and Australian bird species.

He started his Ph.D. at the Netherlands Institute of Ecology in April 1998. There he studied the genetic background of behavioural strategies and the coherence of different personality traits in great tits. This project was part of the NWO financed program on the "Functional significance, heritability and plasticity in coping styles in a free living bird". The results from this Ph.D. are presented in this thesis.

In the final phase of his Ph.D. he had the opportunity to expand this project in two four-month Postdoc periods, in which the first steps were made for future research on marker-trait association, 'candidate genes' for variation in personality (with the Max Planck Institute, Seewiesen) and the relation between cognition and personality (with the University of Edinburgh).

# LIST OF PUBLICATIONS:


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**Van Oers, K., De Jong, G., Drent, P. J. & Van Noordwijk, A. J.** Genetic correlations of avian personality traits: correlated response to artificial selection. *Submitted to Behavior Genetics*. **(chapter 6).**