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## The maintenance of variation in avian personality

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# Chapter3

Negative frequency-dependent selection is not strong enough to maintain variation in personality

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**Abstract**

Several hypotheses have been suggested to explain the maintenance of genetic variation in personality, but supporting empirical evidence is still needed. Next to environmental heterogeneity, negative frequency-dependent selection (nFDS) is considered to be an important factor. We aimed to investigate yearly selection acting on personality with a focus on nFDS. We analysed the impact of own personality and the personalities of direct neighbours on survival of adult breeding great tits (*Parus major*) and their reproductive success. We detected FDS on individual but not on population scale. Differential survival selection pressures related to neighbour personality acted on personality. The selection pressures varied between years and could not be explained by yearly fluctuations in the environmental and population characteristics. Neighbour personality was also associated with the reproductive success and total fitness. The effect on reproductive success and fitness varied between years, but was independent of the personality of the parents. These results suggest that the variation in personality is maintained by a combination of different selection mechanisms, with nFDS being one of them but not strong enough to maintain variability in personality.

## Introduction

The maintenance of quantitative variation is one of the key themes in evolutionary biology. One of the processes actively maintaining genetic polymorphism is balancing selection (Turelli & Barton 2004; 2007). Nowadays a genetic and an environmental form of balancing selection are considered to maintain variation. The first genetic effect of balancing selection is heterozygote advantage (Dobzhansky & Pavlovsky 1960). Heterozygotes may reappear through disassortative mating, when mated pair-members are less similar to each other than expected by chance. Variation is then maintained by Mendelian rules and heterozygote advantage (Penn & Potts 1999; Takahashi & Hori 2008). In contrast, assortative mating, whereby individuals choose a partner with a more similar form of a trait, may lead to reproductive isolation and eventual speciation (Arnqvist *et al.* 1996). With an increasing knowledge about genetic processes genomic mutations (Roff 1998a) and antagonistic pleiotropy were identified as other forms of balancing selection. Antagonistic pleiotropy signifies that genetic polymorphisms have a positive effect on one trait, but simultaneously a negative effect on another trait (Hedrick 1999).

However, since genetic processes alone are often not powerful enough to maintain variation, it has been suggested that environmental heterogeneity and frequency-dependent selection could also be important forms of balancing selection (Hedrick 2007; Penke *et al.* 2007a). Environmental heterogeneity causes selection to vary in space and time in the non-social (Karlsson *et al.* 2008) and social sphere (Kassen 2002; Ernande & Dieckmann 2004) and thus maintains variation through fluctuating selection.

Frequency-dependent selection (FDS) is one of the selective forces that act through social interactions. Thereby, the fitness payoff of a certain phenotype is dependent on the frequency of the interacting phenotypes (Svensson & Sheldon 1998; Sinervo & Calsbeek 2006). In the negative FDS (nFDS) increases fitness of the rarer phenotype at the cost of the more common phenotypes. Frequencies oscillate and establish the balance in a rate leading to the stable coexistence of these phenotypes within a population (Hori 1993; Roff 1998b; Bond & Kamil 2002). In contrast, in positive FDS (pFDS) the fitness benefits are higher in the more common phenotype, which leads to extinction of the rare phenotype (Iwasa & Pomiankowski 1995; Sinervo & Calsbeek 2006). However, we do not have discrete phenotypes but a scale of phenotypes of the focal birds and a scale of social environments surrounding territories of our focal birds. We therefore test how do different combinations of the focal personality and its environment affect fitness of the focal individuals.

Negative FDS is one of mechanisms proposed to maintain variation in animal personality (Dall *et al.* 2004; Wolf *et al.* 2008b). Animal personality is often defined as a suite of correlated traits reflecting individual consistency across situations and time (Koolhaas *et al.* 1999; Gosling 2001; Reale *et al.* 2007). Personality is heritable (Drent *et al.* 2003; van Oers *et al.* 2005a), but development and learning may cause changes in the phenotype along the reaction norm of a genotype (Carere *et al.* 2005; Arnold *et al.* 2007). Personality traits are known to be under natural (Reale *et al.* 2007) and sexual selection

(Van Oers *et al.* 2008). Besides year, sex and age, personality is an important factor influencing individual fitness (Reale *et al.* 2007; Smith & Blumstein 2008). It plays an important role in social interactions (Verbeek *et al.* 1996; Harris *et al.* 2008; McNamara *et al.* 2009) mainly through the use of social information (Marchetti & Drent 2000; Dall *et al.* 2004; Schuett & Dall 2009), learning abilities (Exnerova *et al.* 2010) and social dependence of risk-taking behaviour (van Oers *et al.* 2005b). In socially living species the personality composition of a group is found to be important for both the whole group and its particular members (Sih & Watters 2005; Harcourt *et al.* 2009; Kuruvers *et al.* 2009). Social interactions during competition and cooperation between conspecifics are the mechanisms that are proposed to underlie FDS on personality traits (Dall *et al.* 2004; Wolf *et al.* 2008b; McNamara *et al.* 2009). In Chapter 2 we found that social environment independent of the own personality was associated with reproductive success, but not adult survival of great tits. We proposed that interaction of own individual personality with the neighbour personality (thus nFDS) might be of importance for adult survival. Also theoretical models and laboratory experiments predict nFDS as one of the main forces maintaining variation in personality (Dall *et al.* 2004; Fitzpatrick *et al.* 2007; Wolf *et al.* 2008b; McNamara *et al.* 2009), but these hypothesis have not been tested in wild populations.

Here we investigate the role of nFDS in relation to other selective forces in a long-term population study. We analysed the effect of personality of the direct territory neighbours on survival of adult breeding birds with different personalities and their reproductive success in seven years with varying environmental conditions. With this we can assess the relative importance of nFDS as a selective force maintaining variation in personality compared to other selective forces like environmental heterogeneity. If neighbour personality affects survival differentially for personality types in the wild population, then we expect to find nFDS acting on personality, which would support theoretical models and laboratory experiments. To our knowledge this is the first long-term study investigating the association of FDS with personality traits.

## **Methods**

### *Study species*

The great tit is a non-migratory territorial passerine that breeds in secondary holes and artificial nest boxes (Perrins 1979). In the breeding season adult males are strongly space intolerant, but outside this period they tolerate mixed age and species flocks in their territory. Juveniles are fed by both parents until 2 to 3 weeks after fledging, when they become independent. High mortality occurs in this time (Drent 1984; Naef-Daenzer *et al.* 2001) and reappears in winter, especially with a low seed food supply and/or snow cover (van Balen 1980).

*Local survival and reproductive success*

Data were collected in seven year cycles 2000/1-2006/7 in the nest box population Westerheide, nearby Arnhem, The Netherlands. Nest boxes were checked to determine the breeding pair identity and the hatching date. When nestlings were 10 days old, parents were caught on the nest, nestlings were banded with an individual metal ring, weighed and their tarsus was measured. If adults were caught for the first time weight, tarsus and wing-length (p3) were measured. A week after the expected day of fledging we checked the nest boxes again to assess the exact number of fledglings. Using these data we are able to determine the *annual local adult survival*, which we defined as the presence of the breeding bird at the beginning of the next breeding season. *Reproductive success* was defined as the number of fledglings a pair had in one year that bred locally in any of the next breeding seasons during the period 2000-2007. *Fitness* was defined as the sum of survival (0 dead /1 alive) plus half of the number of recruits.

*Exploratory behaviour*

Birds were caught in mist nets over the whole year (except during the breeding season) and in two roosting controls when sleeping in their nest boxes. Birds caught for the first time were taken into the laboratory and tested for exploratory behaviour in the Novel environment test, a standard personality test (Verbeek *et al.* 1994). The birds were tested the morning after catching, alone in a room (4.0×2.4×2.3 m) with five artificial trees. They were introduced into the room without handling by darkening the cage with a curtain, opening the sliding door and turning on the light in the test room. As a measure of exploratory behaviour the total number of flights (movements between trees) and hops (movements within trees) within the first 2 minutes was recorded. We corrected data for date of capture based on the finding that behaviour changes with capture date within individuals (see Dingemanse *et al.* (2002)). All birds were directly released at the place of capture after all birds were tested.

*Neighbour exploratory behaviour (NEB)*

The data of all nest boxes that were occupied by great tits were used to create individual breeding territories with Dirichlet tessellations. This is a GIS technique assigning space closer to a given point than to any other point as area belonging to this point (here inhabited nest boxes). The calculated GIS territories do not differ from actual territories in the field (Chapter 2).

We calculated the Neighbour exploratory behaviour (NEB) as the mean personality of all neighbours sharing boundary with a territory. This describes the social environment that is present for each nest box as an indication of local frequency of personalities. We were aware that spatial autocorrelation might arise in our case by using an individual as both focal and neighbour (and this multiple times) for one analysis. Spatial autocorrelation is a form of statistical pseudo-replication because of the correlation of a

variable with itself through space (Moran 1950). However, spatial autocorrelation was not detected for this type of analyses in our area (Chapter 2).

### Statistical analyses

We used the software package R 2.7.2 (R Development Core Team 2007) for all statistical analyses. All tests were two-tailed. We analysed the effect of neighbour exploratory behaviour and exploratory behaviour of the focal breeding individual on annual local adult survival and reproductive success between two subsequent breeding seasons.

For *adult survival* we used 516 breeding birds (258 pairs) in a generalized linear mixed-effects model approach (GLMM) with a binomial error structure. Neighbour exploratory behaviour was centred to the mean neighbour exploratory behaviour in the whole area in a given year. The model contained adult survival (survived or died) as the dependent variable with a binomial distribution and logit link function. If FDS plays a role, we would detect an interaction between own individual personality and neighbour exploratory behaviour. Therefore we included neighbour exploratory behaviour, sex, year and the personality of the focal individual as explanatory variables in the model, together with the two and the three-way interactions that included neighbour exploratory behaviour and/or personality. Hence, we tested a specific set of 3-way interactions answering questions, i) Does FDS play a role, and does it differ between years (pers×NEB×year)? ii) Is adult survival dependent on neighbour exploratory behaviour and does this effect differ between years and sexes (NEB×year×sex)? iii) Does FDS differ between sexes (pers×NEB×sex) and iv) although our model has the intention to test the role of FDS on personality we also included an interaction testing if selection on personality types in adult survival differs between sexes and whether this fluctuates over years (pers×year×sex); this interaction was found significant by Dingemanse *et al.*(2004) who analysed the impact of personality on adult survival in the same population.

Control variables were age of breeding (1 year old or older), quality of the nest box of breeding (mean fledgling weight of fledglings produced over 10 years in a nest box) and local density (the centralized distance of the third closest occupied nest box from the focal nest box in the given year). We used age of breeding (1 year old or older) because life history of juvenile and adult tits greatly differs (Perrins 1979). Nest box quality shows success of the territory owners in the long-time span and local density determines interactions between individuals and also survival (Tinbergen *et al.* 1985). Since several nest boxes were used in more than one year, we included nest box identity as a random factor to avoid pseudoreplication. Because we corrected yearly local survival of birds for age, each data point of the same individual is an independent data point.

We ran post-hoc analyses to evaluate which specific year added a significant amount of variation to the yearly variation in FDS (the interaction pers×NEB×year). We thereby compared the final model using the dataset including all years with the model when using the dataset without the target year. To calculate p values, we tested the delta deviance of the

models in a chi-square distribution and the difference in variables between the models as degrees of freedom.

To test, which environmental conditions could explain the year effect in FDS, we ran ANOVA's using estimates for the interaction (pers×NEB×year) as a dependent variable and beech-mast crop (beech-mast crop was calculated following Perdeck *et al.* (2000) as the number of full nuts per m<sup>2</sup>. Using these values, years were divided in rich/poor year) and breeding population density (high/low), as explanatory variables. These variables are characteristics of our study population, generally assumed to be important for survival.

In the analyses of *reproductive success* individual juvenile personality was not used, but only the number of recruits per breeding pair, since we did not have an exploration score for all fledglings. Moreover, we used only the data of pairs having fledged at least one juvenile, because in nest boxes where all juveniles died already as nestlings, there was logically no reproductive success. We were interested in the impact of neighbour exploratory behaviour on reproductive success independent of nestling mortality. 15 out of 258 pairs had no fledglings. The 243 successful pairs did not differ from pairs without fledglings in the neighbour exploratory behaviour (ANOVA,  $F_{1,256}=2.08$ ,  $p=0.151$ ). Birds having recruits survived better (58.6%) than the birds without recruits (38.7%) (Contingency tables,  $\chi^2=15.89$ ,  $df=1$ ,  $N=486$ ,  $p<0.001$ ). The model of reproductive success contained number of recruits per pair as the dependent variable with a Poisson error distribution. We tested the same 3-way interactions as in the adult survival, using local density, hatching date and quality as covariates, and nest box as random effect. We chose these covariates because adult density affects juvenile recruitment (Both *et al.* 1999), hatching date correlates with the caterpillar peak which is important for the nestling condition (Naef-Daenzer & Keller 1999) and nest box quality shows success of the territory owners in the long-time span right on number of fledglings.

In the analysis of *fitness* we used the same dataset as for reproductive success and the model contained fitness as the dependent variable, the same 3-way interactions as in the adult survival and reproductive success models, using age of breeding, density, quality and hatching date as covariates, and nest box as random effect with a normal error structure. Because fitness is a measure of survival and reproductive success together, we used covariates from both former models.

#### *Mate choice*

To test the effect of the *mate choice* on the maintenance of variation, we needed to know, if mating was assortative or disassortative. Correlation between personality of males and females in the breeding couples was analysed by Pearson correlation coefficient, because the data met the requirements for parametric testing.

#### *Standardized selection differential (SSD)*

To test for hypotheses that FDS could act on the population scale, we calculated selection for personality using standardized selection differentials. The SSD determines which



phenotype has relative fitness advantage over the others. The SSD for adult survival was calculated as difference between mean personality score of the breeding birds survived till next year and mean personality score of all breeders, divided by the standard deviation of mean personality score of all breeders (Falconer 1981). Then we used regression to relate the SSD estimates with the mean personality score in the given year. If there was FDS acting on population scale we should detect relationship of SSD and mean personality score.

## Results

### *Adult survival*

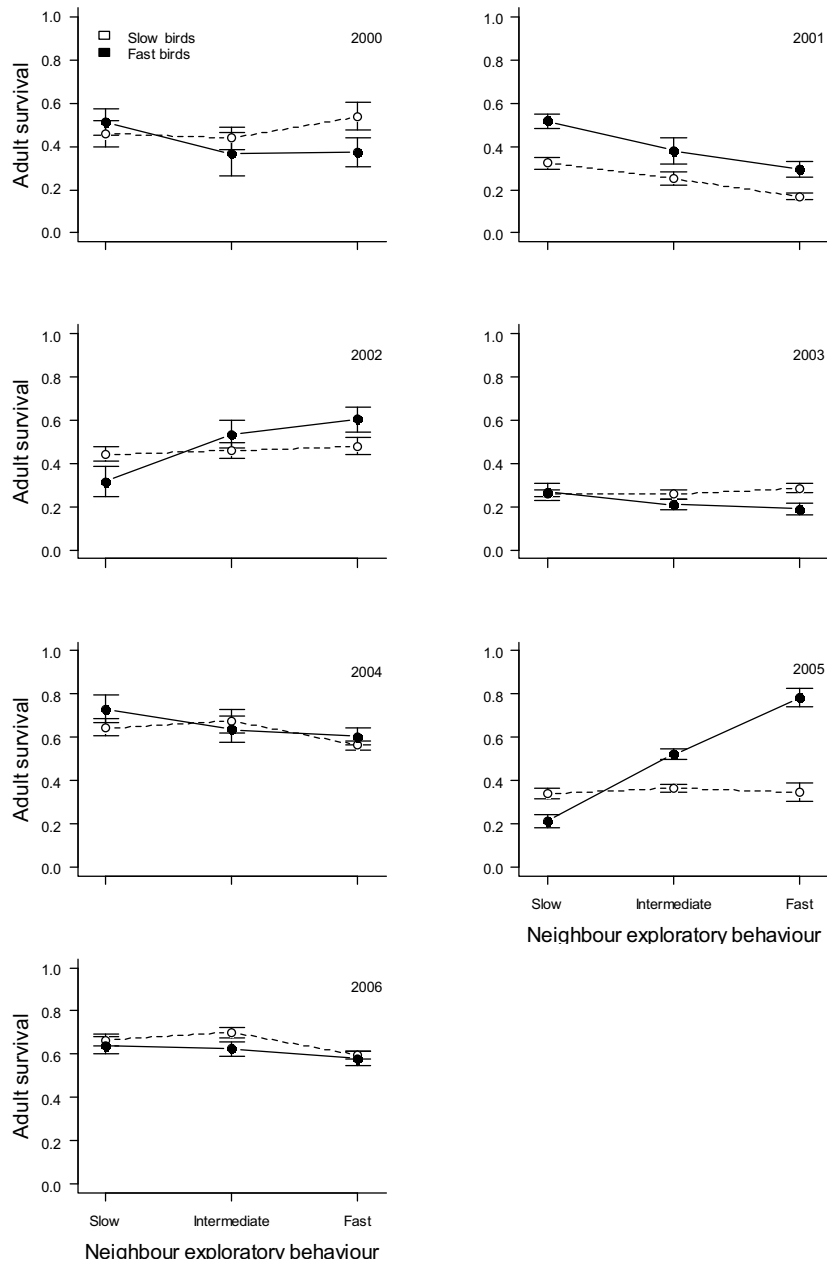
Adult survival differed significantly between personality types and years due to variation in local frequency, which indicates FDS to differ between years (GLMM, pers×NEB×year,  $\chi^2=13.33$ , df=6, p=0.038). In the post-hoc analyses we detected FDS only in the years 2000 and 2005. Year 2000 showed a non-significant interaction with a negative estimate for FDS, (GLMM, pers×NEB×year,  $\chi^2=2.35$ , df=1, p=0.125, negative estimate = -0.012, Figure 1). Year 2005 explained a significant amount of variation that signifies positive FDS (GLMM, pers×NEB×year,  $\chi^2=9.32$ , df=1, p=0.002, positive estimate = 0.045, Figure 1). Other years did not add any significant amount of variation to the interaction and only directional or no selection appeared in these years (GLMM, pers×NEB×year, P>0.17, Figure 1).

We tried to explain the significant factor year in the interaction pers×NEB×year by variation in annual environmental factors. Beech-mast crop weight (ANOVA,  $F_{1,5}=0.19$ , p=0.68) and population density (ANOVA,  $F_{1,5}=1.06$ , p=0.35) and also the interactions of these variables (all p>0.35) could not explain the yearly variation in the direction of FDS.

Selection on personality types in adult survival fluctuated between sexes depending on year, but this was independent on neighbour exploratory behaviour (GLMM, pers×year×sex,  $\chi^2=18.04$ , df=1, p=0.006, Figure 2).

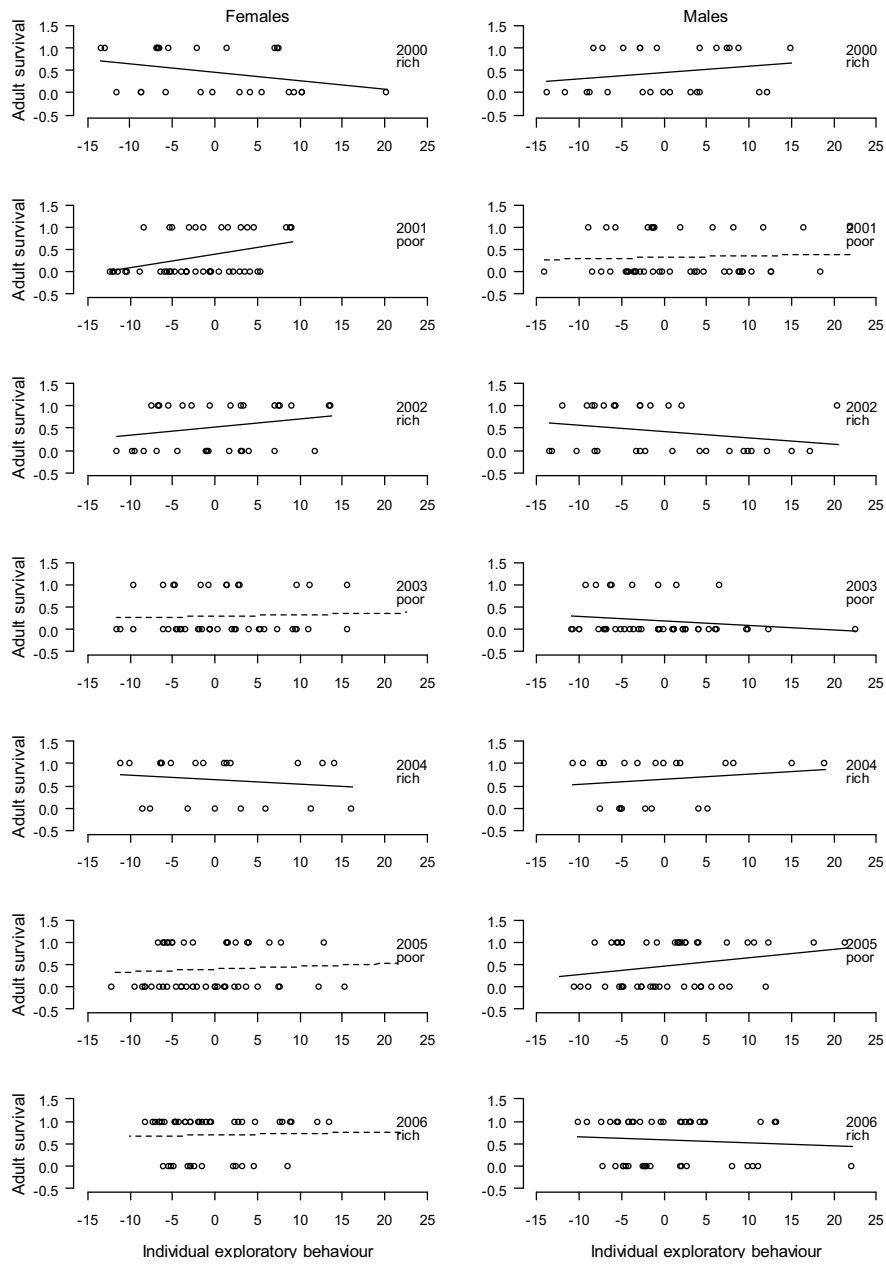
### *Reproductive success and Fitness*

In contrast to adult survival, we did not detect FDS (pers×NEB×year) in reproductive success (GLMM, pers×NEB×year,  $\chi^2=8.83$ , df = 14, p=0.84) and also not when combining reproductive success and survival in a composite fitness measure (GLMM, pers×NEB×year,  $\chi^2=12.99$ , df = 14, p=0.53). However, we found that neighbour exploratory behaviour affected both reproductive success (GLMM, NEB×year,  $\chi^2=15.93$ , df=1, p=0.014) and fitness (GLMM, NEB×year,  $\chi^2=14.23$ , df=6, p=0.03) independent of the personality of the parent. Overall there was no proof of FDS in reproductive success (pers×NEB,  $\chi^2=0.01$ , df=2, p=0.998) and fitness (pers×NEB,  $\chi^2=1.01$ , df=2, p=0.60).



**Figure 1.** Selection acting in different years on survival of personality types in dependence on frequency; 2000 negative FDS, 2001 negative selection on frequency, 2002 positive selection on frequency, 2003,2004,2006 no selection on frequency, 2005 positive FDS. For illustrative purposes personality and neighbour exploratory behaviour are divided in groups. The error bars are calculated for fitted vales from the model of FDS in adult survival on mean frequency (Slow, Intermediate and Fast environment). Compared to classical studies are both exploratory behaviour and NEB continuous variables. We therefore tested the association of different combinations of the focal personality and its environment on fitness of the focal individuals.

Chapter 3



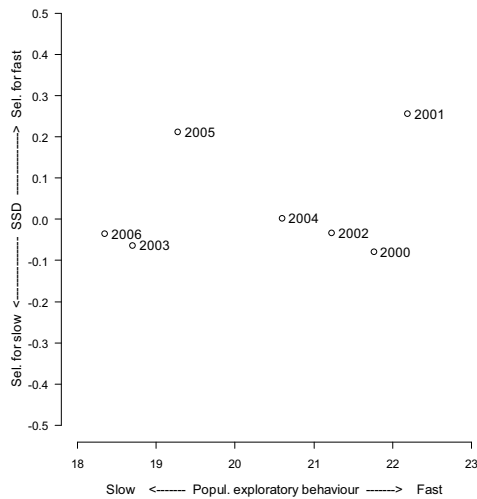
**Figure 2.** Selection acting in different years on survival of sexes in dependence on personality types but independent of neighbour exploratory behaviour. Data points are raw data of adult survival. As visible from lines in graph, selection on personality between sexes did not always differ between sexes and years following beech-mast crop distribution (poor and rich years) as proposed by Dingemanse *et al.* (2004). Low exploratory behaviour reflects slow birds, high reflects fast birds. Lines are regression lines of fitted values of reproductive success from the final model for adult survival on neighbour exploratory behaviour. Solid lines indicate significant and dashed lines non-significant relationships.

*Mate choice*

We found that there was a strong tendency for disassortative mating in our population when taking all breeding attempts in these seven years into account ( $r_p = -0.174$ ,  $N=518$ ,  $p < 0.001$ ).

*Standardized selection differential (SSD)*

The relationship of Standardized Selection Differentials (SSD) with mean population personality which represented population frequency was not significant (lm,  $F_{1,5}=0.28$ ,  $R^2=0.05$ ,  $\beta = 0.02$ ,  $p=0.62$ , Figure 3). Hence, we did not detect FDS on population scale.



**Figure 3.** Frequency-dependent selection on did not act on population scale. The x-axis shows population frequency represented by mean population exploratory behaviour. The y-axis is Standardized Selection Differential - difference between mean personality score of the breeding birds survived till next year and mean personality score of all breeders, divided by the standard deviation of mean personality score of all breeders.

**Discussion**

We found that the direction and strength of selection on personality through adult survival was dependent on neighbour exploratory behaviour, indicating frequency-dependent selection. However, the direction of this relationship varied between years. We identified years with nFDS and pFDS. In years without signs of FDS we found directional selection or absence of selection on personality related to frequency. We did not detect FDS in reproductive success and fitness.

Reproductive success and fitness were affected by neighbour exploratory behaviour (NEB), but not by the personality of the parents, which are the same results as of Chapter 2 but gained by different analyses. We used parental personality in our analyses instead of the recruit's own, since we were primarily interested in the fitness consequences of the parents. We deduce from the results of this chapter that perhaps interaction of own juvenile's personality with the neighbour exploratory behaviour might demonstrate FDS in

juvenile survival as happened in adult survival. Further studies including measures of the personality of the fledglings are therefore needed.

We found that seven years differed in the type and in the direction of selection dependent on frequency that acted on personality by adult birds. Positive FDS is supposed to lead to loss of variation. However, pFDS may be important in cultural transmission, which is highly essential for animals using social learning (Sih *et al.* 2004a; Sinervo & Calsbeek 2006). Great tits differ in social learning in dependence on their personality type (Marchetti & Drent 2000), which may affect their survival (Brown & Laland 2001). For example, learning faster within certain phenotypes could give an important advantage in critical situations such as becoming independent of the parents or gathering new sources of food.

We also detected directional selection (2001, 2002) and in some years did not detect any selection (2003, 2004, 2006). Although directional selection is generally considered to decrease the genetic variation, variation may be maintained by temporal and spatial heterogeneous conditions, which may generate fluctuating selection consisting of short or weak directional selections (Roff 1998a; Robinson *et al.* 2008). Fluctuating selection is an important form of balancing selection, because it represents habitat heterogeneity in time and space. Fluctuating selection was for example found in Soay sheep (*Ovis aries*) living in an unpredictable environment (Robinson *et al.* 2008). Males investing into body condition had higher fitness under poor environmental conditions, but males investing into horn growth did better under good environmental conditions. Disturbance events lead to polymorphism in pygmy grasshoppers (*Tetrix subulata*) by changing selection on colour morphs (Karlsson *et al.* 2008). Our results are in line with Quinn *et al.* (2009) who found that exploration behaviour of great tits is affected by gene $\times$ environment interactions and is under heterogeneous fluctuating selection. Although the selection is often weak and context dependent, environmental heterogeneity affects selection on personality traits and thus may maintain variation.

High variation in selections between years in our population signifies that also our birds experience habitat heterogeneity and fluctuating selection. We tried to explain habitat heterogeneity by a combination of non-social and social population characteristics influencing individual fitness. We found a general effect of the beech-mast crop on survival independent of personality or neighbour exploratory behaviour, which concurs with the literature (van Balen 1980; Drent 1984; Perdeck *et al.* 2000), but beech-mast crop could not explain the variation in forms of social selection acting on personality. Hence, although beech-mast crop might be an important factor explaining between year variation in selection pressures (Dingemanse *et al.* 2004) it cannot explain the presence and strength of FDS in our dataset. Also density, which is an important factor influencing competition (Bleay *et al.* 2007), had no effect on the year difference in selection on personality and FDS. Hence, the year differences may result from other environmental characteristics that we did not measure in this study. For example long-term plant composition changes

resulting from forest management and succession, the dynamics in the insect and seed cycles, weather conditions or predation rate, all vary between years.

The variation of personality may be also strongly maintained by the different impact of selection on age and sex categories. The survival of different personality types differed in our population for the sexes independently of neighbour exploratory behaviour and this relationship varied over years, which also found Dingemanse *et al.* (2004). However, contrary to Dingemanse *et al.* (2004), which included three years only, the direction of selection was not always opposite for sexes in our seven years. Selection appeared in both sexes only in three years, in three years it appeared only in males and in one year only in females. The direction within one sex also did not alter in the beech-mast crop poor and rich years as proposed by Dingemanse *et al.* (2004). Hence, based on our additional years of data, the impact of the beech-mast crop on selection on personality needs to be reconsidered. It is more probable that although the beech-mast crop is an important environmental characteristic, it interacts with the other characteristics and this interaction may cause different survival between years.

The aim of this study was to investigate the role of nFDS in relation to other selective forces. Because great tits are territorial birds we concentrated at the impact of nFDS on an individual scale which represented interaction of individual personality with personality of its direct neighbours. We also tested for nFDS on population scale through relation of population frequency and selection on personality (standardized selection differential – SSD). However, we detected nFDS only on individual scale and not on population scale. Great tits as territorial holders interact actively mostly with their direct neighbours because of the territories and so are probably the interactions on individual scale more important than interactions with all the individuals in the population.

We detected nFDS in year 2000, but other selection pressures dependent on frequency were also detected in the other six years. Research that focused on nFDS acting on morphological traits found an interaction between nFDS and other mechanisms that maintain variation. For example Bleay *et al.* (2007) found nFDS acting on the alternative mating strategies in the side-blotched lizard (*Uta stansburiana*) on individual scale. However, they concluded that nFDS is not strong enough to maintain variation. Also density and strong intrasexual competition are likely to play an important role. Variation in the major histocompatibility complex (MHC) is maintained by nFDS and disassortative mating (Penn & Potts 1999). Takahashi & Hori (2008) found that the combination of disassortative mating with nFDS is more effective than a single mechanism to maintain polymorphisms in the mouth opening direction in Tanganyikan cichlid fish (*Perissodus microlepis*). Also birds in our population mated disassortatively in regard to their personality, which confirms existence of combinations of selection pressures in our study system. Therefore, we conclude that although the contribution of nFDS on adult survival may be present in some years in the combination with other selection forms, it is most likely too weak to maintain variability in personality.

To the best of our knowledge this is the first study showing the impact of negative frequency-dependent selection on personality in the wild. Our findings demonstrate that the potential of nFDS to maintain variation in personality is considerably smaller in complex natural conditions in the wild than previously expected. Contrary to the theoretical models we show that nFDS may play a role as one of the forms of balancing selection, but it is not strong and constant enough to maintain variation in personality on its own. We conclude that the combination of fluctuating selection caused by environmental heterogeneity with age and sex differences, disassortative mating and occasional FDS are responsible for the maintenance of variation in trait complexes like personality.

### **Acknowledgments**

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