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The maintenance of variation in avian personality

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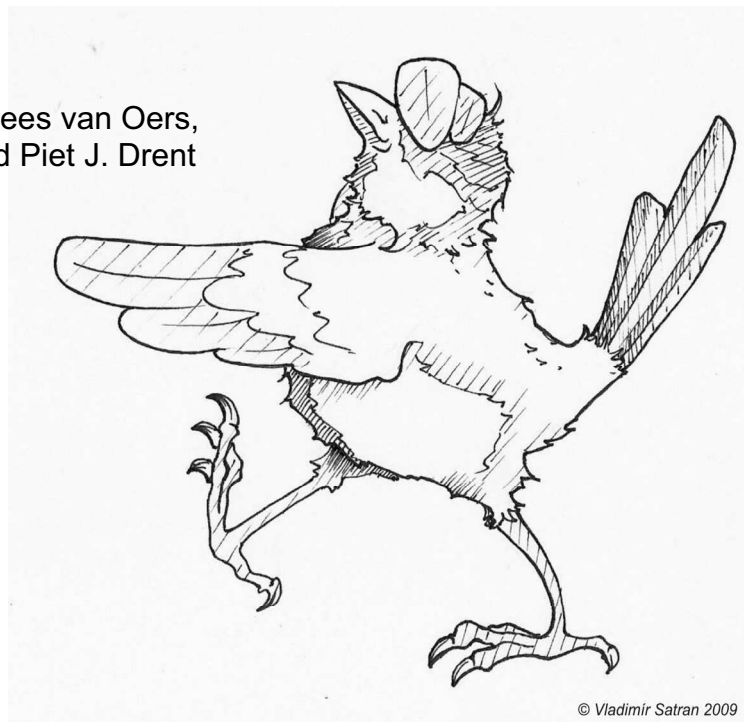
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Chapter 6

Frequency-dependent selection on personality in the great tit: a field experiment

Eva Fucikova, Kees van Oers,
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Abstract

The maintenance of variation in personality traits is of major interest in evolutionary biology, but which mechanisms are underlying this maintenance in the wild remains unclear. Theoretical models and laboratory studies show that negative frequency-dependent selection (nFDS) might be an important mechanism explaining the maintenance of this variation. However, studies in heterogeneous natural environments have shown that various mechanisms might contribute to maintain variation while nFDS is not often detectable. To test the causal relationship between personality frequency and selection, we experimentally manipulated the personalities in the social environment around breeding pairs of different personality phenotypes. In spring 2007 and 2008 we experimentally removed great tits with extreme exploratory scores from two adjacent plots of the experimental area. In 2007 we removed extreme fast explorers in one plot and extreme slow explorers in the second plot, creating a slower and faster social environment for the remaining birds. In 2008 we reversed the experiment for the two plots. We subsequently measured whether such experimentally changed frequency of personalities had consequences for the survival of the territory owners and their reproductive success (number of recruits a pair produced). Based on the results of our former long-term study we expected that changes in neighbour exploratory behaviour could cause differential selection on personality types only in some years and other selection mechanisms, including positive FDS, directional selections and absence of selection dependent on frequency, may act in other years. We found evidence for FDS acting on personality in the adult survival of breeding birds in 2008 only, but not for reproductive success in either year. The effect in adult survival differed between the sexes: we found nFDS in females and positive FDS in males. There was no nFDS in 2007. We can explain this year dependent effect by environmental heterogeneity. Our field experiment supports the hypothesis that frequency-dependent selection plays a role in the maintenance of variation in personality, but it is only detectable in combination with certain environmental conditions and more interactive selection pressures maintain variation in personality.

Introduction

A topic that has received a great deal of attention in evolutionary biology is how individuals vary in their personality and how variation in personality is maintained. Personality is defined as a suite of traits that are consistent across situations and through time (Koolhaas *et al.* 1999; Rodenburg *et al.* 2004; Sih *et al.* 2004a). Personality traits are heritable (Drent *et al.* 2003; van Oers *et al.* 2005a; Bell 2007) and genetically correlated (van Oers *et al.* 2004a). Variation in personality has been studied in a number of animal species (Gosling 2001). Studies on fitness consequences have shown that personality is under natural (Reale *et al.* 2007; Smith & Blumstein 2008) and sexual selection (Van Oers *et al.* 2008). However, how variation in personality is maintained in the wild is still unexplained (Sih *et al.* 2004b; Boon *et al.* 2007; Wolf *et al.* 2008a).

One of most frequently proposed mechanisms to maintain genetic variation is negative frequency-dependent selection (nFDS) (Turelli & Barton 2004; Sinervo & Calsbeek 2006; Penke *et al.* 2007b; McNamara *et al.* 2008; Wolf *et al.* 2008b; McNamara *et al.* 2009). Negative FDS is a type of balancing selection. It operates against common phenotypes and thus rare phenotypes are in advantage. The rarer phenotype experiences for example lower competition (Hori 1993; Bleay *et al.* 2007) and has therefore the highest fitness. When the frequency of this phenotype increases, another phenotype with a lower frequency gains advantage of lower competition and thereby increases its fitness. In this way nFDS balances fitness and creates a certain rate for all the phenotypes in the population. Negative FDS plays a role in many evolutionary models like the maintenance of variation in antibodies, predator-prey interactions or alternative mating strategies. Variation in the major histocompatibility complex (MHC) is known to be maintained by nFDS; pathogens adapt themselves for the most frequent alleles in the population (Penn 2002; Borghans *et al.* 2004) making less frequent alleles less vulnerable to disease. In another example, polymorphisms in the human leukocyte antigen (HLA) which helps to determine specificity in immune response shows nFDS; there is a differential immune response towards HIV disease with an advantage for the rare HLA alleles (Trachtenberg *et al.* 2003). In an example from predator-prey interactions where nFDS acts on prey, blue jays (*Cyanocitta cristata*) searched cryptic, computer generated digital moths (2002). Jays failed to find atypically coloured cryptic prey more often than the more common cryptic prey. A case where nFDS acts on a predator was shown in scale-eating cichlid fish (Hori 1993), where the mouth of these fish can be right or left orientated and individuals approach prey in dependence on this orientation. Because prey usually adapt to the side where attacks take place more frequently, the rarer phenotype of the predating fish is more successful and thus favoured by nFDS. However, descriptive and laboratory studies may overestimate the role of nFDS in natural settings (Bleay *et al.* 2007; Takahashi & Hori 2008). Habitats of different animals may differ e.g. in environmental factors, which are important for trait expression. It is not possible to properly estimate the strength of selection because of the heterogeneity and fluctuation in environmental factors in descriptive studies (see e.g. Dingemanse *et al.* (2004)). Another problem is that the actual trait under selection

may not be among the traits measured in a descriptive study (Schluter *et al.* 1991). Therefore experimental manipulations in the wild are needed to separate cause and effect of alternating frequencies (Schluter *et al.* 1991; Bleay *et al.* 2007; Reale *et al.* 2007).

Few experiments exist that test the role of nFDS in the wild (Olendorf *et al.* 2006; Bleay *et al.* 2007). One of the rare examples was conducted on guppies (*Poecilia reticulata*). When frequencies of males with different colour patterns were manipulated, the rare male phenotypes had lower chance of getting predated (Olendorf *et al.* 2006). One of these studies shows that nFDS may interact with other types of selection and that only these combinations of selections are strong enough to maintain variation (Bleay *et al.* 2007). An experiment of Bleay *et al.* (2007) showed that nFDS acts on mate choice. They manipulated frequencies of the three male colour morphs in side-blotched lizards (*Uta stansburiana*) and tested for female choice. Females chose the rarer phenotype. However, Bleay *et al.* (2007) propose that not only rare male mating advantage (a form of negative-frequency dependence) is responsible for maintenance of variation but probably also e.g. density-dependent selection, territory quality or other mechanisms act on fitness of side-blotched lizards.

Hence, nFDS acts from the genetic to the inter-population level at different contexts and it may interact with other selection mechanisms. It is shown that nFDS is able to maintain polymorphisms coded by a few major loci (Turelli & Barton 2004; Penke *et al.* 2007b) as in the examples mentioned above. However, experiments on the maintenance of variation by nFDS in multiple quantitative behavioural traits coded by many genes, like personality, are lacking.

Personality is a suite of traits and each of these traits may be affected by different selection pressures/mechanisms. A confounding factor here could be that pleiotropic effects of the genes involved in one personality trait could make detection of nFDS more challenging, since they might be subject to different selection forces in other traits (Burger 2002). Until now, there have only been laboratory based studies that test nFDS for personality traits. One was done with *Drosophila melanogaster* larvae (Fitzpatrick *et al.* 2007), although strictly speaking not really on a personality trait. Conditions in this experiment were controlled and the larvae differed only in alleles of a foraging gene, which divides their activity into rovers (move more) and sitters. If both larvae types are in the low nutrient conditions and thereby under high competition, the rarer type has a higher fitness. Brelin *et al.* (2008) tested to what extent populations of brown trout (*Salmo trutta*) differ in behaviour in the personality tests and if the frequency of these phenotypes differed in a laboratory. They tested fish from four different rivers for personality. Frequency of personalities differed between populations and Brelin *et al.* (2008) explain this by a combination of nFDS, habitat heterogeneity or individual choice early in life. However, they did not specifically test this hypothesis.

Hence, it is very difficult to test the role of nFDS on maintenance of variation in personality, especially in the wild. In wild populations conditions are much more complex, e.g. because of high environmental heterogeneity in time and space, or other selection

pressures like density-dependent selection which could interact with nFDS (Sinervo & Calsbeek 2006; Robinson *et al.* 2008). There are not discrete phenotypes but a scale of phenotypes of the focal birds and a scale of social environments surrounding territories of focal birds in our wild population. We therefore had to test how do different combinations of the focal personality and its environment affect fitness of the focal individuals. This combination of personalities is more usual in the wild than discrete phenotypes. In our descriptive study on great tits (*Parus major*) where we used a long-term dataset, we found that survival and reproductive success were dependent on the frequency of personalities surrounding breeding pairs and that for adult survival this varied with personality types. We also showed that not only nFDS acted on personality varying over years (Chapter 3). In seven years, we found evidence for weak nFDS, pFDS (common type has highest fitness), directional and a lack of selection depending on frequency. However we did not detect nFDS in reproductive success (Chapter 2; Chapter 3) and also not in the juvenile survival while using their own personality (Chapter 5). We hypothesised that different selection mechanisms can be detected depending on environmental conditions, and that this together with habitat heterogeneity and disassortative mating may act in the complex situation in the wild, thereby maintaining variation in personality.

The aim of this chapter is to test experimentally the role of nFDS on personality in a natural population, independent from other selection pressures and if this role varies due to variation in environmental circumstances. From the results of our descriptive long-term study we expect that nFDS only acts on adult survival and not on reproductive success. Based on the results of our former long-term study we expect that changes in frequency could cause differential selection on personality types only in some years. Other selection mechanisms including pFDS and absence of selection dependent on frequency may act in other years.

Methods

Population studied

The experiment was done in the Westerheide population near Arnhem, the Netherlands (5°50'E, 52°00'N). This area with mixed plots of different tree species has about 250 nest boxes regularly distributed. We ran an intensive catching program from the time when fledglings reached independence of their parents (mid-June) until March the next year to determine the personality of (later) breeding birds. Moreover, we ran night controls of the roosting birds (roosting controls) in autumn and early spring (great tits roost in nest boxes during the autumn and winter). If birds were caught for the first time we transported them to the aviary facilities to test birds for their exploratory behaviour in the novel environment test.

From April onwards we checked nest boxes twice a week until females were incubating full clutches and each day from the expected hatching date onwards. Nest boxes were checked again a week after the expected day of fledging to assess the exact number of

fledglings. No correction has been used for capture probability of breeding birds, since it is extremely high in our population (97.5% for females and 86.3% for males; see also Both *et al.*(1999)). Nestlings were banded with an individual metal ring on day 10 after hatching, measured and weighed. Parents caught in the nest box with a spring trap were also ringed (if unmarked), measured and weighed but not tested for exploratory behaviour since bringing them to the lab for one day could interfere with their breeding activities.

Novel environment test

Exploratory behaviour was measured using a novel environment test the morning after catching (Verbeek *et al.* 1994). Individuals were tested alone in a room (4.0×2.4×2.3 m) with five artificial trees. We used the total number of flights (movements between trees) and hops (movements within trees) within the first 2 min as a measure of exploratory behaviour. Faster explorers have higher scores compared to slower explorers. We corrected data for date of capture based on the finding that behaviour changes with capture date within individuals (Dingemanse *et al.* 2002). After all birds had been tested and weighed, they were directly released at the place of capture.

FDS experiment

The experiment was carried out from March 2007 until the end of the breeding season of 2009. We checked roosting birds in autumn and one month prior to start of the experiment to establish the identity and exploratory behaviour of birds in our population. On basis of this knowledge we divided the study area in two plots which had similar composition of personalities (t-test, $t = -1.25$, $df = 415.58$, $p = 0.21$) and habitat. We also checked if FDS did not differ between these two plots in years 2000-06. If this happened already in years before the experiment, our experimental effect could be hidden behind this effect. We did not find difference in FDS between the plots (GLMM, $\text{pers} \times \text{NEB} \times \text{plot}$, $df = 36$, $\chi^2 = 3.98$, $p = 0.41$).

The experimental change in the composition of personalities in the future breeding populations was done during one night in the end of March 2007 and 2008. All birds of extreme and unknown personality caught during the roosting in a nest box were brought to the laboratory. Birds of unknown personality were tested next morning. After personality of all birds was known we selected 25% of males and females with extreme personalities (both fast and slow exploring). These birds were released at a place 40 km from the experimental area. Although only male neighbours affect adult survival and reproductive success (Chapter 2), we removed also females (using the same experimental design) to create equal conditions for both sexes. The birds which were brought to the lab in the night control and did not belong to the 25 % extremes were released close to the nest box where they had been found in the roosting control former night. We created a faster environment in the northern plot and slower environment in the southern plot in 2007 (Figure 1) and we reversed the treatments in 2008. We followed survival and reproductive success of the remaining birds for next two breeding seasons. Hence, our determination of plots was done

at the population level and extreme birds were removed randomly. We removed neighbours randomly at the individual level but not equally from individual territories.

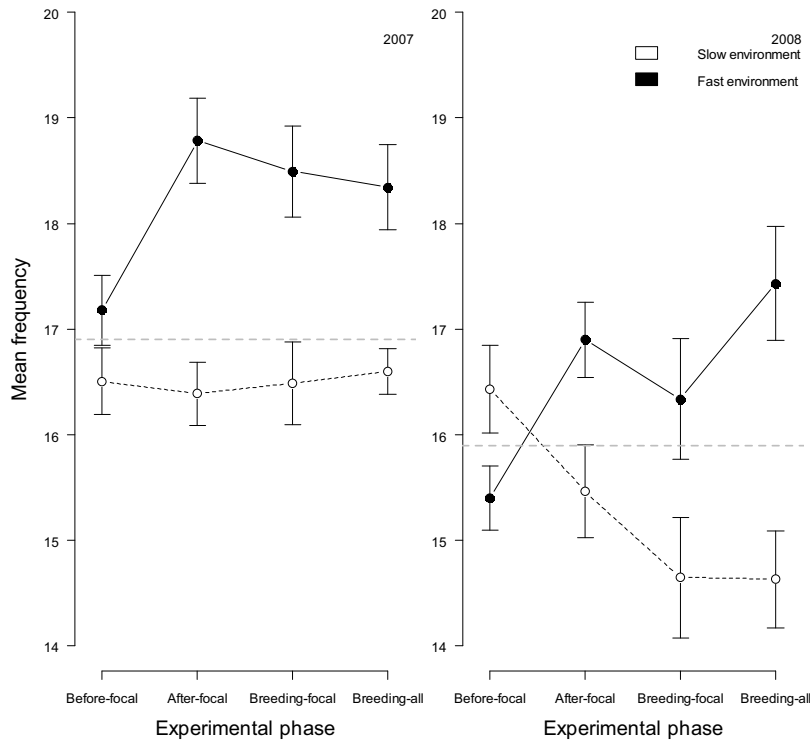


Figure 1. Success of the experiment in two plots of our experimental area in years 2007 and 2008. Neighbour exploratory behaviour (NEB) in different phases of removal of fast/slow exploring neighbours around focal individuals. In 2007 we were successful in creating relatively slower and faster environment between plots. However, the removal in the slow plot in year 2007 was not successful, because the neighbour exploratory behaviour in the breeding season was not lower than the mean NEB before the experiment (absolute neighbour exploratory behaviour). The removal in year 2008 was successful on both levels – relative and absolute. Before-focal: NEB around focal individuals before the experiment, After-focal: NEB around focal individuals after the experiment, Breeding-focal: NEB around focal individuals in the breeding season (starting point for the analyses), Breeding-all: NEB around all individuals in the breeding season.

Territory assessment

To detect a change between the social environment of focal birds before the experiment and in the subsequent breeding season, we calculated territories around these birds using the GIS technique of Dirichlet tessellations. This technique assigns space to a given point (here an inhabited nest box) that is closer to this point than to any other point (another inhabited nest box) and thus fills the experimental area/population with theoretical territories.

Territories created by the Dirichlet tessellation do not differ significantly from real territories and therefore may be used for analyses (Chapter 2). Because of using individuals as both focal and neighbour (multiple times) for one analysis, our variables might be under spatial autocorrelation. Spatial autocorrelation is a form of statistical pseudo-replication because of the correlation of a variable with itself through space (Moran 1950). However, we found no proof for spatial autocorrelation in neighbour exploratory behaviour (Chapter 2).

Neighbour exploratory behaviour (NEB) and Δ Neighbour exploratory behaviour (Δ NEB) - measurement of social environment

We calculated *Neighbour exploratory behaviour (NEB)* as the mean personality of all neighbours sharing a boundary with the territory of a focal bird. This describes the social environment that is present for each nest box as an indication of local frequency of personalities. We used this technique to calculate NEB around i) individuals before the experiment, ii) right after the experiment (removal of birds with extreme personality) and iii) in the subsequent breeding season. For analyses we used birds which were found both roosting (before the experiment) and breeding in our area (focal birds). Because we changed their social environment by our experiment, we were interested how this change affected their survival and the reproductive success. Thereby we used *Δ Neighbour exploratory behaviour (Δ NEB)* which is a difference between NEB before the experiment and NEB in the breeding season for the analyses.

Statistical analyses

We used the statistical software R version 2.7.2 (R Development Core Team 2007) for all statistical analyses. All tests were two-tailed. The analyses were done with focal birds that were found in both plots before the experiment and which were also breeding there after the experiment.

To compare NEB around the focal animals in the northern and southern plot before the experiment we used a t-test. To test success of the experimental removal we compared NEB around the focal animals before the experiment and NEB in the breeding season by conducting a paired t-test.

We analysed the impact of FDS on local adult survival of focal breeding individuals and their reproductive success between breeding seasons 2007-08 and 2008-09. The impact of FDS was analysed for both years combined and separate for each year. In the analyses of *adult survival* 102 birds (48 males and 54 females) were used in 2007 and 88 birds (44 males and 44 females) in 2008. *Adult survival for years 2007 and 2008 combined* was analysed with a generalized linear mixed-effects model approach (GLMM; lmer in R) with a binomial error structure. The model contained adult survival (survived or died) as dependent variable with a binomial distribution and logit link function. Analyses were done on individual basis. To detect FDS, we used same analyses as in Chapter 3, but we used Δ NEB instead of NEB for the experimental data. This enabled us to test if the experimental

change in frequency affected selection. Hence, we included Δ NEB, and personality of the focal individual as explanatory variables in the model, together with sex and year known to be important from former analyses (Chapter 3). We tested the same set of 3-way interactions (pers \times Δ NEB \times year, Δ NEB \times year \times sex, pers \times Δ NEB \times sex, pers \times year \times sex) as in Chapter 3. Control variables were age of breeding (1year old or older), and local density (the centralized distance of the third closest occupied nest box from the focal nest box in the given year) since they are variables known to affect adult survival. Personality was also centralized to the mean personality for a given year. Since several nest boxes were used 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.

Adult survival in a particular year was analysed with a generalized linear model approach (GLM) with a quasibinomial error structure to avoid overdispersion. Because this analysis was run within one year, we tested a 3-way interaction answering the question: Is adult survival dependent on neighbour exploratory behaviour and does this effect differ for personalities and sexes (pers \times Δ NEB \times sex)? Control variables were identical compared to the analyses with years combined. Personality scores and density were not centralized to the year mean for the within year analyses.

The impact of FDS on *reproductive success* was also analysed for both years together and within each particular year. Reproductive success was defined as the number of juveniles which a pair produced and which recruited in our area next year. We used birds having at least one fledgling, because we were not interested at the impact of the nestling mortality on reproductive success. We used 99 birds (47 males and 52 females) for 2007 and 86 birds (43 males and 43 females) for 2008. The response variable was the number of recruits a breeding pair produced and which bred in our area. Hence, we analyzed data including male and female parent exploratory behaviour since we were interested in the number of recruits the focal individuals produced. The model of reproductive success included the number of recruits per pair as the dependent variable with a Poisson error distribution. The model of the *reproductive success with years clumped* together was based on 185 focal birds. We tested the same 3-way interactions as in the adult survival using local density and hatching date as covariates, and nest box as a random effect. *Reproductive success in a particular year* was analysed with a generalized linear model approach (GLM) with a quasipoisson error structure. The interaction and control variables were same as in the analyses of adult survival for separate years.

Because we switched the treatments for the two experimental plots from year 2007 to 2008, we describe the experimental plots as a slow and a fast plot. Because we actually did not analyse Δ NEB separately for the plots but we concentrated at Δ NEB around the focal individuals we describe change of social environment of the focal individuals as slow and fast environment in the following text.

Results

Neighbour exploratory behaviour (NEB) in the two plots of the study area was similar before the experiment (t-test, $t = -1.47$, $df=99.9$, $p=0.14$). Figure 1 visualizes the differences we achieved after removal of the birds compared to the situation before removal. Removal in the slow plot in year 2007 was not successful, because our removal in this plot did not cause a change in NEB in the breeding season compared to NEB before the experiment (paired t-test, $t=0.04$, $df=49$, $p=0.97$; Figure 1). In contrast, the removal in the fast plot was successful (paired t-test, $t = -3.08$, $df=51$, $p=0.003$; Figure 1). In 2008, before the removal, there was a tendency for the two plots to differ in overall frequency (t-test, $t = 1.9$, $df=85.5$, $p=0.06$) as a consequence of the experiment in 2007. Because we did a reverse experiment and we therefore switched treatments between the plots, the frequency before the experiment in 2008 was higher in the slow compared to the fast plot. We were successful in changing the social environment in 2008 (slow plot: paired t-test, $t = 4.13$, $df=51$, $p < 0.001$; fast plot: paired t-test, $t = -2.62$, $df=35$, $p=0.01$, Figure 1). Although we were not completely successful in creating a slower environment in 2007, the slow and the fast plot significantly differed in neighbour exploratory behaviour in the breeding season: 2007 (t-test, $t = -3.45$, $df=99.33$, $p < 0.001$) and 2008 (t-test, $t = -2.09$, $df=83.01$, $p=0.04$).

Adult survival

Looking at overall survival and reproductive success differences for the whole population we found a strong difference between the two years. The adult survival rate was higher in 2007 than in 2008 (2007: 51% and 2008: 11.4%; t-test, $t=7.03$, $df=236.02$, $p < 0.001$). This was also true for recruitment rate of all the juveniles born in our area (2007: 33.3% and 2008: 10.5%; t-test, $t=5.33$, $df=154.32$, $p < 0.001$).

We found that the difference between the neighbour exploratory behaviour before and after removal (Δ NEB) affected adult survival differentially in both years (LMER, Δ NEB \times year, $df=1$, $\chi^2=7.13$, $p=0.007$). We also found two trends detecting selection pressures on personality phenotypes. The first trend showed that adult survival was affected by personality of the focal birds themselves and this effect differed between years (LMER, pers \times year, $df=1$, $\chi^2=3.31$, $p=0.07$). We also found a trend for an effect of a change in local frequency (Δ NEB) in relation with the personality of the focal and hence FDS (LMER, pers \times Δ NEB, $df=1$, $\chi^2=3.30$, $p=0.07$). If either of these two non-significant trends were used in separate models without the other trend (either only pers \times year or pers \times Δ NEB), then both of them became significant (LMER, pers \times Δ NEB, $df=1$, $\chi^2=4.02$, $p=0.045$) and (LMER, pers \times year, $df=1$, $\chi^2=4.03$, $p=0.045$). This result indicates that both frequency-dependent selection (pers \times Δ NEB) and selection on personality fluctuate between years (pers \times year).

To identify the effects within the years, we continued with analysing the years separately. In 2007 survival in general was higher in a slower compared to a faster individual environment (GLM, Δ NEB, $df=1$, $p=0.005$; Figure 2), but since there was no interaction with the personality score of the focal individual itself (GLM, pers \times Δ NEB, $df=1$, $p=0.10$),

there was no indication for FDS in 2007. In 2008 we detected FDS, and this differed between the sexes; we detected nFDS for females and pFDS for males (GLM, $\text{pers} \times \Delta\text{NEB} \times \text{sex}$, $\text{df}=1$, $\text{estimate}_{\text{female}} = -0.05$, $\text{estimate}_{\text{male}} = 0.12$, $p=0.02$; Figure 3).

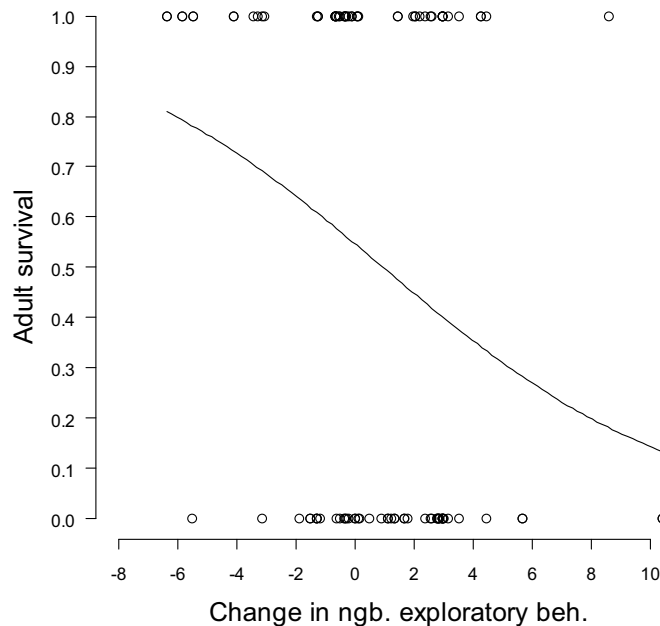


Figure 2. The change in neighbour exploratory behaviour (ΔNEB) between the spring and breeding season affected adult survival in 2007. Axis y shows raw values: zero are birds which died between the breeding seasons 2007 and 2008 and one are the birds which survived. The curve is a line going through fitted values from the final model of dependence of adult survival on (ΔNEB).

Reproductive success

Overall reproductive success (number of recruits a pair produced) was higher in 2007 than 2008 (GLMER, year, $\text{df}=6$, $\chi^2=4.3$, $p=0.03$), which confirms the results of analyses on differences in overall survival between years shown before. When analysed for the years combined, juveniles recruited more successfully if their parents bred in slow compared to fast environment (GLMER, ΔNEB , $\text{df}=6$, $\chi^2=8.22$, $\text{estimate} = -0.26748$, $p=0.004$).

Similar results were found when years were analysed separately: juveniles in both 2007 and 2008 recruited more successfully if their parents bred in slow compared to fast environments (2007; GLM, ΔNEB , $\text{df}=95$, $\text{estimate} = -0.13$, $p=0.007$; 2008; GLM, ΔNEB , $\text{df}=83$, $\text{estimate} = -0.24$, $p=0.01$; Figure 4). There was no indication for FDS via reproductive success in year 2007 (GLM, $\text{df}=98$, $\text{pers} \times \Delta\text{NEB}$, $p=0.86$) nor 2008 (GLM, $\text{df}=82$, $\text{pers} \times \Delta\text{NEB}$, $p=0.65$).

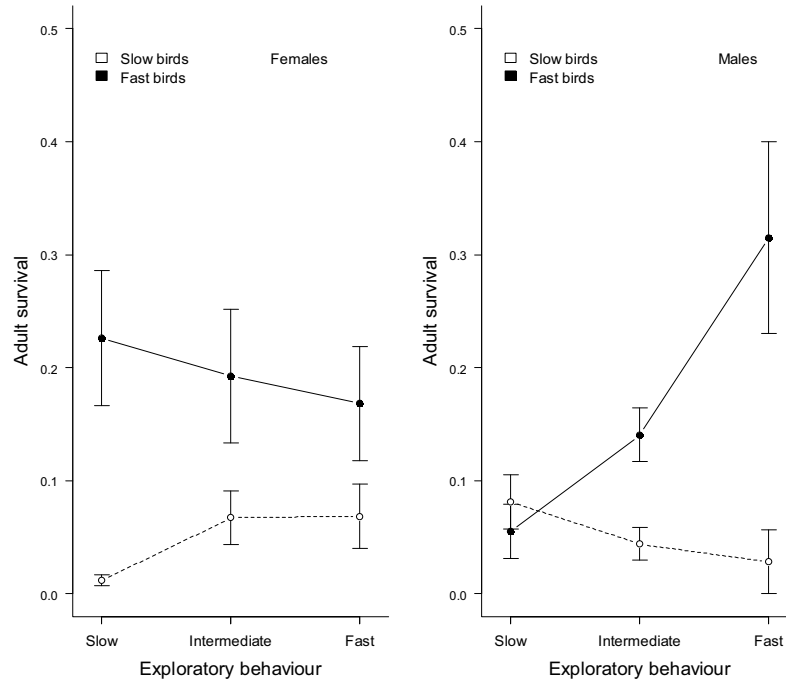


Figure 3. FDS detected in year 2008 in adult survival differed between sexes; birds of different personalities and sexes survived differently in dependence on their neighbour exploratory behaviour. Females were under weak nFDS and males under pFDS. Although the lines in the female part of the graph do not cross, their direction is opposite. The estimate in the final model for the female survival is negative which represents nFDS. The x-axis shows individual personality scores divided in three groups from slow to fast exploring birds. The y-values are fitted values calculated from the estimates of the final model for survival of birds with different personalities.

Discussion

We experimentally tested the presence of frequency-dependent selection on personality by altering the mean personality scores around breeding territories. We found weak nFDS acting on females and pFDS acting on males via adult survival in one out of two experimental years. Apart from this, adult survival in one and reproductive success in both experimental years were affected by the frequency of the personality of the direct social environment. Overall numbers of surviving and recruiting birds differed strongly between years.

We were successful in creating a faster environment in both years but a slower environment only in 2008. Although the removal in the slow plot in 2007 was not successful, since the analyses were done on the individual level, a relatively low experimental change would be expected to yield a low effect. Moreover, the success of the removal most likely did not affect our results, since the neighbour exploratory behaviour in the breeding season differed between plots relative to each other in both years.

If nFDS acted in both experimental years, fast birds should have survived better in a slower environment and slow birds better in faster environment. We did not find evidence for the presence of nFDS in 2007. In 2008 we detected FDS, which differed for the sexes: males were under strong pFDS and females under weak nFDS. This result differs from Chapter 3, where strong pFDS was found, but FDS did not differ between sexes. In Chapter 3 we found also selection on personality which differed between sexes and years but was independent of FDS. A possible explanation for this discrepancy might be that the long-term analyses did not detect the sex difference in FDS because it was masked by another, maybe stronger factor. This also shows that an experiment was needed to detect the opposite frequency dependent selection pressures for the sexes. These sex differences are in line with previous findings where selection on personality was shown to act differently and opposite on survival of the sexes (Dingemanse *et al.* 2004; Both *et al.* 2005; Smith & Blumstein 2008; Quinn *et al.* 2009). One plausible reason for this might be because sexes differ in their life history. Great tits are territorially living birds in which males occupy and defend territories and females choose mates. Within the hierarchical system in flocks males dominate females and adults dominate juveniles (Perrins 1979; Drent 1984). This could cause differences in selection between sexes, status and age categories. So sex might be a very imperative factor for FDS and the difference in FDS acting on sexes in a particular year could be one possible masking factor for the lack of FDS in observational studies (Chapter 3).

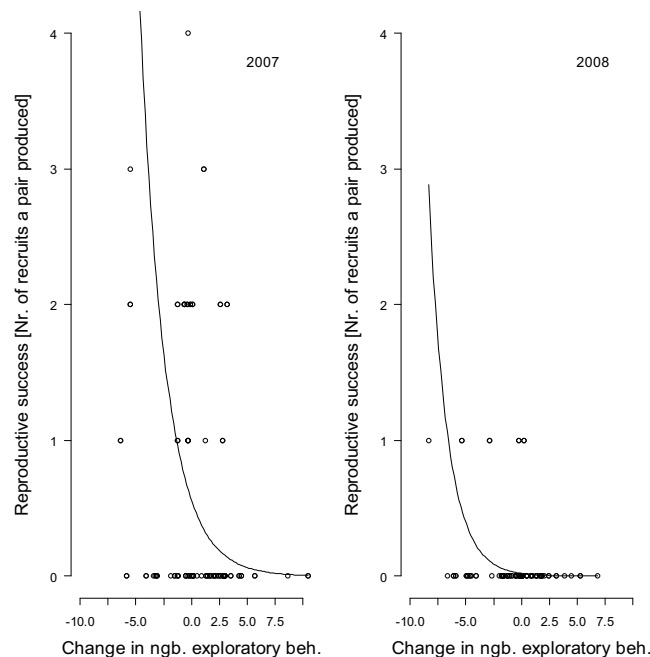


Figure 4. Reproductive success (number of recruits a pair produced) varied with the change in neighbour exploratory behaviour (Δ NEB) in 2007 and 2008. Figure shows raw data with the curve calculated from the model. Low values of Δ NEB are change into slower environment and higher values into faster environment.

Our experimental results for reproductive success confirmed our results of the long-term analyses of reproductive success, showing that there was no indication for FDS acting on the reproductive success (Chapter 2; Chapter 3). Reproductive success is affected by the social environment of the parents, but independent of the personality of the parents. In our analyses of the long-term data set, the number of recruits was higher for birds that were surrounded by mainly fast birds in some years, but lower in other years (Chapter 2). For both experimental years, reproductive success was higher when juveniles grew up in a slower environment. We offer three hypotheses explaining this finding. First, this chapter is based on an experimental change of neighbour exploratory behaviour and hence removal of birds with extreme personality, while our long-term analyses were based on observational studies. Because in both experimental years the number of recruits was higher for birds breeding in the slow environment, experimental procedure as a big change in population could have triggered selection for these individuals. Then juveniles born in slow environment could have an advantage against the ones from fast environment because slow exploring neighbours could interfere less strongly with parents after the experiment when all the birds were stressed by changes caused by the experiment (Verbeek *et al.* 1996; Dingemanse & de Goede 2004). The experiment was a large disruption for the whole population because individuals had to re-establish their territory borders just before the breeding season, which could have strongly affected their breeding conditions (Newton 1992; Amy *et al.* 2010). Moreover, the experiment could have changed additional life history traits that we could not detect by our analyses. Second, by chance we could have hit two years with higher reproductive success of juveniles born in slow environments. In our long-term studies (Chapter 2; Chapter 3) in two consecutive years out of seven parents from slow environments had higher reproductive success. The third hypothesis is that both the experimental effect and environmental conditions could have caused equal reproductive success in two experimental years. In our opinion, the first hypothesis is the most likely one. However, there is also a high possibility that hypothesis three showing a combination of more factors might occur. More long-term experiments are needed to solve this question.

We found that survival of focal birds and reproductive success was three times higher in 2007 compared to 2008. Moreover, we did not find nFDS in 2007 but we found weak nFDS in 2008 acting on adult female and pFDS acting on male personality. The most likely cause for this is that the experimental years 2007 and 2008 differed strongly in the severity of the winter. Winter 2007 was much milder for the birds, because the winter was warm in combination with low predation by sparrowhawks and rich beech-mast crop. Winter 2008 was extremely harsh, because of a higher number of days with temperatures below zero, higher predation and almost no beech-mast crop. These parameters are known to be of great effect on the survival of great tits (van Balen 1980; Perdeck *et al.* 2000). Birds are strongly handicapped in search for food in the case of the presence of snow cover and the absence of large seed supplies of beech mast. Moreover, they need more energy for thermoregulation (van Balen 1980; Perdeck *et al.* 2000). These differences between the two experimental years probably had an impact on competition and thus on the strength of

selection and the probability of detecting FDS. In mild years the selection on personality of settled adults is thereby low since competition is very low and all personality types will be able to survive. In contrast, in the more harsh situation the competition will be high which will increase the importance of personality in relation to the direct social environment (Dingemanse *et al.* 2004).

Harsh conditions in 2008 could have enhanced FDS and the detection of it in our population while mild conditions in 2007 might have hidden FDS. This is comparable with the finding in the experiment on rovers and sitters in *Drosophila* larvae. No nFDS could be detected if food was abundant, and nFDS was detected in extreme conditions only (Fitzpatrick *et al.* 2007). In the case of *Drosophila* was density a very important factor, because in high densities the number of interactions between individuals within a population increases and thereby so does competition, which is needed for nFDS. Moreover, although nFDS seems to be a powerful mechanism maintaining polymorphism in right and left orientation of mouth and thereby approach to prey in cichlid fish, Takahashi & Hori (2008) found that a combination of nFDS with disassortative mating and heterozygote advantage was needed to explain the maintenance of the side-polymorphism. If fish were mated assortatively, according to their lateralisation preference, the ratio of juveniles would be skewed toward left or right orientated animals since the handedness is coded genetically. This shows that the combination of disassortative mating with nFDS is a stronger mechanism than nFDS alone. Bleay *et al.* (2007), who manipulated frequencies of the three colour forms of males in side blotched lizards, proposed that the combination of density-dependent selection together with nFDS is probably responsible for the maintenance of variation in colour forms. Side-blotched lizards may use context-dependent mate choice rules or females may discriminate between males through success in increased intra-sexual competition. Similarly the maintenance of colour morphs in damselflies was explained (*Ischnura elegans*), for which nFDS together with selective mechanisms depending on variation in population conditions (Van Gossum *et al.* 2001; Ting *et al.* 2009) are responsible. We also found disassortative mating in our population (Chapter 3). Combination of the sometimes operating nFDS and disassortative mating could be also important for maintenance of variation in personality in great tits in the wild. Hence, FDS is an important mechanism explaining variation in several polymorphisms, but often in interaction with other selection mechanisms (Bleay *et al.* 2007; Takahashi & Hori 2008; Ting *et al.* 2009). These polymorphisms create usually discrete phenotypes. However, personality is a suite of quantitative traits with continuous variation and which are expected to be coded by many genes (van Oers *et al.* 2005a). Particular behavioural traits may be affected by different selection mechanisms, which may have impact on different genes. Therefore variation in personality might be maintained and at the same moment nFDS could lose its effect on personality traits.

It is possible that in the wild a variety of selection mechanisms may play a role on personality traits. However, nFDS is an unimportant factor which may occur in some years but is not strong enough to maintain variation in the wild. Also, models testing maintenance

of variability in personality on the genetic level show that genetic mutations or pleiotropy alone are weak for the maintenance of variation in quantitative polymorphic traits. This shows that balancing selection represented by a combination of genes interacting with environmental heterogeneity, sometimes nFDS or other selections may efficiently maintain variation in personality in the wild. Our experiment tested the presence of nFDS on personality in the wild. Our results show that evolutionary processes may depend upon local conditions, which depend on habitat heterogeneity in place and time. This experiment denies the hypothesis of nFDS as the main mechanism maintaining variation in personality. To our knowledge this is the first experiment testing the role of nFDS on a quantitative behavioural trait in the wild. More experiments are needed to assess its role and relative importance for maintaining variation in behavioural traits in more systems and contexts.

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