Personality and its fitness consequences in the Seychelles warbler (*Acrocephalus sechellensis*)

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Abstract of thesis

In this thesis, I investigate whether differences in behaviour among individuals, termed personality, are genetically determined and/or shaped by state and whether personality influences reproductive success and strategy. Using the Cousin Island population of Seychelles warbler (*Acrocephalus sechellensis*), I measured five personality traits: exploration of a novel object, exploration of a novel environment, obstinacy, stress response and escape response. I estimated the repeatability of each trait, the heritability of the repeatable traits and tested for correlations among repeatable traits. I found that exploration of a novel environment and object were repeatable and correlated, and novel environment exploration was heritable, suggesting that it may be under selection in the population. I then used a candidate gene approach to further investigate the genetic variation associated with personality, specifically targeting *SERT* and *DRD4*. I found no genetic variation in *DRD4*, but identified four polymorphisms in *SERT* that did not correlate with the novel environment or novel object exploration. These results suggest there was no association between these behaviours and variation in the candidate genes tested in this population, and that a genome-wide study might be beneficial to detect the relevant genes underlying personality. I then looked at how personality is potentially generated and maintained in a social living species by investigating whether personality is social state-dependent or reproductive state-dependent. I found that the novel environment and novel object exploration were not correlated with social status and behavioural consistency was unaffected by social status. Novel object exploration was instead associated with the interaction between insect abundance at year of birth and age (a proxy for reproductive state). Lastly, I investigated the fitness consequences of personality, particularly looking at its influence on reproductive behaviour. I found that disassortative pairs for novel environment exploration were more likely to have females engage in extra pair parentage, and that novel environment and novel object exploration were not associated with the number of offspring sired or the ratio of within to extra group young. Overall my results show that there are consistent among individual differences in behaviour in wild cooperative breeders that may be generated by future fitness potential and are associated with reproductive behaviour within the social pair.

Acknowledgements

“I'm making a list

I'm making a list of things I must say

For politeness,

And goodness and kindness and gentleness

Sweetness and rightness:

Hello

Pardon me

How are you?

Excuse me

Bless you

May I?

Thank you

Goodbye

If you know some that I've forgot,

Please stick them in your eye!”

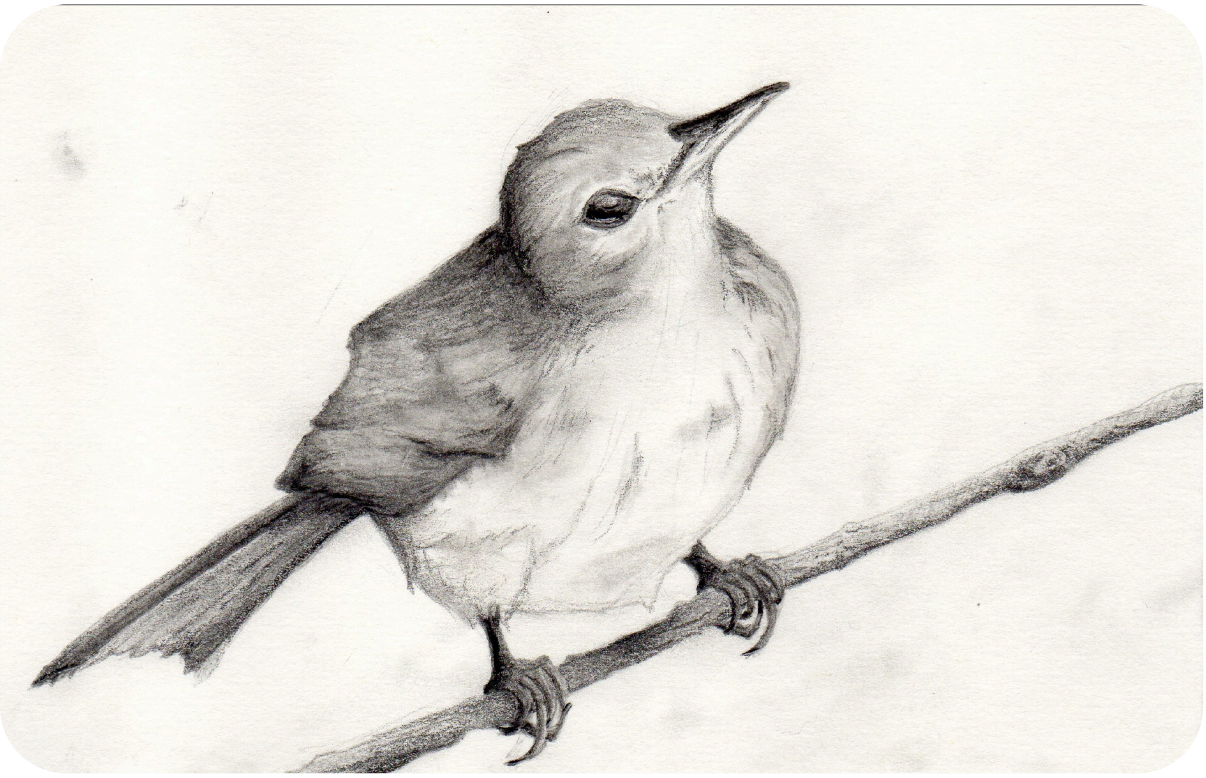
-Shel Silverstein, Where the Sidewalk Ends

Thank you to everyone who played a part in the making of this thesis.

Contents

Chapter 1

General Introduction



1. Individual behavioural variation

Individual organisms continuously respond, both intrinsically and extrinsically, to their surrounding environment to maximise fitness potential (Nettle 2006; Wolf and Weissing 2012; Holtmann *et al.* 2015). In their responses, individuals are expected to balance benefits such as obtaining mates, and associated risks such as predation. For the response to be adaptive, theoretical optimisation models suggest a 'norm of reactions' within which the optimum strategy is adjusted to suit the surrounding environment (Wilson *et al.* 1994). This can explain differences in behavioural responses that evolve between species and populations experiencing different environments. However, variation in behavioural responses also exist between con-specifics in populations where the surrounding environment is generally uniform (Wilson *et al.* 1994; Gosling 2001). When this variation in an individual's behavioural response is consistent across time and contexts, and differs between con-specifics, it is known as personality (Briffa and Weiss 2010). From an adaptive perspective, the evolution of animal personalities remains a conundrum because behavioural plasticity enables individuals to adapt to environmental change (Wolf *et al.* 2007). This conundrum has encouraged research into why personality is consistent through time and across contexts, and why different personalities are maintained in a population.

1.1 Evolutionary theory of personality

A number of theories have been put forward describing how personality could be maintained in a population. Theoretical models suggest that the co-existence of different personality phenotypes in a population could be maintained if the fitness pay-offs associated with a behaviour are dependent on the frequency of the behaviour in a population (Dall *et al.* 2004). This, however, will only give rise to behavioural consistency if positive feedback mechanisms (for example learning) or social responsiveness (for example adjustment of behaviour based on changes in the social environment) exist in the population (Wolf and McNamara 2012). For example, in a frequency-dependent hawk and dove game, incorporation of social responsiveness strategies gave rise to individual differences in behaviour (Wolf *et al.* 2010).

An alternative theory suggests that personality could be maintained if it is state-dependent (Dall *et al.* 2004; Wolf *et al.* 2007; Luttbeg and Sih 2010; Sih *et al.* 2015). State represents the features of an individual at a certain point in time, such as energy reserves or reproductive potential. Differences in state are postulated to generate behavioural differences, consistency of the behaviour is then maintained by positive feedback mechanisms such as learning or the potential costs incurred if the behaviour is changed (Wolf *et al.* 2010). For example, individuals with high future reproductive states should be shy, slower exploring and risk averse and remain this way to reduce the likelihood of predation, compared to those individuals with low future reproductive states (Wolf *et al.* 2007).

Finally, individual differences in behaviour may occur if variation in behaviour is genetically determined. Frequency dependence or mutation selection balance could maintain the genetic variation and the behaviour becomes consistent if flexibility is costly or constrained (Dall *et al.* 2004; Bell 2005). Personality is both heritable and affected by certain genes that may result in behavioural differences (van Oers and Sinn 2013; Garamszegi *et al.* 2014; Mueller *et al.* 2014). Responding inappropriately or being slow to respond to environmental change are examples of such costs that may then limit behavioural flexibility (Dall *et al.* 2004). On the other hand, proximate mechanisms may constrain behavioural flexibility through physiological mechanisms via metabolic rate (e.g. Houston 2010), morphology via growth rate (e.g. Stamps 2007) and linkage with other behaviours in a behavioural syndrome (e.g. Sih *et al.* 2004*a*).

1.2 Defining personality

Personality can describe consistent differences in single and multiple behavioural traits. Multiple correlated differences, can also be referred to as a behavioural syndrome (Sih *et al.* 2004*a*). Behavioural syndromes can be a characteristic of individuals in a population or a characteristic of a species (Sih *et al.* 2004). For example, one individual/species may be more bold and aggressive than another individual/species when defending a territory and when guarding a mate. A bold-aggressive phenotype such as this would be defined as the individual's/species' behavioural syndrome.

To assess the evolutionary significance and maintenance of personality in populations, we need to establish if personality is repeatable and heritable (Dingemanse 2002). Repeatability (R) describes the proportion of the total phenotypic variance (V_P) explained by the difference between individuals (V_I);

1) $R = V_I / V_P$ (Lessells and Boag 1987)

Repeatability gives an indication of how consistent differences between individuals are over time or context (Bell et al. 2009). On average across a range of taxa, 37% (S.E = 0.01) of the variance in behaviour is explained by differences between individuals, with estimates being higher for wild studies (0.39, S.E. = 0.01) when compared with captive studies (0.36, S.E. = 0.01, Bell *et al.* 2009).

Narrow sense heritability (h^2) describes the proportion of the total phenotypic variance (V_P) explained by the genes inherited from an individual's parents, also known as the additive genetic variance (V_A);

2) $h^2 = V_A / V_P$ (Falconer and Mackay 1996).

On average across a range of taxa, 26% of the variance in behaviour is explained by the genes inherited from one's own parents, with estimates also being higher for wild compared to captive studies (van Oers and Sinn 2013). The heritability of personality can further be estimated by the exclusion of temporary environmental effects, such as measurement error, from V_P. Using this second heritability measure, on average 52% (S.E .= 0.09) of the variance in personality is explained by the genes inherited from one's own parents (Dochtermann *et al.* 2015).

1.3 Genetic basis

Personality can have a large heritable component (van Oers and Sinn 2013) but the genetic loci underlying personality are relatively unknown. Promising candidate genes for personality include *DRD4* associated with novelty seeking behaviour in humans (Kluger *et al.* 2002) and mice (Rubinstein *et al.* 1997), and *SERT* associated with both anxiety related behaviour and novelty seeking behaviour in humans (Canli and Lesch 2007) and mice (Holmes *et al.* 2003). Detecting these associations in wild species is important in preventing the alteration of gene expression and selection on genetic variation that may occur in controlled laboratory conditions (Weigensberg and Roff 1996; Smith and Blumstein 2008; Archard and Braithwaite 2010). Although the association has not always been found (Rollins *et al.* 2015), a number of polymorphisms in *DRD4* have been associated with exploratory and neophobic (novelty avoidance) behaviour in wild avian populations (Korsten *et al.* 2010; Garamszegi *et al.* 2014; Mueller *et al.* 2014), with the association differing between populations of the same species (Korsten *et al.* 2010). Associations between *SERT* polymorphisms and exploratory and neophobic behaviour have also reported mixed results, with a higher prevalence of *SNPs* in a neophobic, rural population of blackbirds (*Turdus merula*, Mueller *et al.* 2013) and no association with exploration of novel stressors in Rhesus macaques (*Macaca mulatta*, C.E. Fleener, pers. comm., Holtmann *et al.* 2015).

1.4 Personality and fitness

Personality may also be generated and maintained if frequency–dependent or state–dependent. It is therefore of interest to investigate its association with life-history decisions such as investment in growth, reproduction and survival (Sih *et al.* 2004*a;* Biro and Stamps 2008). A meta-analysis by Smith and Blumstein (2008) found general trends across species, where survival rates for bold and fast exploring individuals were lower than their shy and slow exploring counterparts. Bold individuals had higher reproductive success (a combination of annual and lifetime success), than shy individuals and there was a positive effect of aggression on reproductive success. Notably, a large proportion of these studies addressed the fitness associations in captive bred or laboratory reared individuals (Bell *et al.* 2009). Results from captive individuals can cause misinterpretation of long-term fitness consequences, unintentional artificial selection and differential expression of adult phenotypes with the loss of natural genetic change (Charmantier *et al.* 2014). Field based studies will experience natural environmental variation and thus allow for an accurate understanding of the evolutionary dynamics of personality in a population (Kruuk *et al.* 2008; Archard and Braithwaite 2010).

The general trend of high reproductive success and low survival of bold individuals illustrates the various ways selection can act on personality, but this becomes more complicated in wild populations that experience heterogenous environments (Smith and Blumstein 2008). Aggression in western bluebirds (*Sialia mexicana*, Duckworth 2006), bold behaviour in both mature bighorn ewes (*Ovis canadensis*, Réale et al. 2000; Réale et al. 2009) and male black-browed albatrosses (*Thalassarche melanophrys*, Patrick and Weimerskirch 2014), and fast exploratory behaviour in jackdaws (Corvus monedula, Schuett *et al.* 2012), negatively correlated with reproductive success. In contrast, bold behaviour in young bighorn ewes (Réale et al. 2000; Réale et al. 2009), eastern chipmunks

(*Tamias striatus*, Patterson and Schulte-Hostedde 2011) and female black-browed albatrosses (Patrick and Weimerskirch 2014), positively correlated with offspring success. Traits may also correlate in behavioural syndromes and affect reproductive success. For example, exploratory and bold behavioural types produced fewer young in Steller's jays (*Cyanocitta stelleri*, Gabriel and Black 2012).

Personality can also influence extra group parentage (van Oers *et al.* 2008; While *et al.* 2009; Patrick *et al.* 2011; Martin *et al.* 2014), the age of primiparity (Réale *et al.* 2000; Montiglio *et al.* 2014), the level of asset protection (Réale et al. 2009; Dammhahn 2012) and the level of parental care (Hollander *et al.* 2008; Barnett *et al.* 2012; Twiss *et al.* 2012; Mutzel *et al.* 2013). There is also evidence for the interaction of plasticity and personality impacting on reproductive success. Highly plastic and aggressive Ural owls (*Strix uralensis*) had higher reproductive success over changes in vole abundance (Kontiainen et al. 2009). Highly plastic and less aggressive male tree swallows (*Tachycineta bicolor*) fledged more young over temporal changes in environmental conditions (Betini and Norris 2012). It is important to note that the correlation between personality and fitness may be influenced by the social partner (Dingemanse *et al.* 2004; Both *et al.* 2005; Gabriel and Black 2012; Burtka and Grindstaff 2015) and environmental change, therefore to understand selection patterns, long term studies are required (Dingemanse *et al.* 2004; Quinn *et al.* 2009).

1.5 Personality in cooperative breeders

A cooperative breeding system is where an individual can help in the rearing of young that are not their own. Indirect and/or direct benefits that individuals receive from helping are thought to be the evolutionary drivers of cooperative behaviour (West *et al.* 2007). Individuals may increase their fitness by helping relatives, referred to as kin selection (Hamilton 1964), and this can explain indirect fitness benefits. Helpers may also increase their fitness directly by remaining on a good territory, referred to as the pay to stay hypothesis (Kokko *et al.* 2002) and by group augmentation (improved survival and future reproductive success from living in larger groups, Kokko *et al.* 2001; Clutton-Brock 2002).

The different life history strategies cooperative breeders can adopt allows for the investigation of whether the social environment generates and maintains personality. The social niche specialisation theory suggests that social living individuals that repeatedly interact with one another, will benefit by developing social niches (Laskowski and Pruitt 2014). By developing social niches, individuals reduce social conflict and reduce costs associated with changing social niches. Behavioural consistency is then reinforced through positive feedback mechanisms such as learning (Bergmüller and Taborsky 2010; Wolf and Weissing 2010). For example, consistent individual differences in cooperative behaviour have been noted in meerkats (Suricata suricata, English *et al.* 2010; Carter *et al.* 2014) and wild banded mongooses (*Mungos mungo*, Sanderson *et al.* 2015). Furthermore, repeated social interactions may have carry-over effects to non-social behaviours such as personality and cause individuals to deviate from the optimal behavioural strategy (Niemelä and Santostefano 2015). For example, in the cooperatively breeding African cichlid (*Neolamprologus pulcher),* subordinate helping behaviour predicted an individual's exploratory behaviour and aggressiveness (Bergmüller and Taborsky 2007).

Social status and reproductive behaviour are tightly linked in cooperative breeders, whereby subordinates receive a smaller portion of the group reproduction (Koenig *et al.* 2009). It follows that if social status predicts personality, personality has the potential to influence reproductive behaviour. Studies have found that the exploratory or aggressive tendency of an individual can influence the mode of paternity acquisition. Individuals with high levels of aggression and exploration exhibited high rates of extra pair paternity (van Oers *et al.* 2008; While *et al.* 2009; Patrick *et al.* 2011; Martin *et al.* 2014). The personality of the social partner may also affect the personality of the focal individual (Niemelä and

Santostefano 2015) and affect reproductive behaviour, for example, pairs of individuals with similar personalities exhibited high rates of extra pair paternity (van Oers *et al.* 2008; Patrick *et al.* 2011). Taken together these studies suggest that personality could influence sexual selection.

1.6 Study species

The Seychelles warbler (*Acrocephalus sechellensis*) is a small passerine endemic to the islands of Aride, Cousin, Cousine, Denis and Frégate in the Seychelles (Figure 1). The defining feature of this species is its cooperative breeding system. Dominant breeding birds form long-term pair bonds, often until death (average life span 5.5 years), and defend a territory year-round (Komdeur 1992). Habitat saturation on the main study island of Cousin means breeding opportunities are rare and this forces individuals to delay independent breeding and remain subordinates within a territory, generally their natal territory (Komdeur 1991). Natal dispersal is also female biased, with females dispersing further than males (Eikenaar *et al.* 2008). Individuals that delay breeding can help rear offspring, but this is not the case for all subordinate individuals (Komdeur 1991). The rate of extra pair mating for subordinate females is high with 44% gaining maternity by laying an egg in the dominant females' nests (Richardson *et al.* 2001). Subordinate males on the other hand rarely gain parentage, instead 40% of all offspring are fathered by extra-group males (Hadfield *et al.* 2006), which are dominant breeding males (Richardson *et al.* 2001). There is generally a clutch of one egg per breeding season but two to three eggs can occur (Richardson *et al.* 2001).

Seychelles warblers are predominately monitored on the main study island of Cousin (0.29 km^2; 4°20!S, 55°40!E) during the winter (Jan–Feb) and summer (Jun–Sep) breeding seasons. The Cousin population consist of ca 320 individuals which are distributed across 110–115 territories (Komdeur and Pels 2005). The population is monitored closely each year with a 0.98 ± 0.01 probability of re-sighting adult individuals (Brouwer 2009). Eggs and chicks can be predated by fodies (*Foudia sechellarum*), crabs (*Ocypode spp.* and *Coenobita spp.*) and skinks (*Mabuya spp.*) but adults have no natural predators (Komdeur 1991; Veen *et al.* 2000). Adult individuals do however exhibit innate predator recognition (Veen *et al.* 2000). During the field season, individuals are colour-/British Trist for Ornithology (BTO)- ringed, blood sampled for molecular sexing and genotyping, and their social status and group memberships identified by a combination of censuses and nest watches. There is a low level of immigration and emigration between surrounding islands that has allowed data to be collected on most individuals over their lifetime (Komdeur *et al.* 2004; Komdeur *et al.* 2015).

The Seychelles warbler Cousin Island population has several elements that are beneficial for investigating personality and its fitness consequences. There is a genetic pedigree allowing for testing of reproductive strategies associated with personality and the estimation of the heritability of personality. Subordinate social roles, such as helping can bear a cost (Richardson *et al.* 2002; van de Crommenacker *et al.* 2011), resulting in a trade-off with current and future reproduction that may encourage behavioural variation. There is a high rate of extra group paternity enabling the investigation of correlations between personality and extra group parentage (Richardson *et al.* 2001; Hadfield *et al.* 2006). Finally, linkage disequilibrium is thought to be high due to the population bottleneck, low dispersal between islands and small size, both of which increases the chance of detecting correlations between polymorphisms in personality candidate genes and personality (Hansson and Richardson 2005).

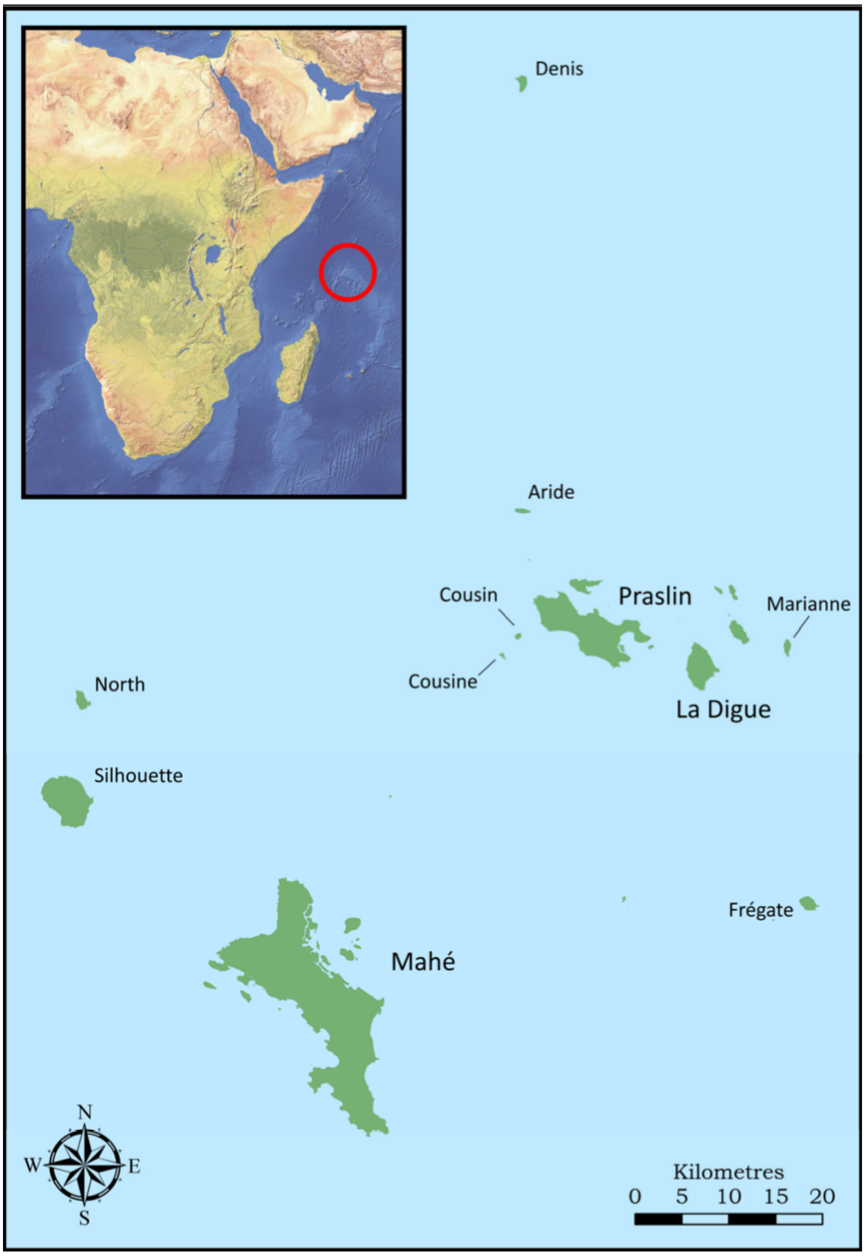


Figure 1: Map of the inner Seychelles islands including the five islands the Seychelles warblers populate; Aride, Cousin, Cousine, Denis and Frégate. Map produced by D. Wright and E.Warren-Thomas.

1.7 Thesis outline

In this thesis I will investigate if personality is present in the Seychelles warbler, look at how it might be generated and maintained in a cooperative system, and investigate its consequences to reproductive success and behaviour. In chapter 2, I calculate the repeatability and heritability of five behavioural assays designed to elicit exploration of a novel environment, exploration of a novel object, obstinacy, stress response and escape response, and investigate whether they correlate in a behavioural syndrome. In the subsequent data chapters, I analyse only the repeatable behavioural traits of novel environment and novel object exploration. In chapter three, I investigate the association of novel environment exploration and novel object exploration, with polymorphisms in two documented personality candidate genes, *DRD4* and *SERT*, to detect the genetic loci underlying personality. In chapter four, I look at whether social state encourages between-individual differences in novel environment and novel object exploration and if social state encourages behavioural consistency. In chapter five, I then investigate whether novel environment and novel object exploration and the social partner's novel environment and novel object exploration affects fitness and the number of extra pair offspring. Finally, in chapter six I discuss my findings from chapters 2 – 5, look at how this expands on the body of work on the ecology and evolution of personality and discuss future directions for personality research.

Chapter 2

Repeatable and heritable variation in a wild cooperative breeder

2.1 Abstract

Quantifying consistent differences in behaviour among individuals is vital to understanding the ecological and evolutionary significance of animal personality. To quantify personality, the phenotypic variation of a behavioural trait is partitioned to assess how it varies among individuals, which is also known as repeatability. If pedigree data are available, the phenotypic variance can then be further partitioned to estimate the additive genetic variance. Quantifying personality traits therefore allows for a better understanding of what natural selection can act upon, enabling evolution. In this study, we measured five personality traits in a wild population of the cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*): novel environment exploration, novel object exploration, obstinacy, escape response, and stress response. We estimated the repeatability of all five behavioural traits and heritability of the repeatable behavioural traits, testing for differences between the sexes and for a behavioural syndrome. We found that compared to estimates in other study species, the exploratory behaviours were moderately repeatable, phenotypically positively correlated, and that novel environment exploration was also moderately heritable. Furthermore, we show that the repeatability and heritability estimates did not differ between the sexes. This study provides further understanding of the additive genetic variance available for selection to act upon in a wild cooperative breeder.

2.2 Introduction

Animal personality is a phenomenon where individuals exhibit consistent behavioural differences between one another (Biro and Stamps 2008; Smith and Blumstein 2008). This can encompass consistent differences in single or multiple traits, and multiple correlated differences are referred to as behavioural syndromes (Sih *et al.* 2004*a*). From an adaptive perspective, the evolution of animal personality remains a puzzle because a plastic behavioural response would allow individuals to adapt to changing environments (Wolf et al. 2007). Personality could be generated and maintained if the fitness pay-offs associated with the behaviour are frequency (Wolf and McNamara 2012) or state-dependent (Wolf *et al.* 2007; Sih *et al.* 2015). However, for personality to evolve under each of these situations, it must be or have been heritable. Measuring personality traits precisely is therefore of huge importance when investigating the potential response of a trait to selection within a population.

To quantify personality, individuals are repeatedly measured for certain traits. From these repeat measures, the proportion of the total phenotypic variance (V_P), explained by the difference between individuals (V_I), can be estimated. Repeatability (R) can then be calculated as: $R = V_I / V_P$ (Lessells and Boag 1987). Repeatability gives an indication of how consistent the differences between individuals are over context or time (Bell et al. 2009). Over a range of taxa, 37% (SE=0.01) of the variance in behaviour is accounted for by consistent differences among individuals (Bell et al. 2009). More importantly, repeatability can be further partitioned to determine the genetic basis of personality and thus its potential evolutionary significance (Dingemanse 2002; Drent et al. 2003; Sinn et al. 2006; Kvarnström 2013). Narrow-sense heritability (h^2_B) describes the proportion of the total phenotypic variance (V_P) that can be explained by additive genetic variance (V_A); $h^2_B = V_A / V_P$ (Falconer and Mackay 1996). Across a range of species, 26%

(SE=0.01) of the variance in behaviour is accounted for by additive genetic variation (van Oers and Sinn 2013). However, a more precise way to estimate the heritability of personality has been described by Dochtermann *et al.* 2015, whereby temporary environmental effects (e.g. measurement error) are excluded from V_P. Using this heritability measure (h^2_P), on average, 52% (SE=0.09) of the variance in personality is explained by additive genetic variation (Dochtermann *et al.* 2015). To date, the Dochtermann *et al.'s* (2015) approach of estimating the heritability of personality has been used in one published study on the wild population of yellow-bellied marmots (*Marmota flaviventris*, Petelle *et al.* 2015)

When investigating the evolution of behaviour, it is important to use natural populations because the artificial, controlled environment of the laboratory may alter the expression of behaviour and selection on behavioural genetic variation (Weigensberg and Roff 1996; Archard and Braithwaite 2010). Furthermore, greater environmental variation in the wild may encourage greater between-individual phenotypic variation through processes such as stimulus generalisation (Bell *et al.* 2009). In line with this, heritability and repeatability estimates are often higher when sampled from natural rather than laboratory populations (Bell *et al.* 2009; van Oers and Sinn 2013). Encouragingly, there are now an increasing number of wild population studies quantifying the heritability of personality (Duckworth and Kruuk 2009; Blumstein *et al.* 2010; Taylor *et al.* 2012; Korsten *et al.* 2013; Poissant *et al.* 2013; Class *et al.* 2014; Petelle *et al.* 2015). To our knowledge, there has been no study investigating the heritability of personality in a wild cooperative breeder and whether there are sex specific differences in heritability. This is surprising given that personality differences have been noted between the sexes (although the direction can vary across species, Schuett *et al.* 2010).

The Seychelles warbler (*Acrocephalus sechellensis*) provides the unique opportunity to investigate personality in a wild cooperative breeder – a breeding system where individuals can raise offspring that are not their own (Cockburn 1998). On the study island of Cousin, a limited number of breeding opportunities caused by habitat saturation, forces some individuals to remain subordinate instead of gaining a dominant breeding position (Komdeur 1991). These dominance-related social niches could generate behavioural differences among individuals in the population (Bergmüller and Taborsky 2010). There is little immigration and emigration between islands (Komdeur *et al.* 2015), allowing for the recapture of individuals for personality testing. The population also has a multi-generational genetic pedigree allowing the heritability of personality to be estimated. Our aim was to investigate the presence of five personality traits in the population: exploration of a novel environment (e.g. Verbeek *et al.* 1994), exploration of a novel object (e.g. Verbeek *et al.* 1994), obstinacy (e.g. David *et al.* 2011), escape response and stress response (e.g. Fucikova *et al.* 2009). We quantified the repeatability and heritability of these five personality traits and investigated whether they showed sex-specific differences and whether they were phenotypically and genetically correlated in a behavioural syndrome.

2.3 Methods

Study system

Seychelles warblers are an endemic facultative cooperative breeding species that occur on five islands within the Seychelles (Wright *et al.* 2014). Habitat saturation on the study island of Cousin (0.29 km^2; 04°20!S, 55°40!E) forces some individuals to assume subordinate roles because of the limited breeding vacancies (Komdeur 1991). There is a carrying capacity of around 320 adult individuals residing in ca. 110 territories (Komdeur *et al.* 2015). A subordinate status is assigned to each bird (>5 months old) that is seen repeatedly on a territory interacting with group members and not exhibiting dominant pair behaviours. A dominant status is assigned to individuals in a pair that are repeatedly seen in the same territory, stay within close proximity, have constant vocal interactions with their mate and either mate guard (if male) or is the object of mate guarding (if female). A territory generally contains a single dominant breeding pair, and approximately 30% of territories also contain subordinates (Komdeur *et al.* 2015).

Seychelles warblers on the main study island of Cousin have been monitored closely since 1981. During summer (Jun–Sep) and most winter (Jan–Feb) breeding seasons individuals are monitored to identify territory boundaries. Individuals are caught with mist nets, a metal British Trust for Ornithology (BTO) ring and colour ring fitted if necessary, morphometric measurements noted, and a blood sample taken for molecular sexing (following Griffith *et al.* 2002) and parentage analyses. There is little migration of birds between islands, and consequently, with the intense monitoring, there is a 0.98 ± 0.01 annual probability of re-sighting adults enabling accurate measurement of survival and fecundity (Brouwer *et al.* 2010; Komdeur *et al.* 2015).

Personality assays

We assayed five behaviours: obstinacy, stress response, exploration of a novel environment, exploration of a novel object and escape response. Supplementary Table S2.1 shows the sample sizes according to sampling intensity for each personality assay.

Individuals were caught by mist net, an active trapping strategy whereby particular individuals are focused upon for capture, suggesting trapping bias caused by individual behavioural differences would have limited impact on our sampling (Michelangeli *et al.* 2015).

After being caught in a mist net the individual was extracted, placed in a bird bag and suspended from a branch out of the wind. Obstinacy or struggle rate was then measured by counting the number of seconds of movement in one minute in the bird bag (Figure 2.1a and Figure S2.1, adapted from Réale *et al.* 2000).

Stress response was tested immediately after extraction from the bird bag, before morphometric measurements. This involved holding the bird in the ringing grip, gently laying the ring finger on the breast and counting the number of breast movements during a one minute period (Figure S2.2, Fucikova *et al.* 2009).

After morphometric measurements were taken, individuals were rested for five minutes in a bird bag and then assayed for novel environment exploration. Exploration of a novel environment was assayed in an Oxygen 4 tent (L322 x W340 x H210 cm; Gelert Ltd Wigan). The tent contained three artificial trees and each tree had two branches that were 45 cm long (one attached at 95cm and one attached to the top of the trunk), and a trunk, 148 cm high. Using tally counters, the number of flights, hops and total number of trees visited were recorded in five minutes through a small opening (6 inches long by 2.5 inches wide) in the gauze of the tent door (Verbeek *et al.* 1994). To test for acclimation to the novel environment we measured the number of hops, flights and trees visited every minute over twenty minutes for twenty individuals (see Supplementary Table S2.2 for break point analysis results). A flight denoted a transfer between branches on the same tree, between trees or between floor and trees, or any movement greater than a branch length, involving flapping of the wings. A hop was described as both feet off the ground with no wing flapping, either on the same branch or on the floor. The numbers of hops, flights and unique trees visited were totalled to give a measure of exploration (Figure 2.1b and Figure S2.3, Dingemanse 2002).

After the novel environment assay individuals remained in the tent and given a two minute break before the novel object assay. A novel pink toy (approximately 15cm x 10cm, and coloured pink because this colour is rarely encountered on the island and therefore novel) attached to a tree branch (95cm long) was inserted and positioned in the centre of the tent (Verbeek *et al.* 1994). We also conducted a control assay with the novel object excluded. The order of the novel object and control assays were randomised using a coin toss, and measured two minutes apart. The behaviour score (summed number of hops, flights and trees visited) was higher in the novel object assay than the control assay (Wilcoxon signed rank test; V=2145, p<0.001), latency time (sec) to move once the novel object or tree branch with no novel object (control) was inserted into the tent was shorter (Wilcoxon signed rank test; n=185, V=3162, p<0.001), and the number of touches to the tree branch was lower (Wilcoxon signed rank test; n=185, V=3162, p<0.001) in the novel object assay than in the control assay. This confirmed that the behavioural reaction resulted from the novel object and not the stick it was attached to. Behaviour scores in the novel object assay were therefore used as a measure of novel object exploration (Figure 2.1c and Figure S2.4). Over the course of the sampling period, tent colour (blue/green), the orientation of the branches of the artificial trees (diagonal and parallel) and the way the bird was released into the tent (by hand and onto tree) varied, and these methodological factors were all controlled for in all statistical analyses.

Escape response was recorded back at the territory of capture. The individual was placed on a man made perch (consisting of a branch 24cm in length attached to a 80cm trunk) and the number of seconds it took to depart from the perch recorded (Figure 2.1d and Figure S2.5). In pilot studies (n=193 birds), the bird was placed in the palm of the hand, but this was changed to prevent hand temperature and movement affecting the measure. This method change was accounted for in the statistical analysis.

To ensure between-observer consistency, two observers measured each behavioural assay simultaneously and the similarity between the results compared.

Figure 2.1: a) obstinacy assay; b) artificial trees in the novel environment/object assay; c) novel object for novel object assay; d) the man made perch for escape response assay

Statistical Analysis

All statistical analyses were performed in R 3.0.1. (R Development Core Team 2013) using MCMCglmm 2.17 (Hadfield 2009). For all univariate models, we specified an expanded prior: V = 1, n = 0.002, alpha.mu = 0 and alpha.V = 1000, because the variance was close to zero (Hadfield 2015). For the novel environment exploration univariate model, we specified an uninformative inverse Wishart prior: V = 1 and n = 0.002. For the bivariate models we specified: V = diag (2), such that both variance priors were set at 1, and n = 1.002.

Furthermore, for the observer identity random effect in the novel object exploration/stress models, we specified the equivalent of a proper Cauchy prior: V = 1, n = 1, alpha.mu = 0, alpha.V = 25^2, due to few observer levels (n=7) (Gelman 2006) and for the sex-specific random effects we specified an expanded prior: V = 1, n = 2, alpha.mu = 0 and alpha.V = 1000. We assessed convergence by inspecting the autocorrelation values (r<0.1) and time series plots of the model parameters and using the heidel.diag and geweke.diag functions.

Repeatability

Generalised linear mixed models (GLMMs) were run using a Poisson distribution with log link for all traits except stress response, where a Gaussian distribution with identity link was used. The dependent variable was the personality trait. We tested fixed effects that have affected personality in other systems: social status (subordinate or dominant, e.g. Bergmüller and Taborsky 2010), sex (e.g. Schuett and Dall 2009), time interval between assays (days, e.g. Dingemanse *et al.* 2012), assay number (e.g. Dingemanse *et al.* 2012), season (number of days from the first of January to account for the minor breeding season and the first of June to account for the major breeding season, e.g. Dingemanse 2002), year (only for obstinacy, stress response and escape response because year was collinear with tent colour/branch orientation/release method in the novel environment and novel environment exploration assay), body mass to account for body size (standardised for time of day, Quinn *et al.* 2011) and age (days, e.g. Fisher *et al.* 2015). Age was mean centred and divided by two standard deviations (Gelman and Hill 2006) and included as both a linear and quadratic term. Weather (sun, cloudy, partly cloudy, rain, sunset) and differences in the method used (tent colour, branch orientation and how the bird was released into the tent for novel environment exploration; tent colour and branch orientation for novel object exploration; release method for escape response) were also included. The random effects of bird identity, and observer identity (obstinacy n=13, stress response n=7, novel environment exploration n=11, novel object exploration n=7, escape response n=13), were included to take into account multiple measures on the same bird and different observers. All of the random effects were also split between the sexes in a separate model to test for sex effects. The variance components were extracted from the GLMM, and the raw phenotypic repeatability of the personality trait captured following Nakagawa et al. (2010). The posterior distribution was sampled every 100 iterations, with a burn-in period of 3000 iterations and a run of 203,000 iterations.

Heritability

Extra group parentage is high in the Seychelles warbler, with 15% of subordinate males siring offspring within the group and 44% of subordinate females having offspring at the dominant pairs' nest (Richardson et al. 2001; Hadfield et al. 2006). Parentage was assigned using 30 microsatellites and Masterbayes 2.52 (Hadfield *et al.* 2006). The pedigree is 10 generations deep and contains 1853 individuals, of which 1809 were offspring and 786 individuals in the pedigree were informative for novel environment exploration, 684 were informative for novel object exploration and 712 were informative for stress response (1487 offspring were assigned a mother and 1554 were assigned a father with at least 80% confidence; Dugdale *et al.* unpublished data).

We extended the univariate models from the repeatability analyses into 'animal' models for the moderately repeatable traits (Kruuk 2004). The fixed effects were the same as in the repeatability analyses. An animal term linked to the pedigree was added as a random effect to account for the additive genetic variance (V_{A}), mother identity to account for the maternal effects (V_{M}), individual identity to account for the permanent environment effects (V_{PE}), and observer identity to account for repeated measures on the same bird and by different observers (V_{Observer}). The random effects were also split between the sexes in a separate model to test for sex effects. The variance components were extracted from the GLMM, and the heritability of behaviour (h^2_B) calculated as, $h^2_B = V_{\mathrm{A}} / V_{\mathrm{P}}$, and the heritability of personality (h^2_P) calculated as, $h^2_P = V_A / V_A + V_{PE} + V_M$, so as to exclude temporary environmental effects (Wilson *et al.* 2010; Dochtermann *et al.* 2015) following Nakagawa & Schielzeth (2010) for Poisson distributions. The posterior distribution was sampled every 500 iterations, with a burn-in period of 30,000 iterations and a run of 1,000,000 iterations.

Behavioural syndrome

To estimate phenotypic and genetic correlation coefficients between the moderately repeatable traits, a bivariate model with the same structure as the univariate repeatability and heritability models was run, respectively. Correlations were calculated by dividing the covariance between the traits by the square root of the product of the variance of the two traits. For the phenotypic bivariate model the posterior distribution was sampled every 100 iterations, with a burn-in period of 3000 iterations and a run of 203,000 iterations. For the genetic bivariate model the posterior distribution was sampled every 500 iterations, with a burn-in period of 30,000 iterations and a run of 1,000,000 iterations.

2.4 Results

Repeatability

Novel environment exploration and novel object exploration had moderate repeatability estimates, compared to the average repeatability of behaviour (Figure 2.2). However, obstinacy, stress response and escape response had repeatability estimates close to zero (Figure 2.2). We ran a sex specific model (Supplementary Table S2.3) but found no differences between the sexes (Supplementary Table S2.4).

For novel environment exploration, exploration scores increased with increasing assay number and age whereas they decreased when measured in the blue rather than the green tent (Figure 2.3). Novel object exploration scores increased with increasing assay number, but unlike novel environment exploration there was a sex-specific effect such that males explored more than females and there was no effect of social status, season or tent colour (Figure 2.4). Obstinacy decreased with increasing assay number but was greater when it was partly cloudy than to sunset, and in 2013 and 2014 than in 2010 (Figure S2.6). Stress response was higher in the year 2015 than 2013 (Figure S2.7). Finally, for escape behaviour, individuals took longer to fly off the perch when it was raining than when it was cloudy, with increasing assay number, in 2013 and 2014 than 2010, with increasing body mass, and when released from the perch rather than the hand (Figure S2.8).

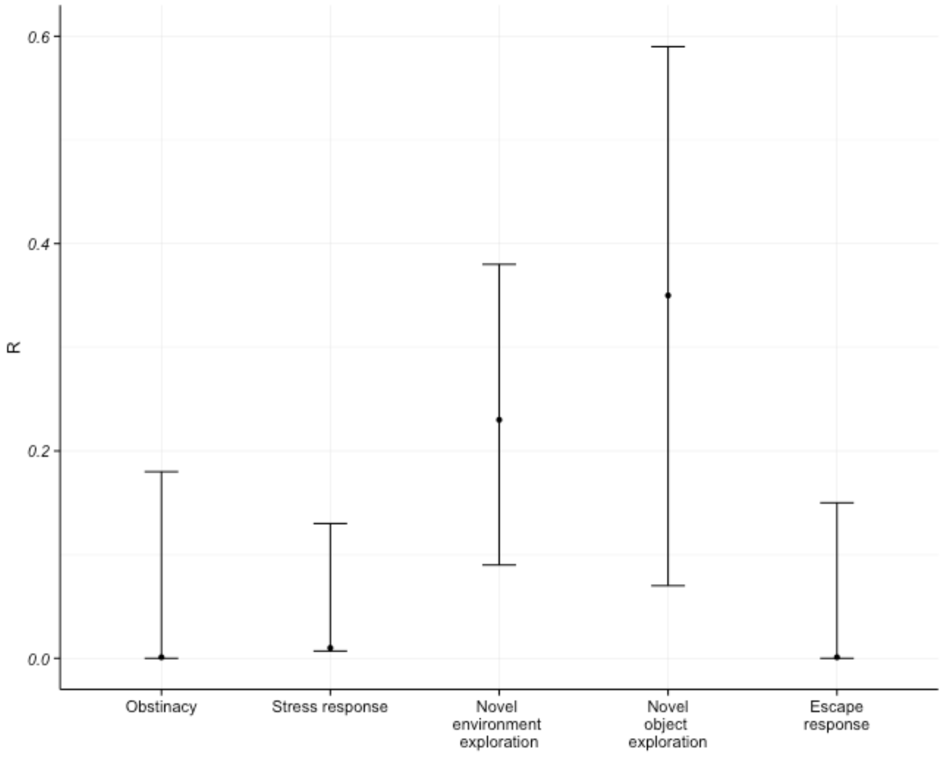


Figure 2.2: Repeatability estimates (posterior mode) for the five behavioural traits, error bars represent the 95% credible intervals (none overlap zero).

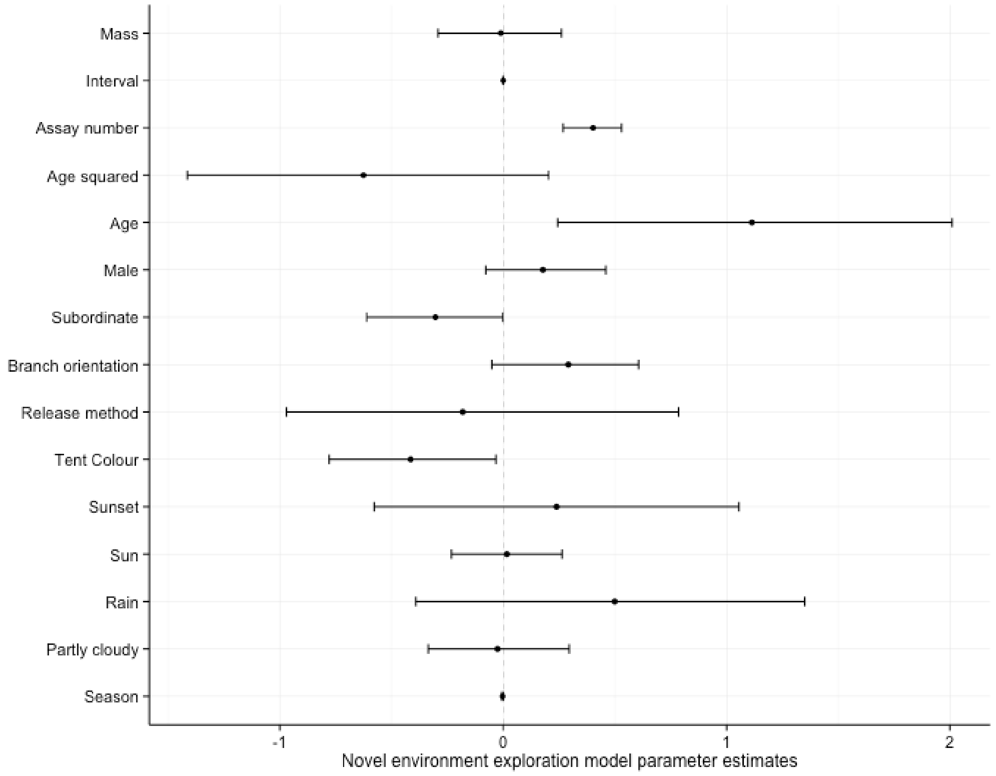


Figure 2.3: Posterior modes of the fixed effects, and associated 95% credible intervals, in the novel environment exploration model: mass (mean centred), interval (days between assay), assay number*, age* (quadratic and linear terms), sex (male = 163, female = 149; contrast = female), social status (dominant = 280, subordinate = 237; contrast =dominant), branch orientation (diagonal = 316, vs. parallel = 201; contrast = diagonal), release method (hand = 64, vs. placed on tree = 449; contrast = hand), tent colour* (blue = 339, vs. green = 178; contrast = blue), weather (partly cloudy = 128, rain = 7, sun = 258, sunset = 9; contrast = cloudy), and season (number of days from the first of January or June).* indicates posterior modes whose 95% credible intervals do not overlap zero.

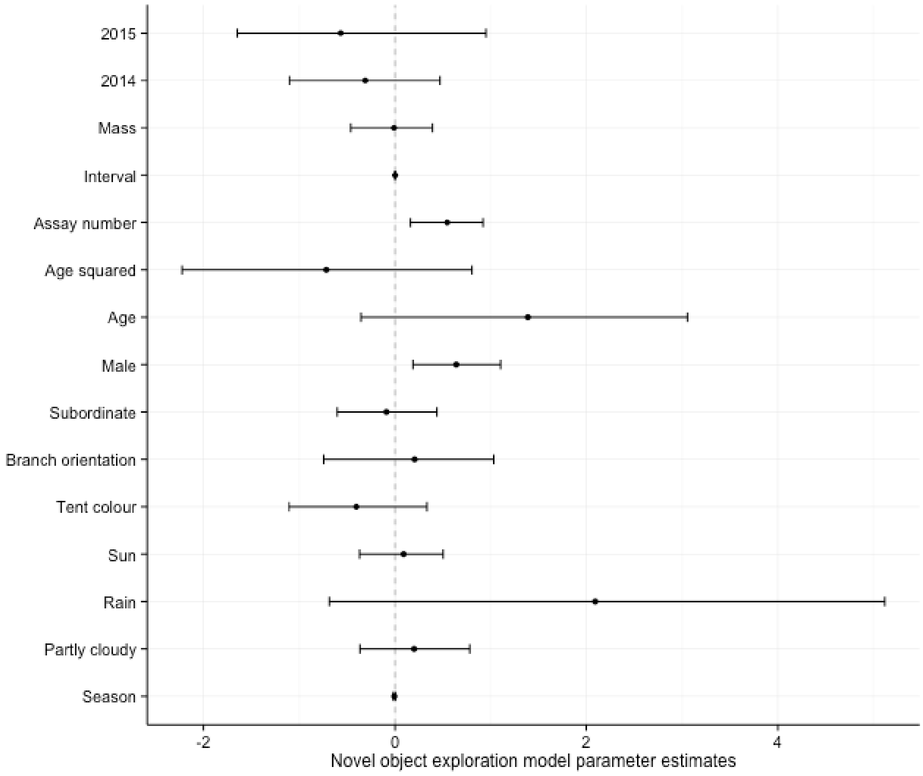


Figure 2.4: Posterior modes of the fixed effects, and associated 95% credible intervals, in the novel object exploration model: year (contrast 2013), mass (mean centred), interval (days between assay), assay number*, age (quadratic and linear terms), sex* (male = 96, female = 81; contrast = female), social status (dominant = 141, subordinate = 99; contrast = dominant), branch orientation (diagonal = 205, vs. parallel = 35, contrast = diagonal), tent colour (blue = 76, vs. green = 164, contrast = blue), weather (partly cloudy = 59, rain = 1, sun = 107; contrast = cloudy) and season (number of days from the first of January or June). * indicates posterior modes whose 95% credible intervals do not overlap zero.

Heritability

The heritability of behaviour, h^2_B, estimates were moderate for novel environment exploration (0.21, Table 2.1), and low for novel object exploration (2e-3, Table 2.1). The heritability of personality, h^2_P, estimates were high for novel environment exploration (0.67, Table 2.1), and low for novel object exploration (0.01, Table 2.1). Permanent environment effects were low for novel environment exploration and novel object exploration (2e-3 & 3e-3, respectively, Table 2.1). Maternal effects were low for novel environment exploration and novel object exploration (1e-3 & 1e-3, respectively, Table 2.1). Observer effects were also low for novel environment exploration and novel object exploration (0.04 & 1e-3, respectively, Table 2.1). We ran a sex specific model (Supplementary Table S2.5) but found no differences between the sexes (Supplementary Table S2.6).

Table 2.1: The heritability of behaviour ($h^2_B = V_A / V_p$), heritability of personality ($h^2_P = V_A / V_A + V_{PE} + V_M$), permanent environment effect ($pe^2 = V_{PE} / V_p$), maternal effect ($m^2 = V_M / V_P$), observer effect ($obs^2 = V_{Observer} / V_P$), residual effect ($res^2 = V_{Residual} / V_P$), additive genetic variance (V_A), and total phenotypic variance (V_p), for each moderately repeatable personality trait. Credible intervals are in brackets.

Personality trait	**h^2_B**	**h^2_P**	**pe^2**	**m^2**	**obs^2**	**res^2**	**V_A**	**V_p**
Novel environment exploration	0.21 (1e-3–0.33)	0.67 (0.05–0.83)	2e-3 (2e-4–0.21)	1e-3 (1e-4–0.07)	0.04 (1e-3–0.23)	0.56 (0.42–0.72)	0.34 (1e-3–0.54)	1.36 (1.13–1.75)
Novel object exploration	2e-3 (7e-4–0.37)	0.01 (1e-4–0.81)	3e-3 (5e-4–0.45)	1e-3 (2e-4–0.12)	1e-3 (2e-4–0.39)	0.46 (0.19–0.72)	0.14 (0.02–0.81)	2.07 (1.39–4.23)

Behavioural syndrome

A positive phenotypic correlation existed between the novel object and novel environment exploration (0.51, 95% credible Interval [Cr.I.]= 0.13–0.68, n = 177). We also calculated between sex correlations and found no differences between the sexes (Supplementary Table S2.7). The genetic correlations between novel environment exploration and novel object exploration (0.55, -95% Cr.I. = -0.05–0.79, n = 177) were not significant. We also calculated between sex correlations and found no differences between the sexes

(Supplementary Table S2.8).

2.5 Discussion

We have shown that novel environment exploration and novel object exploration are moderately repeatable compared to the average repeatability of behaviour (average for field and laboratory studies: 0.37 S.E=0.01, average for field studies: 0.39 S.E.=0.01, Bell *et al.* 2009). This is also the first study to quantify the heritability of personality traits in a wild cooperative breeder. We have shown that novel environment exploration had a moderate h^2_B, compared to the average h^2_B (0.26 S.E=0.01, van Oers and Sinn 2013). These results reveal that there is a possible genetic basis for novel environment exploration in this population and thus this trait has moderate evolutionary potential. Novel object exploration however, had a low h^2_B. Low heritability estimates can suggest that directional selection is depleting genetic variation in these traits if they are linked to fitness (Falconer and Mackay 1996; Kruuk *et al.* 2002). Alternatively, if the behaviours are highly integrated, then the residual variance may co-vary with the additive genetic variance and restrict their independent effects on heritability (Stirling *et al.* 2002). As predicted, our heritability estimates increased for novel environment exploration and novel object exploration when temporary environmental effects were excluded to estimate h^2_P (Dochtermann *et al.* 2015). Removing temporary environmental effects emphasises how factors such as measurement error or short-term variable effects can contribute substantially to the expression of behaviour, and could cause underestimation of the contribution of additive genetic variation to personality (Dochtermann *et al.* 2015).

Observer effects in our study were small for novel object and novel environment exploration showing that observers did not differ considerably in their ability to measure these traits. However, it is crucial to account for observer effects and other confounding variables in behavioural studies (e.g. Altmann 1974). Although permanent environmental effects in our study were small for both traits, they have been notable in previous studies (Taylor *et al.* 2012; Poissant *et al.* 2013; Petelle *et al.* 2015). Particularly in territorial species, it is postulated that territory quality can represent such a permanent environmental effect, leading to long-term consequences on personality (Taylor *et al.* 2012; Petelle *et al.* 2015). Maternal effects were also low for both traits. The low estimates are in line with the majority of work studying the heritability of personality traits in wild populations (Duckworth and Kruuk 2009; Réale *et al.* 2009; Blumstein *et al.* 2010; Poissant *et al.* 2013). Recent work however, has found that maternal effects, possibly through early hormonal exposure, can explain a proportion of the variation in personality (Taylor *et al.* 2012; Petelle *et al.* 2015). Maternal effects also have long-term fitness consequences in our study species (Brouwer *et al.* 2007). Furthermore, indirect genetic effects, such as the social partner, can also contribute to the heritable variance available for selection, (Bijma 2014), but we did not have the power to test for these. Thus where possible, social genetic, maternal and permanent environmental effects should be accounted for in personality research to avoid a confounded V_A estimate (Kruuk and Hadfield 2007; Taylor *et al.* 2012; Petelle *et al.* 2015).

Novel object exploration and novel environment exploration were also phenotypically positively correlated, suggesting the existence of an exploratory behavioural syndrome. This result is similar to previous behavioural syndrome research where fast exploration of a novel environment was associated with a faster approach to a novel object (Verbeek *et al.* 1994). Furthermore, fast exploratory behaviour has been associated with greater levels of aggression and the formation of routines (Verbeek *et al.* 1996). Two features of behavioural syndromes that can explain why personality is maintained, particularly when behaviours appear sub-optimal, is constrained behavioural plasticity and behavioural correlations across situations (Sih *et al.* 2004*a*). For example, trade-offs between personality and life-history traits could lead to traits being selected together, through correlated selection, resulting in behavioural syndromes (Stamps 2007). Unfortunately we were unable to test for trait correlations over multiple functional contexts, and therefore we do not know how novel object exploration and novel environment exploration relate to one another or how selection acts on these traits.

We estimated genetic correlations between the repeatable behavioural traits and found no correlation. When traits are not genetically correlated it suggests that selection is acting independently upon each trait and thus they are distinct traits (Petelle *et al.* 2015). However, we suggest that these results are treated with caution. A large sample size is needed when testing for genetic correlations between traits (Klein 1974; Kruuk 2004). This is often a limitation for studies that have investigated genetic correlations, and similar to our results, where low power is indicated by large credible intervals (Bell 2005; Petelle *et al.* 2015). This limitation is also most pronounced in wild studies because of the logistical and administrative constraints of collecting samples (Bell 2005; Petelle *et al.* 2015). Long term study of personality in this species would help to confirm the presence of any genetic correlations.

Finally, we found no differences between the sexes, although we cannot rule out that we had insufficient power to detect a difference. A meta-analysis showed that females are more plastic in their behaviour than males in most vertebrates (Bell *et al.* 2009). However this result was caused by the low repeatability estimates of mate preference, predominately measured in females (Bell *et al.* 2009). It was concluded that there was not enough evidence to confirm a difference between the sexes, and this was attributed to the differences in sex specific behavioural strategies established in early life between species (Biro and Stamps 2008; Bell *et al.* 2009).

2.6 Conclusion

In summary, we have shown that there is a genetic basis to personality in a wild cooperative breeder, and that this additive genetic variance does not differ between the sexes. This provides further understanding of the potential variance available for selection in a wild cooperative breeder. Further study should investigate the selective processes that create these individual differences in behaviour and the ecological implications in a cooperatively breeding environment.

Chapter 3

No association between personality and candidate gene polymorphisms in a wild bird population

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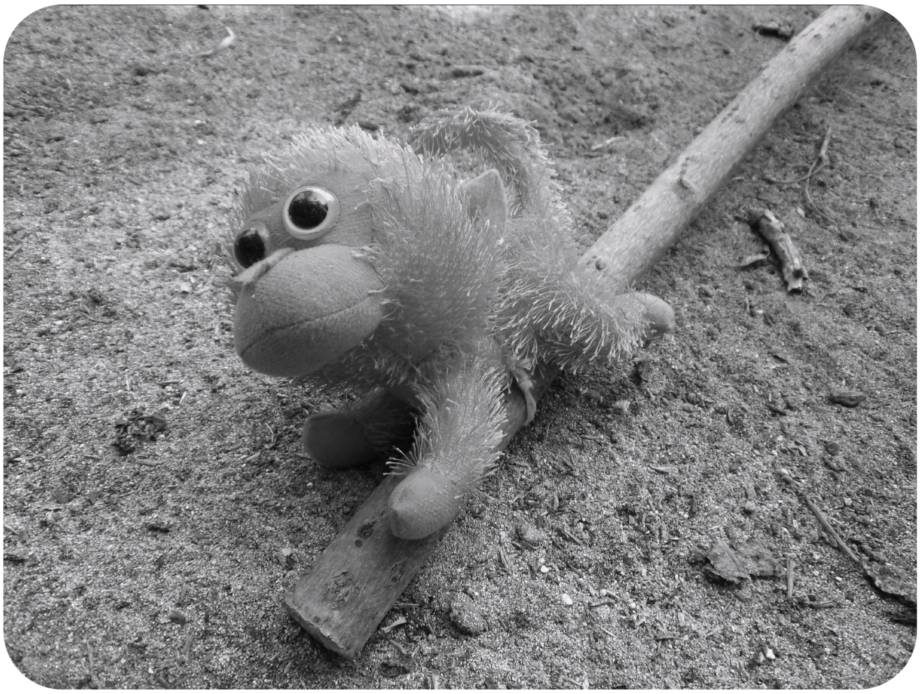


3.1 Abstract

Consistency of between-individual differences in behaviour or personality is a phenomenon in populations that can have ecological consequences and evolutionary potential. One way that behaviour can evolve is to have a genetic basis. Identifying the molecular genetic basis of personality could therefore provide insight into how and why such variation is maintained, particularly in natural populations. Previously identified candidate genes for personality in birds include the dopamine receptor D4 (*DRD4*), and serotonin transporter (*SERT*). Studies of wild bird populations have shown that risk-taking and exploratory behaviour are associated with polymorphisms in both *DRD4* and *SERT*. Here we tested for polymorphisms in *DRD4* and *SERT* in the Seychelles warbler (*Acrocephalus sechellensis*) population on Cousin Island, Seychelles, and then investigated correlations between personality and polymorphisms in these genes. We found no genetic variation in *DRD4*, but identified four polymorphisms in *SERT* that clustered into five haplotypes. There was no correlation between novel environment and novel object exploration and *SERT* polymorphisms/haplotypes. The null result was not due to lack of power, and indicates that there was no association between these behaviours and variation in the candidate genes tested in this population. These null findings provide important data to facilitate representative future meta-analyses on candidate personality genes.

3.2 Introduction

Between-individual differences in behaviour that are consistent through time/context are referred to as personality (Stamps and Groothuis 2010). Personality can be heritable (the average heritability estimate across 209 studies was 0.26 (SE=0.01, van Oers and Sinn 2013), correlated across contexts and can have the potential to influence how populations adapt and evolve (van Oers and Sinn 2013). Why personality persists in populations is hard to explain, given the assumption that behaviour should be centred on an optimum strategy or co-existing evolutionary stable strategies (Wilson *et al.* 1994). It is thought that personality could be maintained if the fitness pay-offs associated with a behaviour were frequency-dependent (Wolf and McNamara 2012) or state-dependent (Wolf *et al.* 2007; Sih *et al.* 2015). Despite the large heritable component personality can have (Dochtermann *et al.* 2015), the genetic loci underlying personality are relatively unknown. Identifying these genetic loci may therefore be pivotal to our understanding of why personality occurs, and its ecological and evolutionary significance.

The most promising candidate genes for human personality traits are the dopamine receptor D4 (*DRD4)*, involved in the mediation of the hormone dopamine in the dopaminergic system, and the serotonin transporter (*SERT)*, which controls the uptake of the hormone serotonin in the synaptic clefts of the neurones (Savitz and Ramesar 2004). Although null results have been found in candidate gene association studies (Savitz and Ramesar 2004), variation in novelty-seeking behaviour in humans is generally associated with polymorphisms in *DRD4* (Kluger *et al.* 2002), and low levels of novelty seeking behaviour have been recorded in *DRD4* deficient mice (Rubinstein *et al.* 1997). Additionally, variation in measures of anxiety related behaviour in humans has been associated with polymorphisms in *SERT* (Serretti *et al.* 2006; Canli and Lesch 2007), and high levels of anxiety related behaviour and low levels of novelty seeking have been reported in *SERT* deficient mice (Holmes *et al.* 2003; Murphy *et al.* 2008).

It is important to investigate these associations in natural populations. Although human personality is not directly comparable to non-human personality, cultural determinants of behaviour are thought to be less influential in natural animal populations, and can provide insight into why individual variation exists (Savitz and Ramesar 2004). In captive animal populations, the controlled, artificial environment of the laboratory can alter the expression of, and selection on, genetic variation (Weigensberg and Roff 1996; Smith and Blumstein 2008; Archard and Braithwaite 2010). In one of the first non-human studies of a wild population, a single nucleotide polymorphism (SNP) in exon 3 of *DRD4* (*SNP830*) was identified and the genotype *SNP830T* correlated with faster exploratory behaviour in a novel environment in one of four populations of great tits, *Parus major* (Korsten *et al.* 2010). A similar association was found in a wild population of collared flycatchers (*Ficedula albicollis*), where *DRD4 SNP554* was linked with neophobia and *DRD4 SNP764* with risk-taking behaviour (Garamszegi *et al.* 2014). *DRD4 SNP449* in two wild populations of invasive yellow-crowned bishops (*Euplectes afer*) was also found to be associated with neophobic behaviour (Mueller *et al.* 2014). More recently, in free ranging Rhesus macaques (*Macaca mulatta*), *DRD4* polymorphisms have also been associated with risk taking behaviour (Coyne *et al.* 2015). Conflicting results have been found in association studies investigating *SERT* in wild populations. Individuals heterozygous for *SERT* at *SNP758* and *SNP988* were more prevalent in rural, less neophobic populations of wild blackbirds (*Turdus merula*) when compared to urban populations, only 23-45 km apart in the same country (Mueller *et al.* 2013). Also in New Zealand dunnocks (*Prunella modularis*), *DRD4* and *SERT* polymorphisms were associated with risk taking behaviour (Holtmann *et al.*

2015), and in great tits, *SERT SNP234* was associated with risk taking behaviour (Riyahi *et al.* 2015). In contrast, free-ranging rhesus macaques did not show an association between their exploration of novel stressors and their *SERT* genotype (C.E. Fleener, personal communication).

In this study we investigated whether polymorphisms in the candidate genes for neophobia (Réale *et al.* 2007), *DRD4* and *SERT*, are associated with variation in novel environment and novel object exploration in a wild population of Seychelles warblers (*Acrocephalus sechellensis*). The Cousin Island population of Seychelles warblers should prove to be a good model for such a study, because adult individuals exhibit innate predator recognition behaviour (Veen *et al.* 2000). Here we test the prediction that between-individual variation in novel environment and novel object exploration is associated with polymorphisms and/or haplotype in *DRD4* and in *SERT*.

3.3 Methods

Study system

The Seychelles warbler is an endemic facultative cooperative breeding passerine that occurs on five islands within the Seychelles. Dominant breeding pairs are territorial and socially monogamous. Paternity is gained predominantly by dominant rather than subordinate males and around 44% of offspring have extra-group fathers (Richardson *et al.* 2001; Hadfield *et al.* 2006). Due to habitat saturation, individuals are forced to assume subordinate roles (Komdeur and Edelaar 2001). Dominance status was assigned to individuals in pairs that were observed in a territory within close proximity of one another, had frequent vocal interactions and mate guarded (Komdeur 1991). A subordinate status was assigned to single birds consistently seen in a territory interacting with group members but not engaging in dominant pair behaviour.

At the study site of Cousin Island (0.29 km^{2}; 04°20!S, 55°40!E), during the winter (Jan-Feb) and summer (Jun-Sep) breeding seasons in 2010-15, the breeding status of each individual was identified, territories mapped and birds caught with mist nets, colour/BTO ringed if required, and a blood sample obtained from a brachial venipuncture. The blood sample was later used for pedigree analysis and molecular sexing (Griffith *et al.* 2002). This population has been individually monitored intensively since 1981, providing a long-term dataset with accurate measurements of survival and fecundity due to the 0.92 probability of annually re-sighting individuals in their first two years of life, and a 0.98 probability of annually re-sighting adults (Brouwer *et al.* 2010; Komdeur *et al.* 2015).

Personality assays

After morphometric measurements were taken, individuals were rested for five minutes in a bird bag and then assayed for novel environment exploration. Novel environment exploration was assayed in an Oxygen 4 tent (L322 x W340 x H210 cm; Gelert Ltd Wigan) containing three artificial trees. The number of hops, flights and unique trees visited was totalled to give a measure of exploration (Dingemanse 2002).

Exploration of a novel object was tested two minutes after the exploration assay (see acclimation test, Chapter 2). A novel pink toy attached to a tree branch (95cm long) was inserted and positioned in the centre of the tent (Verbeek *et al.* 1994). The summed number of hops, flights and trees visited was used as a measure of exploration of the novel object (Chapter 2).

Primer design

DRD4 exon 3 sequences from the great tit DQ006802, the chicken *Gallus gallus*

NP001136321, blue tit (*Cyanistes caeruleus*) JN986724.1 and blackcap *Sylvia atricapilla* (AEC22814.1), and *SERT* chromosome 19 sequences from the blackbird *Turdus merula* KC584781, collared flycatcher *Ficedula albicollis* AGTO02004766.1 and zebra finch *Taeniopygia guttata* ABQF01026424, were aligned using Mega 5.2 (Tamura *et al.* 2011) to design conserved primers. By looking for conserved sequences, we designed suitable primers, tested their capability in Primer 3 0.4.0, length: 18-22 bp, melting temp: 59-61°C (Untergasser *et al.* 2012), and then ran the FASTA sequence in Genbank BLASTN 2.2.28 (Benson *et al.* 2009). The graphical alignment output from BLASTN for the presence of conserved segments among the sequences was inspected to check that primer sets amplified the DNA products of predicted size and target area (Figure 3.1). Three primer sets resulted: DRD4_395 (709 bp of the end region), DRD4_349 (290 bp of the start region) and SERT_592 (394 bp of the non-coding end region, Table 3.1). Although SERT_592 amplified a non-coding end region (approx 470 bp from the end of *SERT* exon), non-coding regions can alter the level of gene expression and behaviour (Pastinen 2010) and linkage disequilibrium (LD) was expected to be high in the Seychelles warbler.

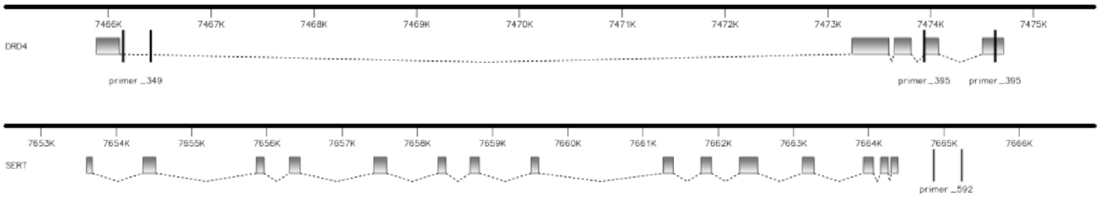


Figure 3.1: Schematic representation of the *DRD4* and *SERT* regions. Grey boxes represent exons and the dotted line introns. The vertical black lines indicate the locations of the primers used in this study.

Table 3.1: Sequence, melting temperature (T_m) and length of product expected from each designed primer in base pairs.

Accession number	Primer ID	Primer sequence 5’-3’	T_m (°C)	Expected product length (bp)
LN833019	DRD4_395	F: GATATTCGCCTTTGCTGTGG	60.6	395
		R: TTCCTGAACTCGGCGTTG	60.6	395
LN833003	DRD4_349	F: CTCGCCCTCCTCGTCCT	60.6	349
		R: GACGGGGATCCCAGGAA	60.6	349
LN833076	SERT_592	F: TGGAACCACAGTGTCAGCAG	60.8	592
		R: CTGGATCACACCCTCTCAGG	60.8	592

SNPs and genotyping

A power analysis (Cohen 1988) using the effect size in the Westerheide population from Korsten *et al.* (2010) revealed that a sample size between 49-56 was sufficient to detect an effect of polymorphisms on behaviour (Table S3.1 of supplementary). Fifty-seven individuals with repeat novel environment exploration assays that belonged in the upper (n=29) and lower (n=28) ten per cent (based on 233 individuals, n=335 assays) were selected for genotyping. Fifty-seven birds were tested for the end region of *DRD4* and when this did not show any variation, we tested nineteen birds for the start region of *DRD4.* An additional twenty-eight birds measured once for novel environment exploration were included in the *SERT* analysis, resulting in a total sample size of eighty-five. The number of individuals measured for novel object exploration was lower because novel object exploration was not assayed in earlier years.

Blood samples were collected and stored in absolute ethanol. DNA was extracted using either a phenol extraction technique (Bruford *et al.* 1998) or a salt extraction method

(Richardson *et al.* 2001). SNP genotyping was performed based on the PCR methods of Kenta *et al.* (2008); modifications included 4 µl of Qiagen PCR master mix, 1 µl of each forward and reverse primer at 5 µM, 1 µl of DNA (~10 ng/µl) and 3 µl of ddH_2O per PCR reaction. The Sanger sequencing protocol was modified using 1/8 of the BigDye® Terminator Cycle Sequencing reagents 3.1 (Applied Biosystems). Sets of primers were used for sequencing on the ABI3730 sequencer.

Sequences were aligned in CodonCode Aligner 5.1.4 (Codon Code Corporation, www.codoncode.com) and visually examined for polymorphisms. Note that 3 base pairs at position 80 were missing in two samples. Construction of haplotypes followed in DNAsp 5.10.1 (Librado and Rozas 2009); sequences are provided in Table S3.2 of supplementary material.

Statistical analyses

Statistical analyses were performed in R 3.0.1 (R Development Core Team 2013) to analyse the *SERT* polymorphisms. We used Haldane's exact test from the Hardy-Weinberg package 1.5.2 (Graffelman and Camarena 2008) to assess whether SNP frequencies deviated from Hardy-Weinberg equilibrium (HWE).

A generalised linear mixed model, GLMM, was run in lme4 1.1-5 (Bates *et al.* 2014) using the function glmer with a Poisson error distribution and log link, adjusted for over-dispersion (Harrison 2014). We ran both overdominant and additive models to investigate different SNP/haplotype effects on the assayed personality traits. For the SNP analyses, the fixed effects were each SNP (*SNP147, SNP209, SNP446, SNP467*) as a factor for the overdominant model (3 levels i.e. Aa, aa, AA) and SNP as a continuous variable for the additive model (0, 1, 2 copies of SNP). For the haplotype analyses, the fixed effects were each haplotype as a factor for the overdominant model (5 haplotypes with 0, 1, 2 copies of a haplotype) and haplotype as a continuous variable for the additive model (0, 1 or 2 copies of the haplotype). Assay number, see Table S3.3 for repeat assay number, (Dingemanse *et al.* 2012), social status, subordinate or dominant (Favati *et al.* 2014), sex (Schuett and Dall 2009) and age (Fisher *et al.* 2015) have been shown to correlate with personality so were included as fixed effects. Age was mean centred and divided by two standard deviations (Gelman and Hill 2006) to account for non-linear relationships, and was included as a quadratic effect. Bird identity and observer identity were included as random effects, as the analyses included birds with repeat personality assays and measurements by more than one observer. The significance of SNP/haplotypes was assessed using a likelihood ratio test (LRT) with a null model excluding the SNP/haplotype effect. The p-values for each model were corrected for multiple testing with false discovery rate (Benjamini and Hochberg 1995). Including personality tent colour did not alter the results.

3.4 Results

DRD4 was monomorphic, however four SNPs were identified in *SERT* at *SNP147, SNP209, SNP446* and *SNP467* in the non-coding end region. None of the four SNPs deviated from Hardy-Weinberg equilibrium (Supplementary Table S3.4). The five haplotype sequences clustered with the blackbird and great tit *SERT* exon one sequences, and the mRNA sequences for the chicken, collared flycatcher and zebra finch clustered together (Figure S3.1).

Overall, there was no effect of haplotype on novel object exploration and novel environment exploration in the overdominant and additive models (Figures 3.2-3.5, accompanying LRT values can be found in Tables S3.5 and S3.6 of the supplement). Similarly, no SNP effect was seen in the overdominant and additive models for novel object exploration and novel environment exploration (Figures S3.2-S3.5, accompanying LRT values can be found in Tables S3.5 and S3.6 of the supplement). There was a positive correlation with age for novel environment exploration and novel object exploration in all models (haplotype models Figures 3.2-3.5; SNP models supplementary Figures S3.2-3.5). Dominant individuals were faster exploring than subordinates in the additive and overdominant models for haplotypes one and two. Individuals also became bolder with increasing assay number in the overdominant model for haplotype one (Figure 3.3).

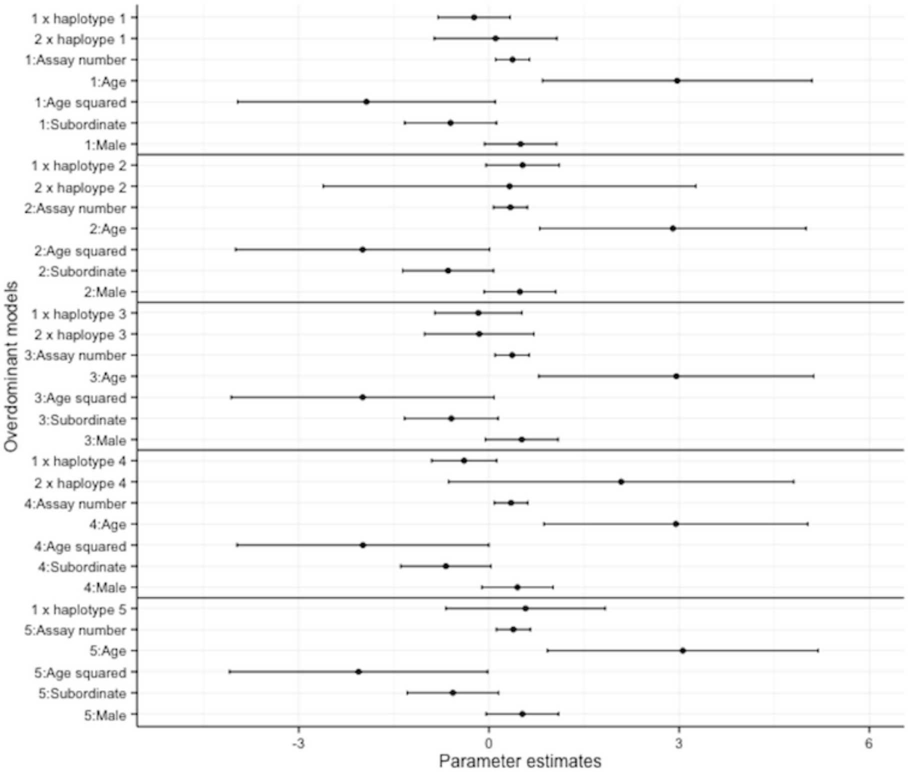


Figure 3.2: The coefficients and associated 95% confidence intervals (CI) of the overdominant haplotype models for novel environment exploration. The models are relative to individuals with no copies of the haplotype, subordinate is relative to dominant, male is relative to female.

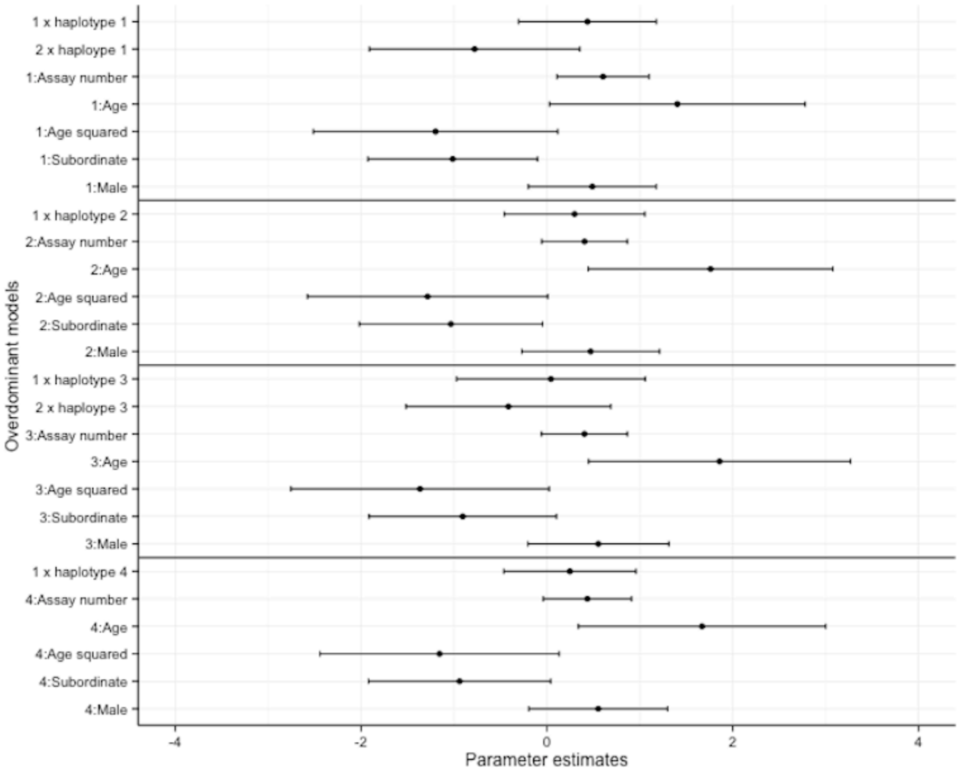


Figure 3.3: The coefficients and associated 95% confidence intervals of the overdominant haplotype models for novel object exploration. The models are relative to individuals with no copies of the haplotype, subordinate is relative to dominant, male is relative to female.

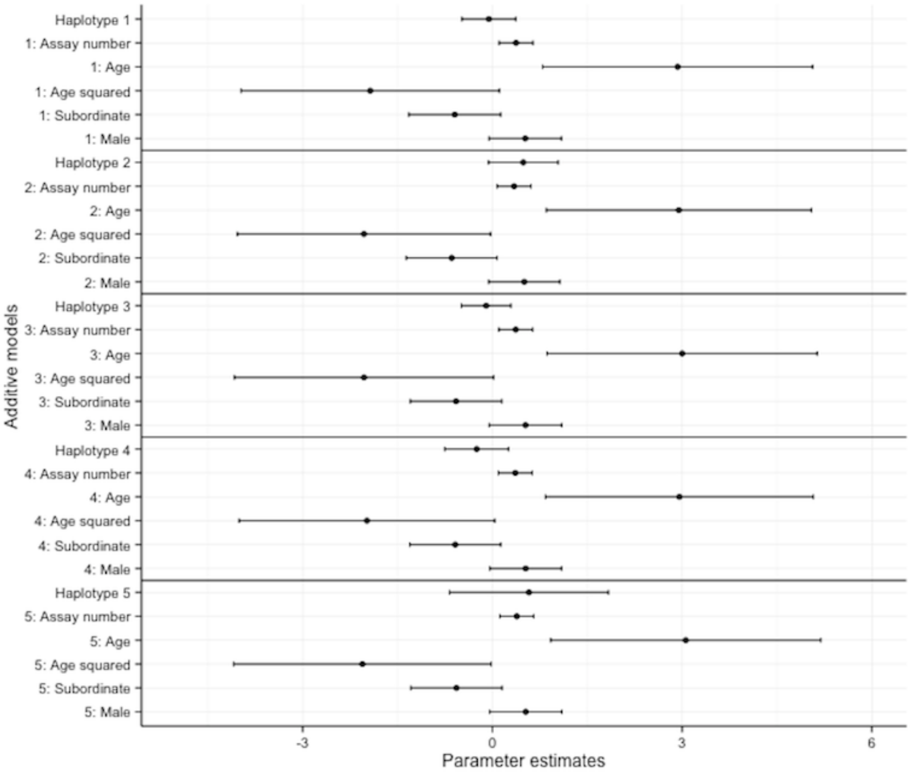


Figure 3.4: The coefficients and associated 95% confidence intervals of the additive haplotype models for novel environment exploration. The models are relative to individuals with no copies of the haplotype, subordinate is relative to dominant, male is relative to female.

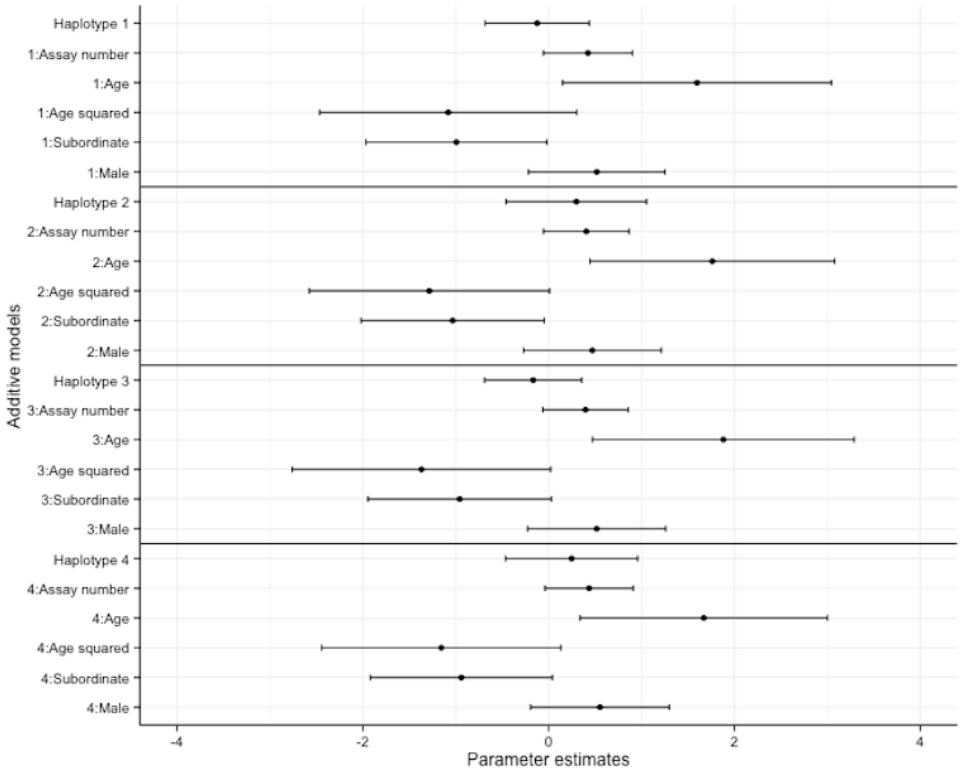


Figure 3.5: The coefficients and associated 95% confidence intervals of the additive haplotype models for novel object exploration. The models are relative to individuals with no copies of the haplotype, subordinate is relative to dominant, male is relative to female.

3.5 Discussion

Identifying the genetic basis of personality can greatly further our understanding of why between-individual differences in behaviour persist in populations. Despite *DRD4* polymorphisms being previously associated with exploratory behaviour (Korsten *et al.* 2010), the portion of this locus that we sequenced was monomorphic in our study population. Having sequenced both the start and end regions, which represents 11% of the length of *DRD4*, from the regions that vary in passerines, it is unlikely that we missed variation in this gene, but without sequencing the whole gene we cannot rule this out. Polymorphisms were, however, found in *SERT,* but these did not correlate with variation in novel environment exploration or novel object exploration.

Despite our null result, we cannot rule out induced and inherited changes in a gene's expression (known as epigenesis) influenced by factors such as age or the external environment (Deans and Maggert 2015). Blue tit nestlings showed genetic correlations between two personality traits (aggression and stress) that disappeared in adults. This was thought to be due to a change in the expression of the genes determining the traits over development (Class and Brommer 2015), highlighting the importance of studying personality longitudinally (Stamps and Groothuis 2010). Furthermore, in wild great tits, an association between exploratory behaviour and *DRD4* genotype was detected in one of the four tested populations. One of the potential reasons given for this result was environmental differences between populations modifying the genetic effects (Korsten *et al.* 2010). In our study species, an additional four populations have been founded from the sequenced population; the first translocation was in 1988 and the most recent was in 2011 (Wright *et al.* 2014). It would be of interest to investigate whether genotype by environment interactions occur within or between the five island populations.

An association between candidate genes and personality does not always imply a direct functional effect (Hansson *et al.* 2004). For example, LD was found between *DRD4* polymorphisms and polymorphisms in the neighbouring *DEAF1*, involved in the regulation of the serotonergic system, in chickens (Flisikowski *et al.* 2009). Although LD is not known in the Cousin population of the Seychelles warbler, it is expected to be high. The population has experienced a relatively recent bottleneck around 120-250 years ago (33-64 generations), reducing the population to around 29-75 individuals (Crook 1960; Spurgin *et al.* 2014), and consequently reducing genetic diversity by 25% and heterozygosity by 19% (Spurgin *et al.* 2014). Therefore the number of recombination events since the bottleneck will be small (Reich *et al.* 2001; Hansson *et al.* 2004). Additionally, it is a small population with a very low dispersal rate between islands (Hansson *et al.* 2004; Komdeur *et al.* 2004).

High LD increases the power to detect a correlation between the *SERT* polymorphisms, and novel object exploration, and novel environment exploration, because of higher linkage with other personality related genes close by.

Being unable to identify the genes underlying focal traits is one disadvantage of the candidate gene approach. Additionally, the candidate gene approach is often biased towards genes with large effect sizes (van Oers and Mueller 2010) and this may be amplified by the publication of mainly positive results (Savitz and Ramesar 2004). As yet, it is unknown how many studies have found a null result and not published the findings. This is why it is important to publish all studies, including studies with null results such as ours, to allow for more representative meta-analyses to be conducted. Nevertheless, we chose the candidate gene approach to maximise the likelihood of detecting ageing effects while minimising the chance of type I and II errors (Tabor *et al.* 2002).

A future direction could be to employ a genome-wide study to look for signatures of selection on personality. The bottlenecked past of our study species may have left signatures of selection at other putatively adaptive relevant loci that genome-wide scans could detect (Steinmeyer *et al.* 2009; van Oers and Mueller 2010). However, it is then necessary to rule out the possibility of pleiotropy, correlated selection or transgenerational epigenetic effects (Barrett and Hoekstra 2011). Alternatively, genome-wide study could look at the partitioning of genetic variance, which would facilitate detection of relevant genes located in genomic regions with small effect sizes (Yang *et al.* 2011).

3. 6 Conclusion

Understanding the molecular genetic basis of personality can ultimately help to explain why behavioural differences between individuals occur in populations. Studies in wild populations that experience natural selective pressures will allow us to address these questions. We found no association between these behaviours and variation in the candidate genes tested in our study population. Future work should account for age or environment effects on *SERT* variants and investigate underrepresented candidate genes that may have an additive or pleiotropic effect on personality. We emphasise the importance of studying personality throughout development in a controlled longitudinal study and the need for the publication of null findings to aid future meta-analyses on personality candidate genes.

Chapter 4

Exploration is not social state-dependent in a wild cooperative breeder

4.1 Abstract

Personality is an intriguing phenomenon in populations because it constrains behavioural flexibility. One theory suggests that personality could be generated and maintained if dependent on asset protection. It is predicted that trade–offs with fitness expectations and survival probability encourage consistent individual differences among individuals (personality). Although not mutually exclusive, the social niche specialisation hypothesis suggests that a group of individuals that repeatedly interact will develop personality to avoid costly social conflict. The point at which behavioural consistency originates in the social niche hypothesis is still unclear, with predictions for development after a change in social status. In the facultative cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*), residing on Cousin Island, breeding vacancies are limited and this forces individuals into different social roles. We used this system to test whether reproductive and social state predicted among–individual differences in exploration. We had two predictions. Firstly, that an individual's start in life can predict personality, whereby young, and/or dominant individuals with a good start to life (associated with early age reproduction and earlier onset survival senescence) are fast explorers, suggesting reproductive state-dependence. Secondly, that an individual's social status can predict personality, whereby dominant individuals will be fast exploreres suggesting that the behaviour is social state–dependent. Neither of the behaviours were associated with social state and social state did not affect behavioural consistency. However, novel object exploration was associated with a proxy of reproductive state. Our results provide support for state being a mechanism for generating individual differences in behaviour.

4.2 Introduction

The occurrence of consistent differences in behaviour among individuals, known as animal personality (Sih *et al.* 2004*a;* Réale *et al.* 2007), is an intriguing phenomenon considering that a flexible behavioural response should enable individuals to adapt to varying environments (Wolf *et al.* 2007; Réale *et al.* 2010). Personality can be highly heritable (Dochtermann *et al.* 2015) and can affect fitness (Smith and Blumstein 2008) but little is known about how it is maintained at the individual or population level (Bell 2007). To gain further evolutionary understanding as to why personality is generated and maintained, we require longitudinal studies of personality in the wild. This is because captive environments can alter an individual's behavioural expression and longitudinal studies on free living organisms in the natural environment are a way to circumvent this (Stamps and Groothuis 2010).

Circumstances or properties that alter the costs and benefits associated with behaviour are known as states (Dall *et al.* 2004; Wolf *et al.* 2007; Biro and Stamps 2008; Dingemanse and Wolf 2010). States are inherently slow changing, but can encourage long–term stability in behaviour if associated in a positive feedback loop (Luttbeg and Sih 2010). Behaviour dependent on asset protection predicts that individuals with a high future reproductive state (i.e. high assets) will be consistently slow explorers and risk averse (behaviours that are often positivey correlated, e.g. Quinn *et al.* 2012), in order to prevent predation, compared with those that have a low future reproductive state (Dall *et al.* 2004; Stamps 2007; Wolf *et al.* 2007). Although more study is needed, a few empirical studies have found support for this prediction. Slow exploratory behaviour was associated with increased survival probability and hence high future reproductive states in wild great tits (*Parus major,* Nicolaus *et al.* 2012) and reduced risk–taking behaviour was exhibited by young individuals with high future reproductive states in grey mouse lemurs (*Microcebus murinus*, Dammhahn 2012).

A less explored avenue of research is the potential for personality to be dependent on social state. Reproductive state and social state are not mutually exclusive because social status is often associated with breeding benefits, although in some species subordinates do have opportunities to breed (Richardson *et al.* 2002). The social niche specialisation hypothesis suggests that a group of individuals in a social group that repeatedly interact will benefit by developing social niches (Bergmüller and Taborsky 2010; Montiglio *et al.* 2013). Social niches, such as social status, cause individuals to behave differently by reducing social conflict and encouraging behavioural consistency through positive feedback mechanisms such as learning and costs incurred by changing social niches (Bergmüller and Taborsky 2010; Wolf and Weissing 2010). The relationship between social status and behavioural differences among individuals is still unclear (Gómez-Laplaza 2002; Fox *et al.* 2009). However, a few studies have shown that a dominant social status correlates with fast exploration and bold and aggressive behaviour in a territorial context (Verbeek *et al.* 1996; Dingemanse and de Goede 2004; Favati *et al.* 2014). Also in a cooperatively breeding system, immature individuals that showed lower levels of a subordinate helping behaviour were aggressive and fast exploring (Bergmüller and Taborsky 2007).

Investigating how social state may affect individual differences in behaviour among individuals does not give any insight into how it may affect within-individual consistency in a population (Dingemanse *et al.* 2010). For example, it is still unclear at what point behavioural consistency within individuals originates under the social niche hypothesis (Carter *et al.* 2014). It has been postulated that, within-individual behavioural consistency could occur after a change in social status, termed the “transition” hypothesis (Carter *et al.* 2014). Further studies are needed, but in support of this theory, meerkat (*Suricata suricata*) subordinate females that later became dominant, exhibited different cooperative personalities after social status change compared to those that remained subordinate (Carter *et al.* 2014). Also, male domestic fowl (*Gallus gallus*) showed increases in vigilance, activity and exploration when changed from a subordinate to a dominant social position (Favati *et al.* 2014).

Facultative cooperative breeders, such as the Seychelles warbler (*Acrocephalus sechellensis*), are an excellent system in which to test the state dependency of personality. In this species, individuals can forego reproduction to raise offspring that are not their own (Cockburn 1998). They are highly territorial and the limited number of breeding vacancies in the Cousin Island population forces many individuals into a subordinate social status indefinitely or until there is a dominant breeding vacancy (Komdeur 1991). A subordinate social status can bear a cost through the loss of direct breeding benefits and reduced body condition when helping to rear young (Richardson *et al.* 2002; van de Crommenacker *et al.* 2011). These social states could consequently encourage behavioural differences among individuals through character displacement and trade-offs with future reproductive state (Bergmüller and Taborsky 2010). Furthermore, Individuals born in years of high food availability (high insect abundance at year of birth), reproduce at an earlier age but have an earlier onset of survival senescence compared to those born into years of low food availability (Hammers *et al.* 2013). This good or bad start could influence an individual's future reproductive state and generate behavioural differences among individuals (e.g. Dingemanse *et al.* 2002; Wolf *et al.* 2007).

In this study, we investigate whether exploration (exploration of a novel environment and exploration of a novel object) is associated with current social state or future reproductive state. In an attempt to tease these two states apart we tested for two predictions. Firstly, that an individual's start in life can predict personality, whereby young and/or dominant individuals that have a good start to life, and thus breed at an earlier age and have early onset survival senescence, will be faster explorers than old and/or subordinate individuals that have a bad start to life, suggesting that personality is associated with future reproductive state. Secondly, that an individual's social state can predict personality, whereby dominant individuals are faster explorers than subordinate individuals, suggesting that personality is associated with current social state (e.g. social conflict and aspects of the social niche environment such as resource holding potential). Furthermore, we will investigate whether social status affects within-individual behavioural consistency, thus providing support to the ''transition'' hypothesis.

4.3 Methods

Study system and site

Seychelles warblers were monitored on the main study island of Cousin (0.29 km^2; 04°20!S, 55°40!E) during the winter (Jan–Feb) and summer (Jun–Sep) breeding seasons in 2010–2015, where they have been monitored intensively since 1981 (Komdeur 1991). During this time, social status and group memberships were identified, individuals were colour/BTO ringed and blood sampled (for sexing and genotyping). The sex of each individual is determined using molecular sexing methods (Griffith *et al.* 2002). The population experiences virtually no immigration and emigration between surrounding islands (Komdeur *et al.* 2004; Komdeur *et al.* 2015) and there is a 0.92 ± 0.02 probability of annually re-sighting in the first two years of life and 0.98 ± 0.01 probability of annually re-sighting in adults (Brouwer *et al.* 2010). Subsequently birds are presumed dead if not seen after one year. The mean life span of an individual is five and a half years and the maximum life span of seventeen years has been recorded (Komdeur 1991; Barrett *et al.* 2013).

To determine territory boundaries, breeding status and to observe interactions with other warblers, dominant females were followed for a minimum of 15 minutes on a weekly basis throughout each summer and winter season. A subordinate status was assigned to individual birds (> 5 months old) that were consistently seen in a territory and interacted with group members, but didn't engage in dominant pair behaviour. Dominant status was assigned when a pair of individuals was observed in a territory over multiple weeks and the individuals within the pair stayed within close proximity of one another and had frequent vocal interactions. The age of the first breeding attempt has been shown to range from one to eight years old, with 48% breeding in their first year (Hammers *et al.* 2013). The Seychelles warblers are insectivorous and take 98% of their food from the underside of leafs of predominately *Pisonia grandis*, *Morinda citrifolia*, and *Ficus sp*. (Komdeur 1991; Komdeur 1994). Insect abundance was thus measured over 14 locations across the island during the main breeding season (Komdeur 1992). Using this data we then averaged insect abundance over these 14 locations per year to get an estimate of annual variation in food availability (Spurgin *et al.* Unpublished manuscript).

Personality assays

Birds were caught in mist nets throughout the winter and summer breeding season of 2010–2015 for exploration of the novel environment, and 2013–2015 for exploration of a novel object. Once a bird was caught in a mist net it was extracted, measured for morphometric traits, given five minutes in a bird bag, and then assayed for personality. Exploration of a novel environment was tested in an Oxygen 4 tent (L322 x W340 x H210 cm, Gelert Ltd Wigan). The tent contained three artificial trees each with two branches 45 cm long (one attached at 95 cm and the other at the top of the trunk), and a trunk 148 cm high (adapted from, Verbeek *et al.* 1994). The number of flights, hops and the total number of trees visited were recorded during a five–minute period. A flight denoted a transfer between branches on the same tree, between trees, or between floor and tree, or any movement greater than a branch length that involved flapping of the wings. A hop was described as both feet off the ground with no wing flapping, either on the same branch or on the floor. The combined number of hops, flights and trees visited was totalled to give a measure of exploration (Chapter 2).

Exploration of a novel object was then tested two minutes after the exploration assay to allow for habituation to the novel environment of the tent (see acclimation test, Chapter 2). A novel pink toy attached to a tree branch (95 cm long) was inserted and positioned in the centre of the tent (adapted from Verbeek *et al.* 1994). The number of hops, flights and trees visited was summed in the novel object assay and used as a measure of novel object exploration (Chapter 2).

Personality assays were collected on 312 individuals (1 measure = 175 birds; 2 = 96; 3 = 25; 4 = 8; 5 = 5; 6 = 3, female=149, male=163) for novel environment exploration and 177 individuals (1 measure = 120 birds, 2 = 52; 3 = 4; 4 = 1, female=81, male=96) for novel object exploration.

Statistical analyses

All statistical analyses were performed in R 3.0.2. (R Development Core Team 2013).

(i) Social or reproductive state-dependence

Generalised linear mixed models (GLMM) using a Poisson error distribution with a log link were run in the package MCMCglmm 2.17 (Hadfield 2009). For all models we specified an Inverse Wishart prior (V=1, n=0.2), the posterior distribution was sampled every 100 iterations with a burn–in period of 3000 iterations, and a run of 203,000 iterations. Convergence was assessed by autocorrelation values ($r<0.1$), visual inspection of time series plots of the model parameters and using the heidel.diag and geweke.diag functions. We ran two models, with the responses of exploration of a novel environment and exploration of a novel object. The fixed effects included variables known to influence personality: social status at testing (subordinate or dominant) and an interaction with insect abundance at year of birth (mean=4.61, variance=3.76), age (novel environment age range: 36-5687 days, novel object age range: 60-1432 days, e.g. Fisher *et al.* 2015) and an interaction with insect abundance at year of birth, assay number (e.g. Dingemanse *et al.* 2012), sex (e.g. Schuett and Dall 2009) and body mass (standardised for time of day). Age (days) was mean centred (Gelman and Hill 2006) and included as a linear term. Tent colour was also included as a fixed effect in the novel environment exploration model because it was shown to have an effect in previous analyses (Edwards *et al.* Under review). The model also included observer identity and bird identity as random effects to account for repeat observations.

(ii) Behavioural consistency at social status transition

Hierarchical generalised linear models (HGLM, Cleasby and Nakagawa 2011; Cleasby *et al.* 2014) allow for individual/group differences in the residual variance to measure how an individual's behaviour changes when measured repeatedly, and hence its predictability. Therefore only individuals with repeat personality measures were included in this analysis. We included assay number, age and sex as fixed effects and fitted the difference of status between measures into the dispersion part of the standard Poisson HGLM using the package HGLM 2.0–11 (Ronnegard *et al.* 2010). The social status differences were grouped as: 1) individuals that remained subordinate (novel environment exploration n=49; novel object exploration n=15); 2) individuals that remained dominant (novel environment exploration n=74; novel object exploration n=31); or 3) individuals that transitioned from subordinate to dominant social status between behavioural measures (novel environment exploration n=36; novel object exploration n=11). No individuals transitioned from dominant to subordinate status. Bird identity was also included as a random effect to control for repeat measures. To assess the effect of social status difference on the residual variance, we compared the fit of a model with and without the social status fixed effect in the dispersion part of the HGLM, using conditional AIC values (Cleasby *et al.* 2014). The model with the smaller conditional AIC value and a difference greater than seven was interpreted as a better fit (Burnham *et al.* 2011). Modelling variance often requires a large sample size(Martin *et al.* 2011; van de Pol 2012). To ensure the small sample size of the novel object exploration assay was not biasing our estimates, we ran a simulation analysis with a Poisson HGLM. We then changed the sample sizes (n=11–500) to investigate the effect on two simulated models parameter estimates. Simulated sample sizes of 11 individuals per group (representative of our social status grouping) did not sufficiently bias the model parameters, suggesting our sample sizes were sufficient to detect an effect (see Supplementary Table S4.1).

4.4 Results

(i) Social or reproductive state-dependence

Social state and insect abundance at year of birth were not associated with novel environment exploration (Figure 4.1). Novel environment exploration instead increased with assay number. The marginal effects explained 0.19 (0.12–0.31) of the variance and the conditional effects explained 0.53 (0.27–0.73) of the variance in the novel environment exploration model. Social state was not associated with novel object exploration but with insect abundance at year of birth and the interaction with age (Figure 4.2). There was a negative relationship between insect abundance and age, whereby young individuals born into years of high food abundance were associated with faster exploration of the novel object (Figure 4.2 & 4.3). Males were faster explorers than females and novel object exploration also increased with assay number and age (Figure 4.2). The marginal effects explained 0.22 (0.15–0.28) of the variance and the conditional effects explained 0.52 (0.39–0.62) of the variance in the novel object exploration model.

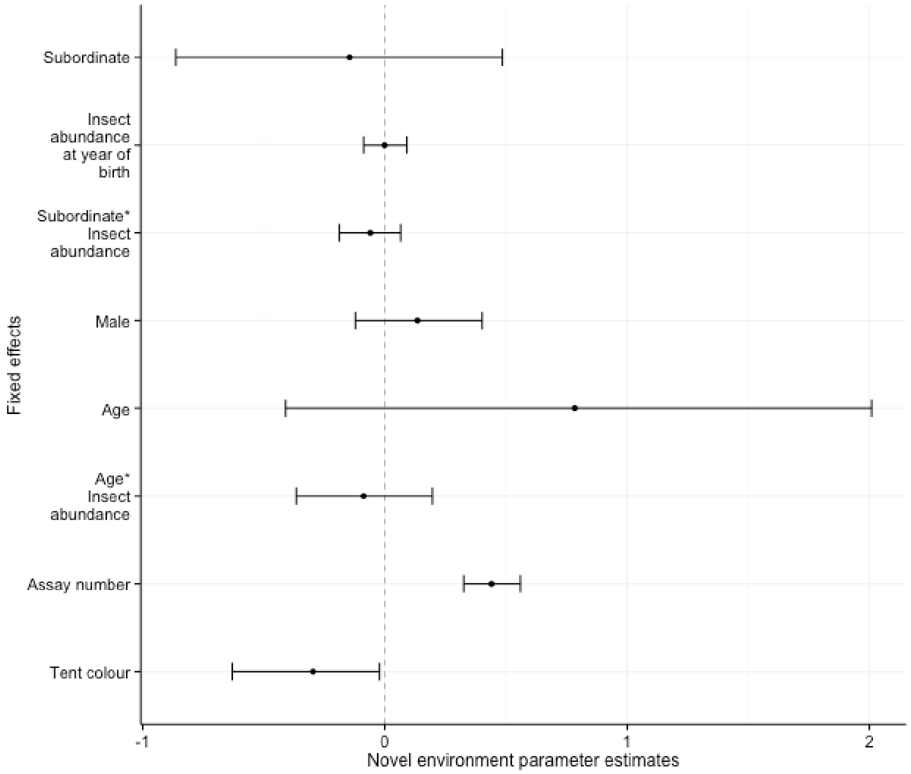


Figure 4.1: Posterior modes and associated 95% credible intervals of social status (contrast level = dominant, N: subordinates = 237, dominants = 280), insect abundance at year of birth and the interaction with social status, sex (contrast level = female, N: female = 149, male = 163), age and the interaction with insect abundance at year of birth, assay number*, tent colour (contrast level = blue, N: blue = 339, green = 178)* and body mass in the novel environment exploration model. * indicates posterior modes whose 95% credible intervals do not overlap zero.

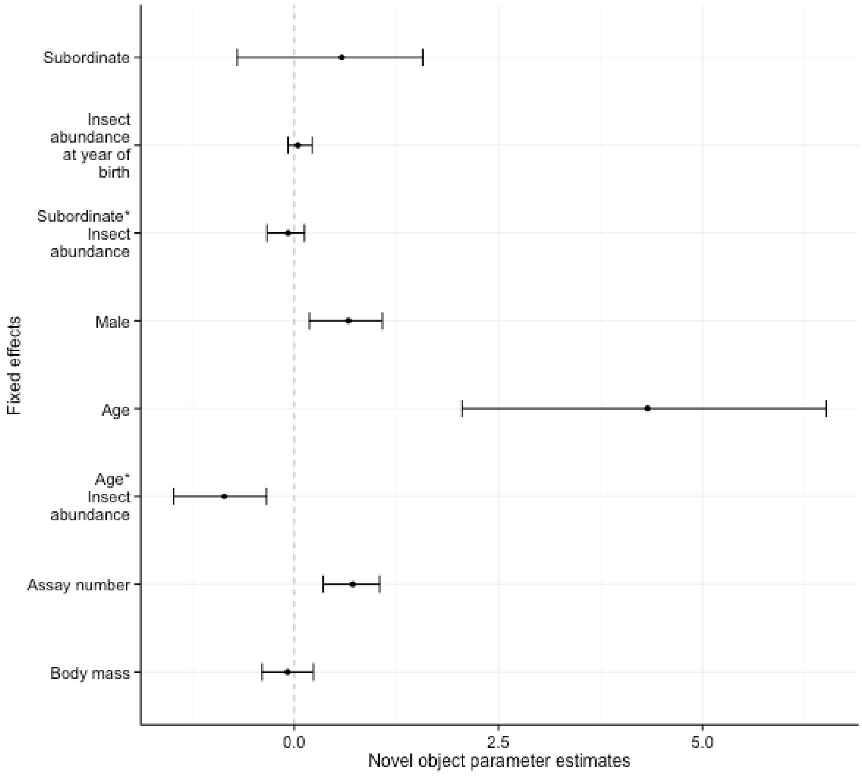


Figure 4.2: Posterior modes and associated 95% credible intervals of social status (contrast level = dominant, N: subordinate = 99, dominant = 141), insect abundance at year of birth and the interaction with social status, sex (contrast level = female, female = 81, male = 96)*, age and the interaction with insect abundance at year of birth*, assay number* and body mass in the novel object exploration model. * indicates posterior modes whose 95% credible intervals do not overlap zero.

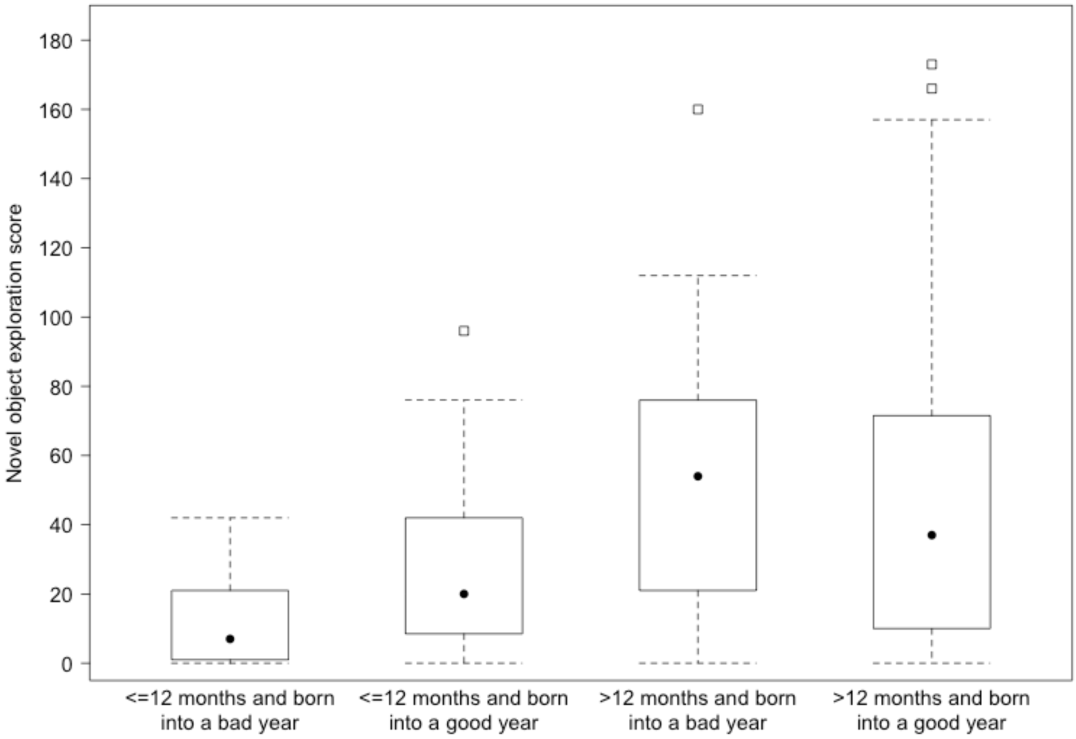


Figure 4.3: The novel object exploration scores of individuals twelve months or younger, born into bad (n = 30) and good years (n = 35) of food abundance (defined as above and below the average (4.35) insect abundance across all years), and individuals older than twelve months born into bad (n = 39) and good years (n = 73). The black dot is the median boldness score, whiskers represent the lower and upper quartiles (25% and 75%) and the black squares are outliers.

(ii) Behavioural consistency at social status transition

For novel environment exploration, the standard Poisson model and the model with social status fixed in the dispersion were of similar fits (Standard Poisson HGLM cAIC= 2864.63, h–likelihood=2891.61, and modified dispersion HGLM cAIC= 2871.01, h–likelihood=2896.68). Similarly for novel object exploration the two models were of similar fits (Standard Poisson HGLM cAIC= 1137.75, h–likelihood=1102.13, and modified dispersion HGLM cAIC= 1135.46, h–likelihood=1105.94). Thus there was no evidence for within-individual behavioural consistency being affected by social state.

4.5 Discussion

Asset protection could encourage consistent behavioural differences through trade–offs with future fitness expectations and survival probability (Wolf *et al.* 2007). The social niche specialisation hypothesis further suggests that in a group where individuals repeatedly interact, consistent behavioural differences will develop in order to avoid costly social conflict (Bergmüller and Taborsky 2007; Laskowski and Pruitt 2014). In our study, we found that between individual differences in exploration were not associated with social state and that within-individual behavioural consistency was unaffected by social state. However, insect abundance at year of birth, a proxy of reproductive state, and the interaction with age did predict novel object exploration.

Young individuals born in years of greater food abundance were associated with fast exploration of the novel object. This finding is in line with the asset protection theory that suggests individuals should adjust their risk–taking/exploratory behaviour when there are trade–offs with future fitness expectations and survival probability (Wolf *et al.* 2007). This relationship has also been confirmed in empirical studies, where slow exploratory and reduced risk–taking behaviour is associated with individuals with high future reproductive states (Dammhahn 2012; Nicolaus *et al.* 2012). In the Seychelles warbler, greater food abundance at year of birth causes individuals to reproduce for the first time at an earlier age but results in earlier survival senescence (Hammers *et al.* 2013). Reproductive tactics are thus modified to suit environmental conditions to maintain survival (Hammers *et al.* 2013) and reproductive output is age–dependent, with an initial increase followed by a decline in old age (Hammers *et al.* 2012). Young individuals born into good insect abundance years (low future reproductive states) may exhibit risky behaviour, such as territory guarding and novel foraging, to ensure the success of current reproductive attempts. Long–term studies should look at how novel object exploration may be linked with aspects of the Seychelles warblers' ecology, such as predator susceptibility or resource holding.

Novel object exploration was also associated with age, with older individuals exhibiting faster exploration. Previous studies have found that among–individual variance in personality traits increase later in life (Roberts and DelVecchio 2000; Fisher *et al.* 2015). It could be that older individuals have experienced greater environmental variation (novel prey or novel con–specifics) and are faster explorers than younger individuals. Furthermore, processes such as stimulus generalisation (transfer of a response learned from one stimulus to a similar stimulus) in older individuals may encourage fast exploratory behaviour. These two processes coupled together could result in age–related behavioural differences.

We also show that assay number affected between-individual differences in exploration. Differences in how individuals habituate to a novel environment are associated with individual differences in learning (Light *et al.* 2011) and fearfulness (File 2001). After repeat testing, individuals are thought to overcome fear and explore novel environments more superficially compared to previous experiences (Verbeek *et al.* 1994). This effect is particularly pronounced in slow explorers (Carere *et al.* 2005). Clearly, repeat testing is conflated with habituation and therefore assay number should be accounted for when repeatedly measuring traits that are associated with learning and fearfulness.

Novel object exploration also differed between the sexes, with males being faster explorers than females. The direction of among–individual differences in exploration between the sexes can vary and it has been postulated that sexual selection may play a role in encouraging these differences (reviewed in Schuett *et al.* 2010). Exploratory behaviour has been correlated with spatial response to territory intrusion, with fast explorers spending more time in proximity to the intruder in great tits (Snijders *et al.* 2015). In the Seychelles warbler, attack frequencies towards a simulated predator were higher in males, than in females (Veen *et al.* 2000). Exploration may therefore be associated with territorial defence and if selected for in males, may result in these sex differences.

Behavioural consistency of exploration was unaffected by social state, although this has been suggested as a mechanism for within-individual behavioural consistency in meerkats (Carter *et al.* 2014). There are, however, some limitations to our study. We did not experimentally manipulate individuals and relied upon natural changes in social status. This means the individuals we measured may have been predisposed to certain changes in social statuses caused by environmental, physiological or experiential factors. Also, behavioural variation within individuals was not captured over a lifetime (personality tested birds had an average age of 2.98 years but Seychelles warblers have a mean life span of 5.5 years, Komdeur 1991). For example, subordinates that remained subordinate between personality measures could potentially transition to dominance at a later stage, and dominants that remained dominant may not have been assayed when subordinate. It is difficult to decipher whether the three social status groups (individuals that remained subordinate, individuals that remained dominant, or individuals that transitioned from subordinate to dominant social status between behavioural measures) were equally plastic or equally consistent, because variation within all the groups was the same. Both scenarios have their advantages for an individual. Behavioural plasticity can be adaptive (Sih *et al.* 2004*a;* Kontiainen *et al.* 2009; Betini and Norris 2012) and allow individuals to display costly behaviours only when required. On the other hand, consistent behaviour can allow individuals to specialise in different social niches and avoid costly social conflict (Bergmüller and Taborsky 2007).

4.6 Conclusion

We have shown that social state does not explain behavioural differences in exploration, nor affect behavioural consistency. Instead we show that a proxy of reproductive state, sex and age affect individual differences in novel object exploration, and repeat testing affects individuals differences in novel environment exploration. Our results provide further support that exploration can be reproductive state–dependent, and that this may be a mechanism for generating individual differences. We suggest that future work should look directly at survival probability as a mechanism for encouraging personality and sex–specific behaviour such as territorial defence, which may encourage sex differences.

Chapter 5

Extra group parentage and personality in a wild cooperative breeder

5.1 Abstract

True genetic monogamy is rare in many socially monogamous systems. To understand why there is variation in extra group parentage the costs and benefits of the behaviour are increasingly investigated in relation to inherent differences amongst individuals . Consistent individual differences in behaviour among individuals, termed personality, can be heritable and affect fitness. An example of a way in which personality can affect fitness is through reproductive behaviour, where it has been shown that fast exploring individuals are usually associated with high rates of extra pair paternity (EPP). In this study, we investigate whether reproductive success and behaviour are associated with personality in a wild cooperative breeder, the Seychelles warbler (*Acrocephalus sechellensis*). We predicted that fast explorers would have a high number of offspring (high mate encounter rate), and a high number of extra-group offspring (EGO) compared to their slower counterparts. We also predicted that within the social pair, fast exploring males would have a high number of EGO, and females paired with fast exploring males would have a high number of EGO, compared to slow exploring males and females paired with slow exploring males. We found that the propensity to have EGO for females was higher in disassortative pairs for novel environment exploration. Novel environment and novel object exploration, were not however associated with the number of offspring sired or the ratio of within group offspring (WGO) to EGO in a season. We conclude that extra group parentage in the Seychelles warbler is associated with personality, but that this does not depend solely on an individual's personality, but also that of the social partner. We suggest that the behavioural compatibility of the mating pair could be a mechanism that determines decisions to engage in extra group parentage.

5.2 Introduction

True genetic monogamy is rare, even in many socially monogamous systems (Griffith *et al.* 2002; Uller and Olsson 2008; Cohas and Allainé 2009). The occurrence of extra group parentage can be seen as an adaptive behaviour if it is associated with greater numbers or quality of offspring (Mays and Hill 2004; Andersson and Simmons 2006; Wilson and Nussey 2010) for example, or as a way to avoid inbreeding (Arct et al. 2015). However, extra group parentage can also be maladaptive and incur costs including lost foraging opportunities (Rowe 1992), increased risk of death (Magnhagen 1991; Rowe 1994; Réale *et al.* 1996) and loss of paternity at the social nest (Petrie and Kempenaers 1998). To understand why there is variation in extra group parentage, the costs and benefits of the behaviour are often averaged across individuals, and increasingly in relation to inherent differences amongst individuals (Eliassen and Kokko 2008).

Although once perceived as noise, consistent individual differences in behaviour, termed animal personality, may impact upon the fitness components of an individual (e.g. Smith and Blumstein 2008). The relationship between personality and reproductive success is often ambiguous in wild populations and can be context-dependent (Réale and Festa-Bianchet 2003; Dingemanse *et al.* 2004; Le Cœur *et al.* 2015) and influenced by the social mate (Dingemanse *et al.* 2004; Both *et al.* 2005; Gabriel and Black 2012; Burtka and Grindstaff 2015). A meta-analysis by Smith and Blumstein (2008) found such trends across species, where survival rates for bolder and fast exploring individuals were lower than their shyer, slower counterparts, and bolder and aggressive individuals had a higher reproductive success rate (a combination of annual and lifetime success) than their shyer, less aggressive counterparts.

Personality can also explain individual variation in reproductive behaviours, such as extra group parentage (Duckworth 2006; van Oers *et al.* 2008; While *et al.* 2009; Patrick *et* *al.* 2011; Martin *et al.* 2014; McCowan *et al.* 2014). Previous research in socially monogamous species has revealed that the exploratory or aggressive tendency of an individual can influence the mode of paternity acquisition. For example, fast exploring males and aggressive, non-docile females engaged in high rates of extra pair paternity (EPP, While *et al.* 2009; Patrick *et al.* 2011; Martin *et al.* 2014). Additionally, the social partner can strongly affect the expression of personality and in turn, reproductive behaviour (Niemelä and Santostefano 2015). For example, within the social pair, the females' personality can affect the probability of EPP (Patrick *et al.* 2011), and high pair behavioural compatibility correlates with high rates of EPP (van Oers *et al.* 2008). Collectively these studies suggest that the personality of both sexes has the potential to influence paternity and that this is likely to be influenced by the sex-related pay-offs of engaging in promiscuous behaviour within a population (Patrick *et al.* 2011).

The cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*) on Cousin Island can be used to test the hypothesis that personality influences the number of extra group offspring (EGO). In this population, dominant pairs are socially monogamous but there is a high rate of extra group paternity (EGP), with 40% of all offspring being sired by dominant males outside of the natal territory (Richardson *et al.* 2001). Extra pair fertilisations can provide genetic benefits for offspring (MHC diversity, Brouwer *et al.* 2010) but they are constrained by the social male mate-guarding the female (Komdeur *et al.* 1999; Komdeur *et al.* 2007). Novel environment exploration and novel object exploration are repeatable and heritable in the Cousin Island population (Chapter 1). These personality traits have been shown to positively correlate with risk-taking behaviour (e.g. Quinn *et al.* 2012) and activity (e.g. Quinn and Cresswell 2005) in other wild bird species. Activity levels of fast explorers may encourage high encounter rates with potential extra-group partners, since an increase in density, and potentially encounter rate, can increase the rate of extra pair copulations (e.g. Kokko and Rankin 2006). Here we predict that fast exploring individuals sire a greater number of offspring and consequently have a higher number of EGO (Patrick *et al.* 2011) than their slow exploring counterparts. Furthermore, there is a risky trade-off between gaining extra group paternity and mate-guarding due to loss of paternity (Komdeur *et al.* 1999; Komdeur 2007), and increased energy expenditure (Komdeur 2001). We predict that fast exploring males have a high probability of gaining EGP and this increases the propensity of their mate to have offspring with an extra-group father.

5.3 Methods

Study system

The Seychelles warbler is a small passerine endemic to the Seychelles. It is a facultative cooperatively breeding system, where individuals can forego reproduction to raise offspring that are not their own (Cockburn 1998). Dominant breeding birds defend a territory year-round and form long-term pair bonds, often until death (average life span 5.5 years, Komdeur 1992). Habitat saturation on the main study island of Cousin means that breeding opportunities are rare and forces individuals to delay independent breeding and remain subordinates within a territory (Komdeur 1991). Subordinates can help raise offspring, and this decision for female subordinates depends on the presence of the dominant female that raised them (Richardson *et al.* 2003). Subordinate females may gain paternity by laying an egg in a dominant female's nest. Subordinate males rarely gain parentage and extra-group offspring are primarily fathered by dominant breeding males outside of the natal territory (Richardson *et al.* 2001). Helper presence not only increases the survival of offspring but also has survival benefits into adulthood (Komdeur 1994; Brouwer *et al.* 2012). In general, there is a single clutch in a breeding season, consisting of a single egg (Richardson *et al.* 2001).

Seychelles warblers are monitored on the main study island of Cousin (0.29 km^2; 4°20!S, 55°40!E) during the winter (Jan–Feb) and summer (Jun–Sep) breeding seasons. During the field season territories are defined and individuals are followed for approximately 15 minutes on a weekly basis to ascertain social status and identify breeding attempts, and from this information birth dates are calculated. A subordinate status is assigned to single birds that did not express dominant pair behaviour, were consistently seen in a territory and interacting with group members. A dominance status is assigned to individuals in a pair that were observed in a territory over multiple weeks that stayed within close proximity of one another and had frequent vocal interactions. Mist nets are used to capture individuals for ringing with colour and BTO rings if required, take morphometric measurements and blood samples for molecular sexing (following Griffith *et al.* 2002) and parentage analysis. Parentage was assigned using 30 microsatellites and Masterbayes 2.52 (Hadfield *et al.* 2006). The complete pedigree was 10 generations deep, and included 1875 individuals, 66 founders and 1809 offspring. 786 individuals in the pedigree were informative for novel environment exploration and 684 were informative for novel object exploration. 1487 offspring were assigned a mother and 1554 were assigned a father with at least 80% confidence (Dugdale *et al.* unpublished data).

Personality assays

Personality was tested during the winter and summer seasons of 2010–2015. Exploration of a novel environment was assayed in an Oxygen 4 tent (L322 x W340 x H210 cm; Gelert Ltd Wigan) containing three artificial trees (Edwards *et al.* 2015). The number of hops, flights and unique trees visited was totalled to give a measure of exploration of the novel environment, which was repeatable (Chapter 2).

Two minutes after the exploration assay to allow for habituation to the novel environment of the tent (see acclimation test, Chapter 2), exploration of a novel object was assayed. A novel pink toy attached to a tree branch (95cm long) was positioned in the centre of the tent. The number of hops, flights and trees visited was totalled to give a measure of exploration of the novel object, which was repeatable (Chapter 2).

Statistical Analysis

All statistical analyses were performed in R 3.0.1. (R Development Core Team 2013) using the MCMCglmm package 2.17 (Hadfield 2009).

i) Individual analysis

For the individual data we tested for the effect of personality on: 1) the total number of offspring that an individual was assigned parentage to per season, regardless of the mode of paternity, using a Poisson distribution with log link; 2) the number of offspring the focal individual was assigned parentage to with a mate from outside the social group and the number of within-group offspring per season, using a multinomial distribution with logit link; and, 3) the likelihood to be assigned EGO (yes/no) per season with a categorical distribution and logit link. We ran separate models with either exploration of novel environment as a fixed effect (n = 171) or exploration of a novel object as a fixed effect (n = 93) for the Poisson, multinomial and categorical models. All models contained the following fixed effects: social status of an individual (subordinate or dominant, Richardson *et al.* 2001), the linear and quadratic term of age (counted as the number of winter and summer seasons up to the season the offspring was born, and then standardised e.g. Koenig *et al.* 2009; Cleasby and Nakagawa 2012), year of birth, insect abundance (annual insect abundance) and personality score. Both the first score and the average score gave similar results, so we used the first score to account for tent colour in the novel environment assay. Since the presence of helpers in a territory can improve nestling survival and recruitment (Komdeur 1994; Brouwer *et al.* 2012), we also included a helper variable in all models. This was calculated as the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season. Tent colour (blue/green) was included as a fixed effect for the models with novel environment exploration only, because it influences novel environment exploration (Chapter two). Bird identity was included as a random effect to account for repeat observations.

ii) Pair compatibility analysis

For the pair data we tested for the effect of personality on: 1) the total number of offspring a female was assigned maternity to per season, with a Poisson distribution and log link; 2) the total number of offspring a male was assigned paternity to per season, with a Poisson distribution and log link; 3) the likelihood of having EGO (yes/no) for the female per season, with a categorical distribution and logit link; and, 4) the likelihood of having EGO (yes/no) for the male per season, with a categorical distribution and logit link. The fixed effects in all models were the linear and quadratic term of age, year of birth, insect abundance, and the helper variable, season of offspring's birth, males' behavioural score and the females' behavioural score (for individuals tested more than once, we used the score closest in time to when the pair were together) and an interaction between them, personality assay number (novel environment exploration), and tent colour (novel environment exploration). Bird identity was included as a random factor to account for individuals with more than one social mate. We ran models 1-4 with exploration of a novel environment (number of: pairs = 76, males = 64, females = 67) as a fixed effect and 1-2 with exploration of a novel object (number of: pairs = 31, males = 29, females = 30) as a fixed effect. We did not include novel object exploration in the categorical model because the sample size was too small.

We used an Inverse Wishart (V=1, n=0.2) prior for the Poisson and multinomial models. We specified V=1 and n=2 for the residual, and an Inverse Wishart structure for the random effects in the individual analysis categorical models, and a parameter expanded structure (V = 1,nu = 1, alpha.mu = 0, alpha.V = 1000) for the random effects in the pair analysis categorical models. We sampled the posterior distribution every 100 iterations, with a burn-in period of 3000 iterations and a run of 203,000 iterations. We assessed convergence by using the heidel.diag and geweke.diag functions and inspecting the autocorrelation values ($r<0.1$) and time series plots.

5.4 Results

i) Individual analysis

The number of offspring, ratio of EGO to WGO and propensity to engage in extra group parentage, were not associated with exploration of a novel environment (Tables S5.1-S5.3) or exploration of a novel object (Tables S5.4-S5.6). An individuals' year of birth affected the ratio of EGO to WGO in a season (Table S5.2 & 5.5). The likelihood to be assigned EGO per season increased in individuals that lived longer and was affected by an individuals' year of birth (Figure 5.1 & Table S5.3).

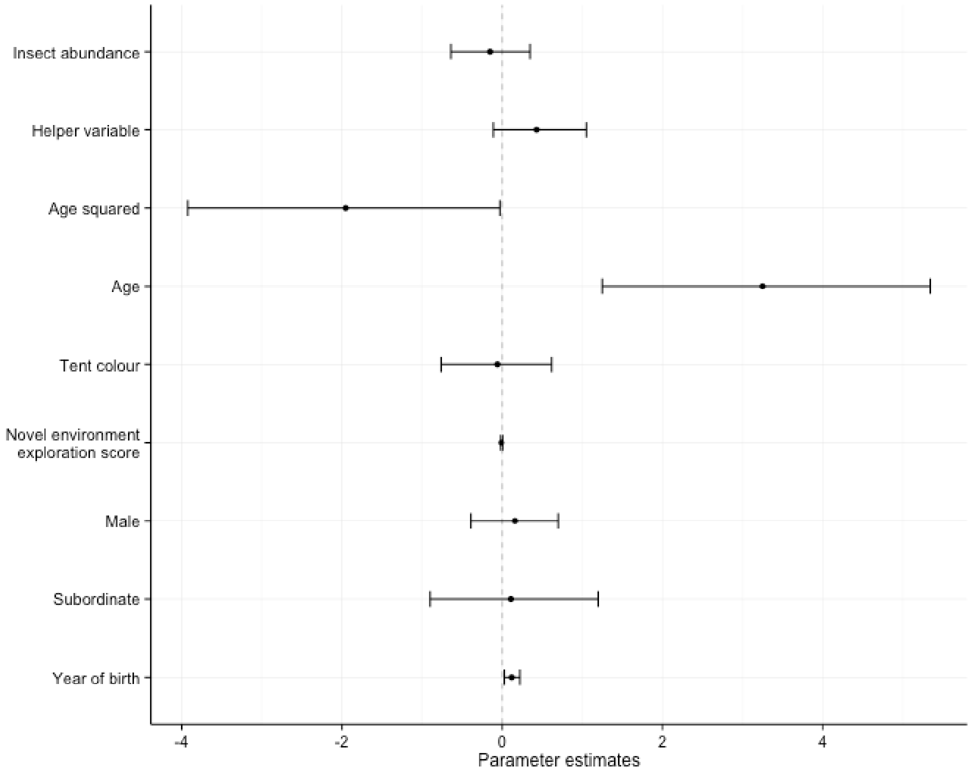


Figure 5.1: The posterior distributions estimates for the fixed effects in the categorical model for the likelihood of extra pair offspring: insect abundance (annual insect abundance averaged over each individuals lifetime), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season), age (quadratic and linear terms)*, tent colour (N: blue = 135, green = 36; contrast level = blue), novel environment exploration score, sex (N: male = 86, female = 85; contrast level = female), ultimate social status (N: dominant = 147, subordinate = 8, sired offspring as subordinate and as a dominant = 16; contrast level = dominant) and year of birth*. * indicates posterior modes whose 95% credible intervals (Cr.I.) do not overlap zero

ii) Pair compatibility analysis

The total number of offspring sired by either the male or female within the pair was not associated with the interaction of the males and females exploration of a novel environment (Tables S5.7 & S5.8). The total number of offspring sired by a male increased with age (Table S5.8). The propensity to have EGO for females was higher in disassortative pairs tested for exploration of the novel environment (Figure 5.2, Table S5.9), but there was no effect on male tendency to gain EGO (Table S5.10). The male tendency to gain EGO was associated with female assay number (Table S5.10). The total number of offspring sired by either the male or female within the pair was not associated with the interaction of the males and females exploration of a novel object (Tables S5.11 & S5.12).

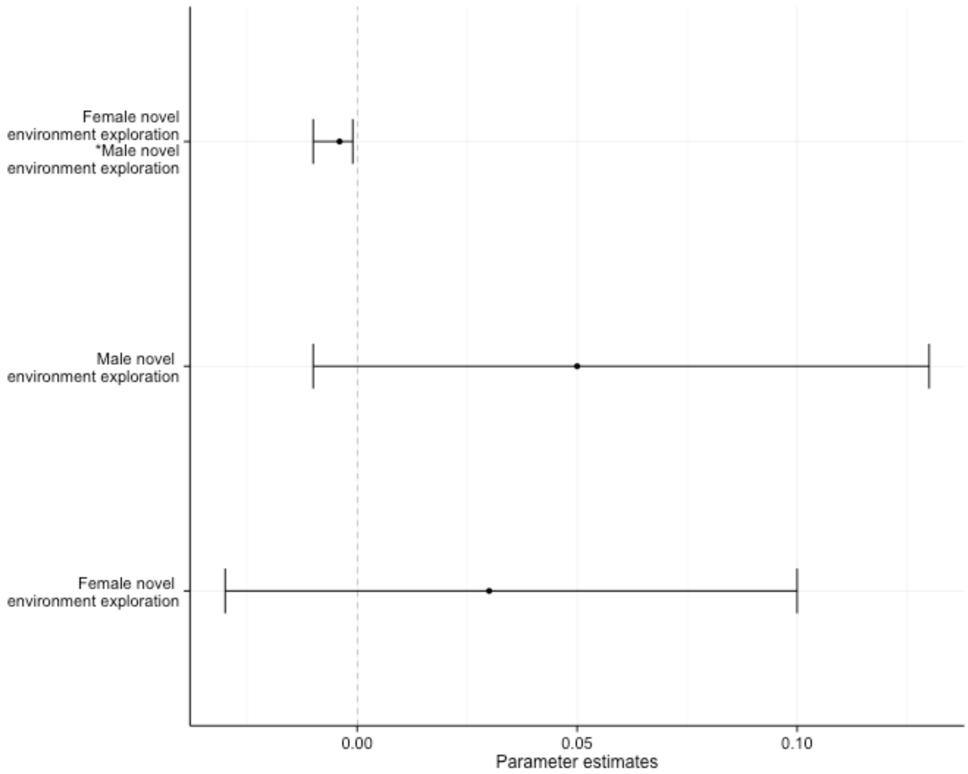


Figure 5.2: The posterior distributions estimates for the novel environment exploration of the male and female in the categorical model for the propensity to gain EGO by the female (posterior modes and associated 95% credible intervals).

5.5 Discussion

We have shown that the likelihood of being assigned EGO in a season increased in older individuals, and that the proportion of EGO in a brood and the likelihood of being assigned EGO was affected by an individuals' year of birth. Furthermore, exploration within a social pair was associated with the propensity to have extra-group young. More specifically, the propensity to have EGO for females was higher in disassortative pairs tested for exploration of the novel environment. Overall however, there was no association between the number of offspring sired by a male or female in a pair, and the pair's personality.

The behavioural incompatibility results differ from previous work that has shown pairs with similar personalities, engaged in high rates of EPP (van Oers *et al.* 2008). It has been suggested that variation in reproductive behaviour could be under correlated selection with personality (Patrick *et al.* 2011), especially if personality is associated with attractiveness and there is a preference for partners with similar personalities (Godin and Dugatkin 1996; van Oers *et al.* 2008). The degree of attractiveness may result in trade-offs with mate-guarding, or to be mate-guarded if EGP provides a benefit to females, causing loss of paternity within the pair (Kokko and Morrell 2005; McCowan *et al.* 2014). Furthermore, personality may be associated with the timing of the social partner's fertile period (Araya-Ajoy *et al.* 2015), encounter rate of extra pair mates and the speed at which breeding attempts are initiated, controlling the rate of extra group parentage within pairs (McCowan *et al.* 2014; Araya-Ajoy *et al.* 2015).

We suggest behavioural incompatibility in our study may encourage engagement in extra group parentage in females because it allows females within a pair to exhibit different reproductive strategies to the male. For example, in great tits (*Parus major*), males paired to dissimilar exploratory females provided less parental effort than males paired with similar females (David *et al.* 2015). Alternatively, behavioural compatibility could be a behavioural mechanism that determines decisions to engage in extra-group parentage (Spoon *et al.* 2007; van Oers *et al.* 2008). Behavioural compatibility within the social pair can influence reproductive success (Dingemanse *et al.* 2004; Spoon *et al.* 2006), and therefore the degree of compatibility may influence a female's decision to obtain an extra group mate and accrue fitness benefits (e.g. indirect genetic benefits), if the extra group mate enhances her reproductive success (Spoon *et al.* 2007). For example, in cockatiels (*Nymphicus hollandicus*), pairs that engaged in EPP had lower behavioural compatibility than pairs that did not (Spoon *et al.* 2007). Although there is no evidence for inbreeding avoidance in the population (Richardson *et al.* 2004; Eikenaar *et al.* 2008), it could be that behavioural compatibility is used as a determinant for engaging in EGP and enhancing reproductive success in another way. To conclude this however, we would need to look at EGP propensity over a lifetime and assess the reproductive success of each individual's social pairing.

Personality is expected to persist in populations if there is balancing selection acting on personality types over time (Dingemanse and Wolf 2010). Although the compatibility of personality within the pair predicted the propensity to have EGO in our study, it did not predict the total number of offspring sired. Thus all pair combinations had equal fitness with respect to the total number of offspring sired. Frequency-dependent selection, a mechanism where the fitness benefits of a reproductive strategy are related to the frequency with which it is expressed, may therefore explain why variation in personality and pair compatibility is maintained (Dingemanse and Wolf 2010; Patrick *et al.* 2011).

We wish to note that the incidence of EPP can be heritable, and this has been shown in female song sparrows (*Melospiza melodia,* h^2 = 0.18; Cr.I. = 0.05-0.31, Reid *et al.* 2010), demonstrating that there can be a genetic constraint on extra group parentage in a mating system (Reid *et al.* 2010). Furthermore, the social partner has the potential to affect the expression of personality and the propensity of extra group parentage in a focal individual, through indirect genetic effects (Niemelä and Santostefano 2015). Future work should therefore investigate whether extra group parentage is a heritable trait and in particular whether there is a heritability component to pair compatibility, and if indirect genetic effects exist.

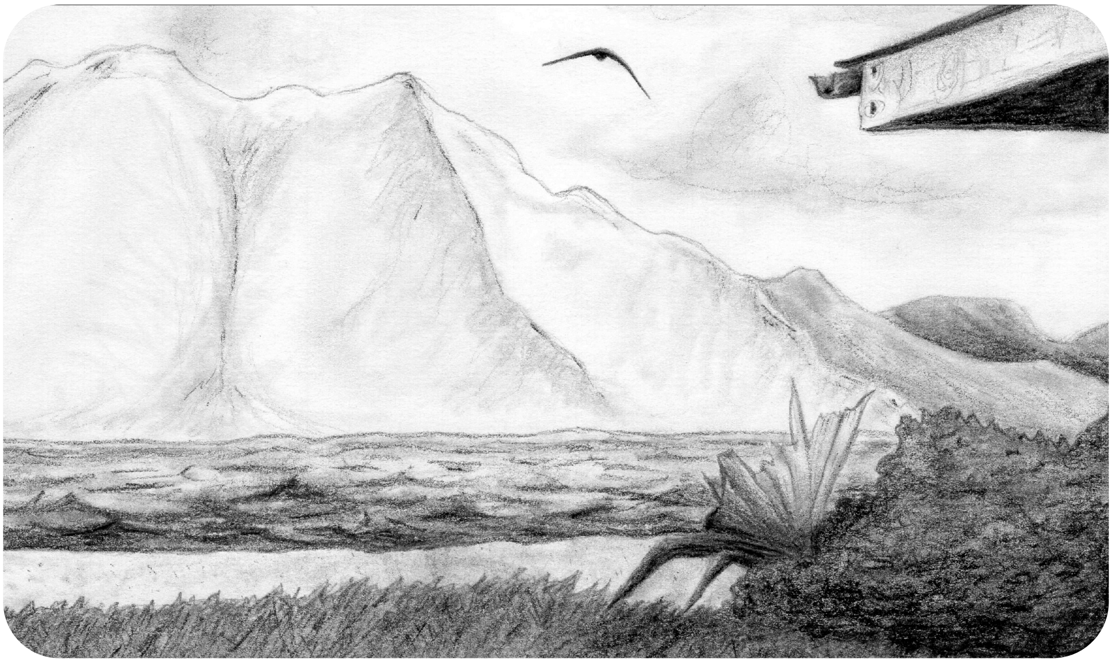
We also found that the likelihood of an individual having extra group parentage increased with an individual's age. It is known that age is an important determinant of parentage in passerines (Griffith *et al.* 2002; Cleasby and Nakagawa 2012) and it has been suggested that females use extra group parentage to gain indirect genetic benefits, if EPP is an adaptive behaviour (Forstmeier *et al.* 2014), from older, better quality males (e.g. Richardson and Burke 1999). However, it is important to note that age could be confounded by the increased ability of older males to display or seek extra group parentage (Griffith *et al.* 2002). In the Seychelles warbler extra group parentage is regulated by mate-guarding (Komdeur *et al.* 2007) and associated with the major histocompatibility complex diversity of the social partner (Richardson *et al.* 2005). It could be that females have extra group parentage to gain indirect genetic benefits from older males causing the observed increased likelihood of extra group parentage in older age.

5.6 Conclusion

To conclude, we have shown that the propensity to engage in extra group parentage for females is associated with pairs that are behaviourally dissasortative for exploration of a novel environment. This provides further evidence that the personality of the social partner can affect the expression of a personality within the pair, and consequently reproductive behaviour. Our results indicate that personality is under correlated selection with reproductive behaviour. We also suggest that behavioural compatibility may be used as a mechanism that can determine decisions to engage in extra group parentage.

Chapter 6

General Discussion



In a range of species individual differences in behaviour are often exhibited, yet it is still unclear how these differences are generated and maintained. The aim of this thesis was to determine if personality is exhibited in a cooperatively breeding system and to then investigate how personality can be maintained and whether personality had fitness consequences. Firstly, I quantified personality by measuring traits over time and estimating their heritability (Chapter 2). Secondly, I investigated how personality may be generated and maintained in the population by investigating candidate genes (Chapter 3), and state-dependent mechanisms (Chapter 4). Finally, I investigated the fitness consequences, specifically the effect on reproductive success and reproductive strategies (Chapter5). In this general discussion chapter, I will discuss the implications of the findings, and suggest future directions for personality research.

6.1 Consistency over time

Differences in behaviour can be consistent among individuals and constrained across contexts (Sih *et al.* 2004*a;* Bell *et al.* 2009). Understanding how selection acts on these consistent individual differences can help to reveal the ecological and evolutionary causes and consequences of personality. In the Seychelles warbler (*Acrocephalus sechellensis*), I found that consistent differences in behaviour among individuals were exhibited during novel environment exploration and novel object exploration (Chapter 2). Novel environment and novel object exploration were positively correlated amongst individuals, suggesting the constraint of an exploratory behavioural syndrome (Chapter 2). Novel environment exploration was also a moderately heritable trait, giving further insight into the variance available for selection (Chapter 2). I also show repeatability and heritability estimates did not differ between the sexes, a difference often overlooked in personality studies. Using the results from this thesis, the following sections will discuss how these consistent individual differences may have been maintained in the population (Chapters 3 & 4), and how they are associated with fitness (Chapter 5).

6.2 Genetic determination

There are a several theories postulating how personality is generated and maintained in a population. One theory proposes that individual behavioural differences could be genetically determined and these individual differences are then maintained because behavioural flexibility is costly or constrained (Dall *et al.* 2004). Although polymorphisms in the personality candidate genes *DRD4* and *SERT* have been associated with personality in wild bird species (Korsten *et al.* 2010; Mueller *et al.* 2013; Garamszegi *et al.* 2014; Mueller *et al.* 2014; Holtmann *et al.* 2015), I found no such association in the Seychelles warbler (Chapter 3). However, I cannot dismiss the possibility of epigenetic effects masking an association. For example, in two populations of great tits, methylation rates of the *SERT* promoter region were higher for fast explorers in an urban rather than a forest environment (Riyahi *et al.* 2015). Since early life conditions can affect methylation rates of *SERT* in humans and monkeys (Wong *et al.* 2010; Ouellet-Morin *et al.* 2013; Kinnally 2014), it was postulated that early life conditions in the urban environment may affect *SERT* methylation rates and in turn adult behaviour (Riyahi *et al.* 2015). A future step would be to test the association between personality and *SERT* methylation rates. Furthermore, a genome wide association study could be conducted to detect putatively adaptive relevant loci (Steinmeyer *et al.* 2009; van Oers and Mueller 2010), or genome partitioning of genetic variation could be used to detect SNPs in relevant genes with small effect sizes (Yang *et al.* 2011).

I did however find that novel environment exploration was moderately heritable suggesting there is some genetic basis for this trait in other genes (Chapter 2). While the documenting of heritability estimates for personality in wild populations is increasing (Duckworth and Kruuk 2009; Blumstein *et al.* 2010; Taylor *et al.* 2012; Korsten *et al.* 2013; Poissant *et al.* 2013; Class *et al.* 2014; Petelle *et al.* 2015), there has been no heritability estimates documented for personality in a wild cooperative breeder. By showing that novel environment exploration is moderately heritable, we can further understand the potential variance available for selection in this wild cooperatively breeding population. It would be of interest to investigate the costs or constraints of flexibility in novel environment exploration. I attempted to estimate genetic correlations between traits but the large credible intervals around the estimates indicated that the sample size was too small (Chapter 1). Continued study of personality will help to reveal the presence of any genetic correlations that may be constraining behaviour.

6.3 State-dependence and behaviour

State-dependency is another theory that proposes how personality is generated and maintained in a population (Dall *et al.* 2004). States are defined as the features of an individual (such as energy reserves or reproductive potential) or the circumstances that alter the costs and benefits of a behavioural trait. It is predicted that individuals with low future reproductive states will be consistently fast exploring and risk taking compared to those with high future reproductive states, and encourage consistent individual differences in behaviour (Dall *et al.* 2004; Wolf *et al.* 2007; Biro and Stamps 2008; Dingemanse and Wolf 2013). States can change slowly, but if associated with a positive feedback mechanism, can encourage long-term stability in behaviour (Luttbeg and Sih 2010). In support of the prediction, empirical studies have found that personality can be social state–dependent (Verbeek *et al.* 1994; Dingemanse and de Goede 2004; David *et al.* 2011; Favati *et al.* 2014) and reproductive state–dependent (Dammhahn 2012; Nicolaus *et al.* 2012).

I found novel environment and novel object exploration were not social state–dependent. Novel object exploration was instead associated with the interaction between insect abundance at year of birth and age (a proxy for reproductive state, Chapter 4, Hammers *et al.* 2013), where fast novel object exploration was correlated with young individuals born into years of high food abundance. I therefore conclude that reproductive state could be a mechanism that encourages and maintains individual differences in this trait. I further postulate that young individuals born into good insect abundance years (therefore having low future reproductive states) may ensure the success of current reproductive attempt by exhibiting risky behaviour, such as territory/nest defence. This builds upon the few empirical studies that have tested for the prediction that state can encourage individual differences in behaviour. Long-term study in this system will allow for the opportunity to investigate the ecological function of novel object exploration, for example, resource holding potential, and the direct test of the association between survival and personality in this long-lived passerine.

6.4 Fitness consequences

Personality is associated with fitness, and the fitness consequences are often context–dependent. For example, personality can be subject to fluctuations in food availability (Dingemanse *et al.* 2004) and the presence of predators (Réale and Festa-Bianchet 2003). Across different species, survival rates for bold and fast exploring individuals were low, while bold and aggressive individuals had a high reproductive success (a combination of annual and lifetime success, Smith and Blumstein 2008). Personality can also explain individual variation in reproductive behaviour (Duckworth 2006; van Oers *et al.* 2008; While *et al.* 2009; Patrick *et al.* 2011; Martin *et al.* 2014; McCowan *et al.* 2014). Studies investigating the association between personality and extra group parentage have found that fast exploring individuals exhibited high rates of extra pair paternity (van Oers *et al.* 2008; While *et al.* 2009; Patrick *et al.* 2011; Martin *et al.* 2014). The social partner can also affect the expression of personality in a focal individual through indirect genetic effects, particularly if the social partner generates permanent environmental effects (Niemelä and Santostefano 2015). Studies have shown that extra group parentage within the social pair is affected by the personality of the female, and the behavioural compatibility of the pair (van Oers *et al.* 2008; Patrick *et al.* 2011).

I found that novel environment and novel object exploration were not associated with the number of offspring an individual was assigned parentage to, and thus the long-term fitness consequences of these traits were equal. However, I did find that the propensity to engage in extra group sexual behaviour in females was higher in disassortative behavioural pairs (Chapter 5). My conclusion is that extra group paternity does not depend solely on an individual's personality, but also that of the social partner. This gives further support to the few empirical studies that have found that the social partner can influence extra group parentage within the social pair. I propose that behavioural compatibility may be a mechanism used to assess the fitness prospects of a partner and thus determines whether individuals engage in promiscuous behaviour (Spoon *et al.* 2007). The next step should be to investigate the effects of indirect genetic effects and the possibility of extra group parentage being a heritable trait.

6.5 Future work

Exploratory behaviour has been associated with risk taking behaviour in other wild bird species (e.g. Quinn *et al.* 2012), and thus exploratory behaviour has been used as a proxy for risk-taking in this thesis. It would be beneficial in the future to test this prediction in the Seychelles warbler. Exploratory behaviour has been associated with non-consumptive effects of predation i.e. changes in morphology and behaviour in relation to perceived predation risk (Abbey-Lee *et al.* 2015). Therefore, playbacks of predator calls or mounted predator decoys could be used to experimentally manipulate the perception of predation risk of exploratory tested individuals (Veen *et al.* 2000) to test if there is an association between the two behaviours.

More broadly, there are still gaps in our knowledge about the ecological and evolutionary significance of personality. With long-term study of personality in this population these gaps could be addressed. Personality could influence population carrying capacity and stability, and be of importance to species of conservation concern such as the Seychelles warbler. Differences in habitat use (e.g. Bonnot *et al.* 2014) and foraging (e.g. Wright *et al.* 2015) are associated with different behavioural types. These differences reduce competition of these limited resources and encourage resource specialisation, optimising resource use. Behavioural variation in a population may also buffer responses to environmental changes through portfolio effects, buffer changes in the frequency of behavioural types through averaging effects, and contain behavioural types able to cope with environmental change (also known as insurance effects, Wolf and Weissing 2012). Although logistically difficult, it would be of great interest to compare the effects of personality on population dynamics and stability in the main study population and in one of the translocated populations.

Personality could be one characteristic that influences dispersal and settlement success (Wolf and Weissing 2012). The tendency for an individual to disperse is not random and often associated with sex and the potential for successful dispersal and settlement (Eikenaar *et al.* 2007; Eikenaar *et al.* 2008). Exploratory behaviour could therefore influence the collection of information and the propensity to take risks, affecting dispersal decisions (Debeffe *et al.* 2013; Debeffe *et al.* 2014). Although dispersal is sex biased in the Seychelles warbler, with females dispersing further than males (Eikenaar *et al.* 2008), personality variation may affect dispersal and settlement success. Personality may also affect where individuals disperse to and in turn the distribution of individuals within a habitat (Wolf and Weissing 2012).

In chapter four, I looked at the state-dependency of personality, in particular social state and asset protection, i.e. predicting that fast exploring/risk taking is associated with low future reproductive state. However, with further long-term study we could address the association between personality and senescence (the reduction in survival and reproductive performance in old age). Although more study is needed, in the wandering albatross (*Diomedea exulans*), there was a sex-specific association between boldness and reproductive performance in late adulthood (Patrick and Weimerskirch 2014). Current telomere work in the Seychelles warbler could provide the opportunity to look at the association between lifespan (correlated with telomere length) and personality, to further study this relationship.

6.6 Conclusion

Personality is important because it can limit behavioural plasticity and encourage non-optimal behaviour, thereby having major effects on fitness, adaptability and the distribution of individuals within a habitat (Sih *et al.* 2004*a*). In this dissertation, I have quantified personality in a wild cooperative breeder, examined potential mechanisms that may have generated these behavioural differences and examined the fitness consequences of these behavioural differences. In summary, novel environment exploration was genetically determined and influenced extra group parentage within the social pair, and novel object exploration was predicted by the future fitness potential of an individual. Further controlled, longitudinal study of personality in this study system would enable changes over development and over varying contexts to be captured, allowing for a greater understanding of the causes and consequences of personality.

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Supplementary materials

Chapter 2: Repeatable and heritable behavioural variation in a wild cooperative breeder

Table S2.1: The number of birds with single or repeat samples for each personality assay.

	Total birds assayed	Number of birds with the following samples						
Assay		**1**	**2**	**3**	**4**	**5**	**6**	**7**
Obstinacy	300	153	100	31	7	7	1	1
Stress	200	129	55	14	1	1	0	0
Novel environment exploration	312	175	96	25	8	5	3	0
Novel object exploration	177	120	52	4	1	0	0	0
Escape	300	165	90	29	7	8	0	1

Table S2.2: Breakpoint analysis. A linear mixed model (LMM) was run in lme4 1.1-5 (Bates *et al.* 2014) with exploration score as the dependant variable, minute and the breakpoint as fixed effects and bird identity as a random effect. The model had random slope variances and a random intercept variance for a break point at minute 10. The R function optimize was used to estimate the breakpoint.

Estimated break point (minutes)	**Upper confidence interval**	**Lower confidence interval**
6.32	1.28	1.21

Table S2.3: Repeatability = V_I/ V_P, between-individual variance (V_I), observer effect ($obs^2 = V_\mathrm{Observer} / V_\mathrm{P}$), residual effect ($res^2 = V_\mathrm{Residual} / V_\mathrm{P}$), and total phenotypic variance, V_P, for each personality trait in the sex specific model. Credible intervals are in brackets.

Personality trait		R	V_I	obs^2	res^2	V_P
Obstinacy	Female:	1.3e-3 (2.7e-8–0.09)	0.01 (1.3e-7–0.78)	0.09 (7.15e-7–0.35)	0.60 (0.41–0.83)	4.59 (3.22–7.17)
	Male:	0.17 (6.5e-7–0.31)	0.57 (4.2e-6–1.87)	0.03 (2.11e-6–0.25)	0.57 (0.35–0.79)	4.42 (3.12–6.43)
	Together:	0.05 (3.0e-3–0.20)	0.86 (0.03–2.19)	0.11 (0.01–0.30)	0.70 (0.48–0.84)	9.37 (7.14–12.79)
Stress response	Female:	1.4e-3 (3.9e-7–0.26)	0.45 (1.2e-4–77.41)	0.33 (0.10–0.81)	0.59 (0.13–0.82)	340.98 (231.70–1164.06)
	Male:	1.1e-3 (2.69e-8 0.21)	0.35 (7.84e-6–61.70)	0.25 (0.01–0.67)	0.75 (0.25–0.94)	339.36 (187.23–606.75)
	Together:	0.02 (2.1e-4–0.19)	39.47 (0.07–136.96)	0.38 (0.11–0.75)	0.51 (0.19–0.80)	621.49 (452.75–1634.23)
Novel environment exploration	Female:	0.22 (6.6e-3–0.36)	0.25 (5.4e-6–0.78)	0.09 (5.0e-3–0.38)	0.53 (0.32–0.79)	1.89 (1.34–2.77)
	Male:	0.29 (0.10–0.49)	0.40 (0.11–0.64)	6.7e-4(3.9e-7–0.15)	0.52 (0.33–0.69)	1.07 (0.84–1.37)
	Together:	0.25 (0.11–0.39)	0.70 (0.30–1.28)	0.08 (0.01–0.31)	0.56 (0.38–0.74)	2.83 (2.38–3.93)
Novel object exploration	Female:	0.45 (0.03–0.73)	1.66 (0.37–3.12)	0.01 (1.5e-7–0.75)	0.19 (0.02–0.57)	2.85 (1.94–6.22)
	Male:	0.02 (7.4e-6–0.42)	0.03 (2.9e-5–0.72)	2.3e-3 (1.6e-7–0.67)	0.72 (0.22–0.96)	1.42 (0.90–4.41)

	Together:	0.38 (0.05–0.59)	1.85 (0.69–2.54)	0.02 (7.8e-5–0.73)	0.42 (0.07–0.67)	4.78 (3.13–10.67)
Escape response	Female:	2.3 e-3 (5.0e-9–0.41)	0.04 (7.7e-8–6.88)	0.39 (0.07–0.74)	0.41 (0.09–0.76)	12.66 (8.56–36.74)
	Male:	1.1e-3 (2.3e-9–0.17)	0.01 (2.8e-8 – 1.72)	0.01 (7.0e-7–0.25)	0.91 (0.63–0.99)	9.01 (5.38–14.81)
	Together:	0.06 (1.1e-4–0.28)	1.52 (2.1e-4–7.62)	0.31 (0.04–0.59)	0.57 (0.29–0.86)	26.93 (16.26–48.38)

Table S2.4: Testing the significance of the differences between the sexes for the repeatability estimate = V_I/ V_P, between-individual variance (V_i), observer effect (obs^2 = $V_{Observer}$ / V_P) and residual effect (res^2 = $V_{Residual}$ / V_P). Calculated by subtracting the posterior distribution of the male trait from the female trait. * indicates posterior modes whose 95% credible intervals do not overlap zero.

Personality trait	***R***	V_I	obs^2	res^2
Obstinacy	0.12 (-0.06–0.31)	0.57 (-0.43–1.88)	-0.04 (-0.33–0.11)	-0.05 (-0.29–0.19)
Stress response	-0.01 (-0.23–0.23)	-1.46 (-55.34–60.96)	-0.17 (-0.59–0.31)	0.09 (-0.33–0.58)
Novel environment exploration	0.11 (-0.15–0.37)	0.02 (-0.55–0.45)	-0.09 (-0.34–0.08)	-0.08 (-0.32–0.25)
Novel object exploration	-0.36 (-0.71–0.12)	-1.19 (-2.98– -2e-4)	0.29 (-0.15–0.74)	-1.1e-3 (-0.77–0.49)
Escape response	4.1e-3 (-0.41–0.15)	0.01 (-6.99–1.38)	-0.42 (-0.75– -0.03)	0.42 (-0.01–0.8)

Table S2.5: The heritability of behaviour ($h^2_B = V_A / V_p$), heritability of personality ($h^2_P = V_A / V_A + V_{PE} + V_M$), permanent environment effect ($pe^2 = V_{PE} / V_p$), maternal effect ($m^2 = V_M / V_P$), observer effect ($obs^2 = V_{Observer} / V_P$), residual effect ($res^2 = V_{Residual} / V_P$), additive genetic variance (V_A), and total phenotypic variance (V_p), for each moderately repeatable personality trait in the sex specific model. Credible intervals are in brackets.

Personality trait		h^2_B	h^2_P	pe^2	m^2	obs^2	res^2	V_A	V_P
Novel environment exploration	Female:	0.02 (2.3e-8–0.29)	3.5e -3 (1.1e-7–0.76)	1.6e-3 (3.3e-8–0.26)	8.4e-3 (7.0e-8–0.13)	0.10 (1.7e-4–0.34)	0.54 (0.32–0.73)	3.5e-3 (4.5e-8–0.64)	1.78 (1.39–2.72)
	Male:	1.0e-3 (5.9e-7–0.36)	3.1e-3 (1.5e-6–0.71)	1.7e-3 (2.1e-9–0.29)	1.4e-3 (2.4e-7–0.31)	7.5e-4 (2.3e-7–0.15)	0.53 (0.32–0.65)	2.3e-3 (7.8e-7– 0.48)	1.09 (0.86–1.44)
	Together:	0.12 (1.2e-4–0.28)	0.55 (4.5e-4–0.72)	0.02 (2.9e-4– 0.22)	0.02 (2.1e-5–0.16)	0.04 (1.6e-3–0.27)	0.55 (0.39–0.69)	0.29 (1.5e-3– 0.92)	3.02 (2.41–3.95)
Novel object exploration	Female:	2.8e-3 (3.6e-6–0.49)	3.9e-3 (9.1e-7–0.84)	2.7e-3 (2.9e-6– 0.63)	8.4e-4 (3.6e-4–0.20)	5.2e-4 (3.6e-6–0.75)	0.22 (0.01–0.52)	0.01 (1.69e-6–1.98)	3.24 (1.83–13.14)
	Male:	1.5e-3 (5.7e-5–0.28)	3.5e-3 (3.3e-7–0.72)	1.4e-3 (1.69e-4– 0.30)	1.5e-3 (5.4e-5–0.28)	1.6e-3 (2.4e-7–0.61)	0.52 (0.17–0.86)	2.4e-3 (1.32e-7–0.51)	1.53 (0.95–3.86)
	Together:	0.01 (1.4e-4–0.37)	0.06 (3.3e-4–0.74)	0.08 (1.3e-3– 0.48)	5.7e-3 (6.2e-5–0.18)	0.04 (4.8e-5–0.72)	0.35 (0.08–0.60)	0.04 (8.5e-3 –2.18)	4.88 (3.25–16.51)

Table S2.6: Testing the significance of the differences between the sexes for the heritability of behaviour ($h^2_B = V_A / V_p$), heritability of personality ($h^2_P = V_A / V_A + V_{PE} + V_M$), permanent environment effect ($pe^2 = V_{PE} / V_p$), maternal effect ($m^2 = V_M / V_P$), observer effect ($obs^2 = V_{Observer} / V_P$) and residual effect ($res^2 = V_{Residual} / V_P$). Calculated by subtracting the posterior distribution of the male trait from the female trait. * indicates posterior modes whose 95% credible intervals do not overlap zero.

Personality trait	h^2_B	h^2_P	pe^2	m^2	obs^2	res^2
Novel environment exploration	-1.4e-3 (-0.23–0.34)	-1.0e-3 (-0.59–0.59)	1.6e-3 (-0.23–0.28)	-1.5e-3 (-0.14–0.29)	-0.06 (-0.33–0.07)	-0.01 (-0.30–0.23)
Novel object exploration	-1.9e-3 (-0.48–0.26)	1.4e-3 (-0.74–0.64)	2.6e-3 (-0.63–0.19)	7.3e-4 (-0.22–0.30)	1.3e-3 (-0.79–0.52)	0.26 (-0.19–0.64)

Table S2.7: The between sex phenotypic correlation between the moderately repeatable personality traits. Credible intervals are in brackets.

Personality trait	**Correlation coefficient**
Novel environment exploration and novel object exploration	Female: 0.53 (0.36–0.88) Male: 0.63 (-0.07–0.89)

Table S2.8: The between sex genetic correlation between the moderately repeatable personality traits. Credible intervals are in brackets.

Personality trait	**Correlation coefficient**
Novel environment exploration and novel object exploration	Female: 0.42 (-0.76–0.95) Male: 0.81 (-0.75–0.96)

Figure S2.1: Frequency distribution for the obstinacy assay (n = 300).

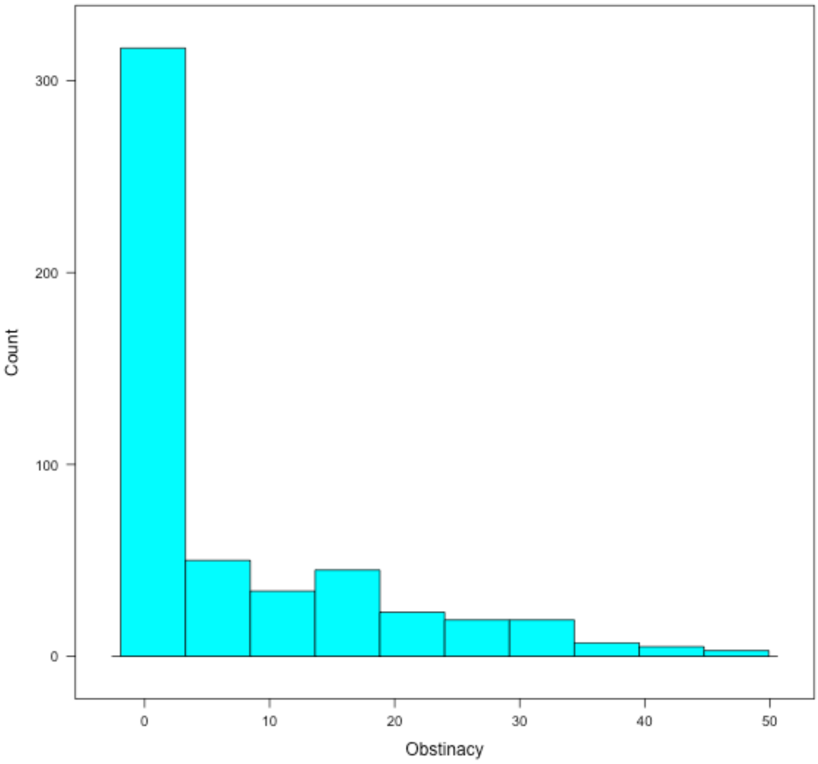


Figure S2.2: Frequency distribution for the stress response assay (n = 200).

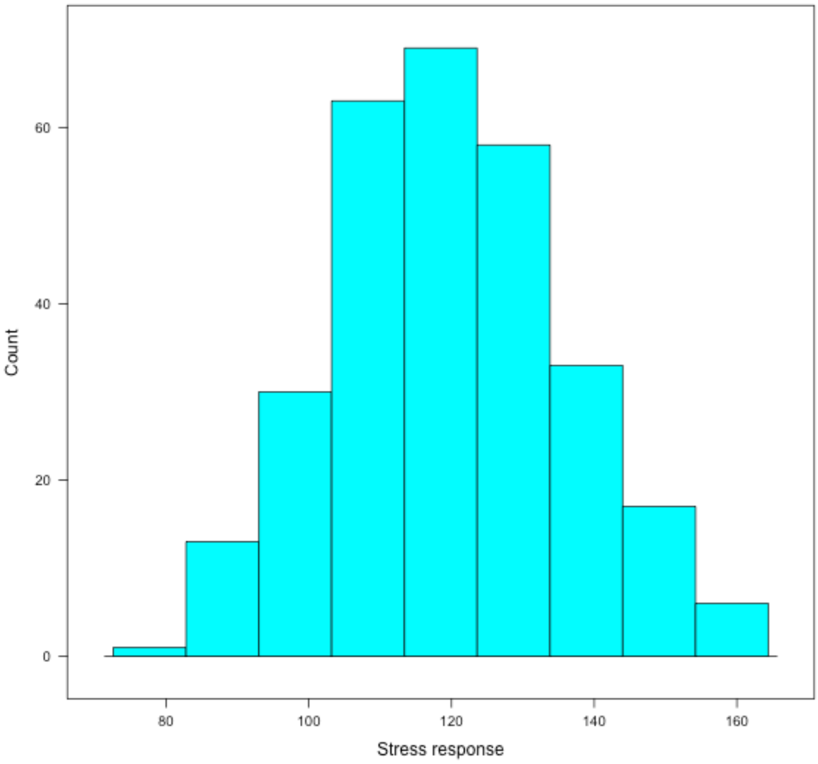


Figure S2.3: Frequency distribution for the novel environment assay (n = 312).

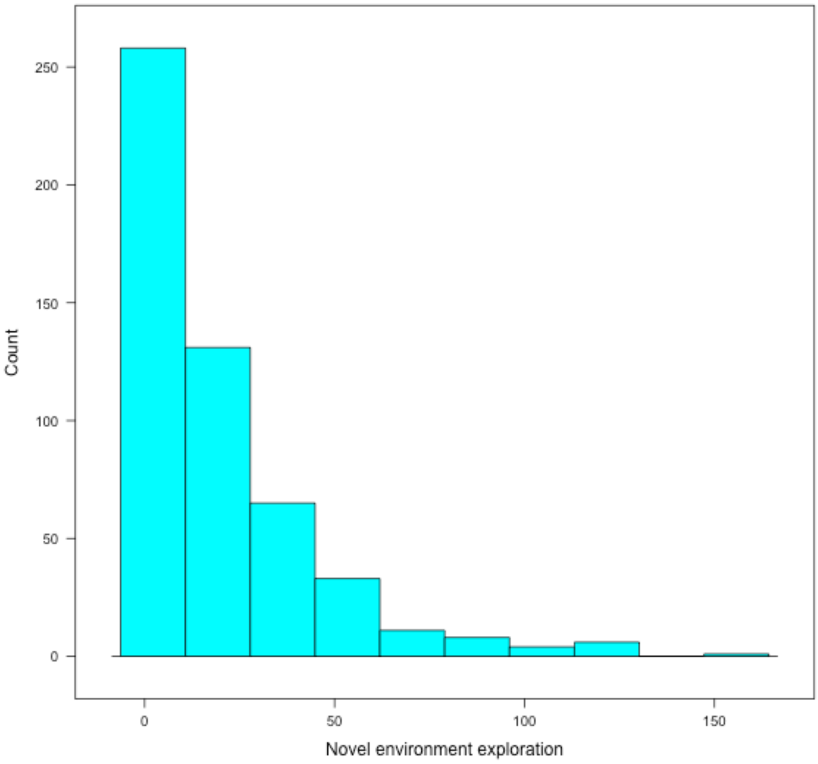


Figure S2.4: Frequency distribution for the novel object assay (n = 177).

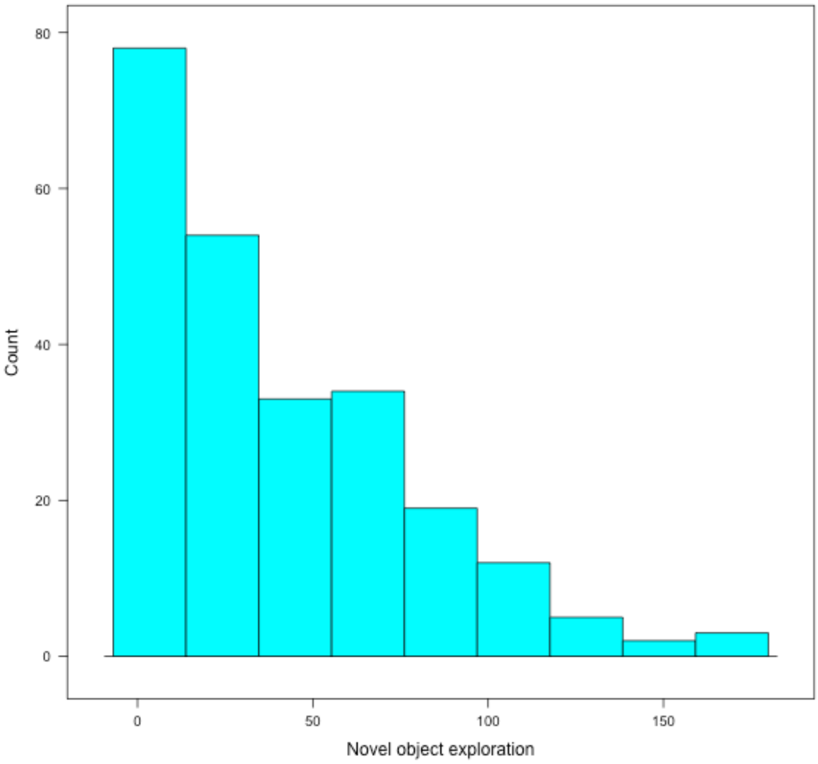


Figure S2.5: Frequency distribution for the escape response assay (n = 300).

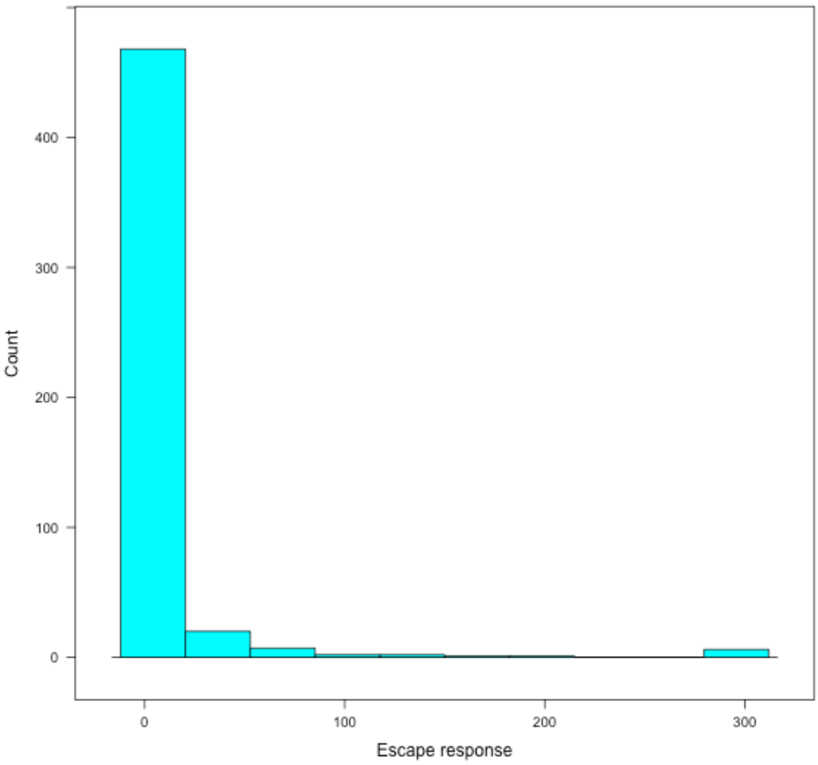


Figure S2.6: Estimates of the posterior mode distributions of the fixed effects in the obstinacy model: year* (2012 = 200, 2013 = 200, 2014 = 57; contrast level = 2010), mass (mean centred), interval (days between assay), assay number*, age (quadratic and linear terms), sex (male = 149, female = 151; contrast = female), social status (dominant = 294, subordinate = 228; contrast = dominant), weather* (partly cloudy = 118, cloudy = 124, rain = 7, sun = 265; contrast = sunset) and season (number of days from the first of January or June). * indicates posterior modes whose 95% credible intervals do not overlap zero.

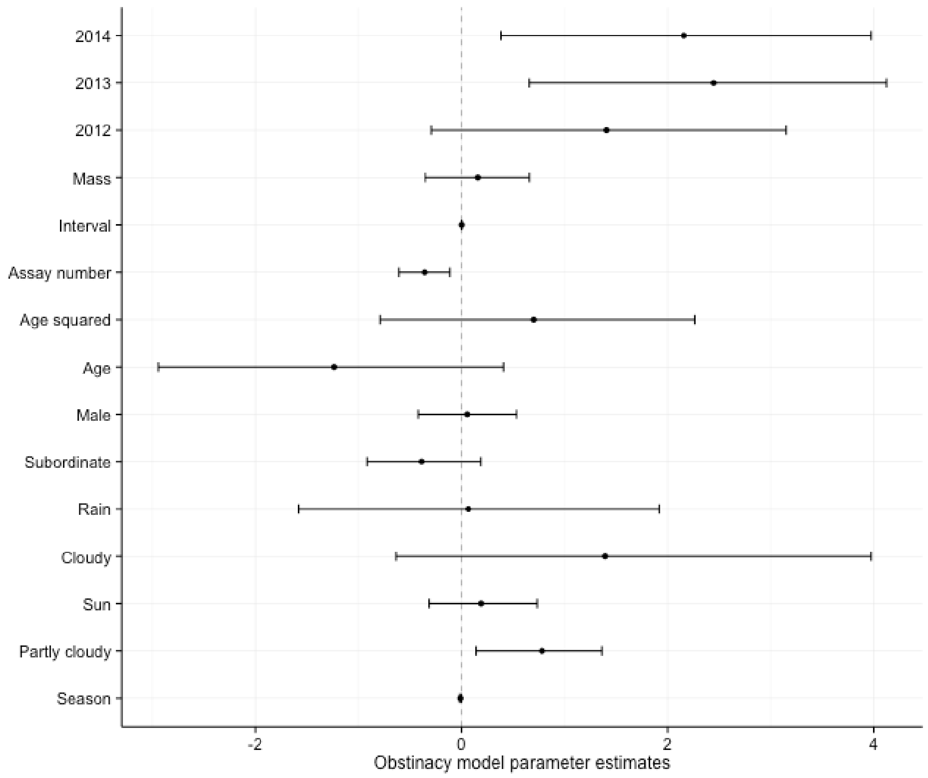


Figure S2.7: Estimates of the posterior mode distributions of the fixed effects in the stress response model: year* (2014 = 51, 2015 = 35; contrast = 2013), mass (mean centred), interval (days between assay), assay number, age (quadratic and linear terms), sex (male = 106, female = 94; contrast = female), social status (dominant = 163, subordinate = 127; contrast = dominant), weather (partly cloudy = 75, sun = 117, sunset = 1; contrast = cloudy) and season (number of days from the first of January or June). * indicates posterior modes whose 95% credible intervals do not overlap zero.

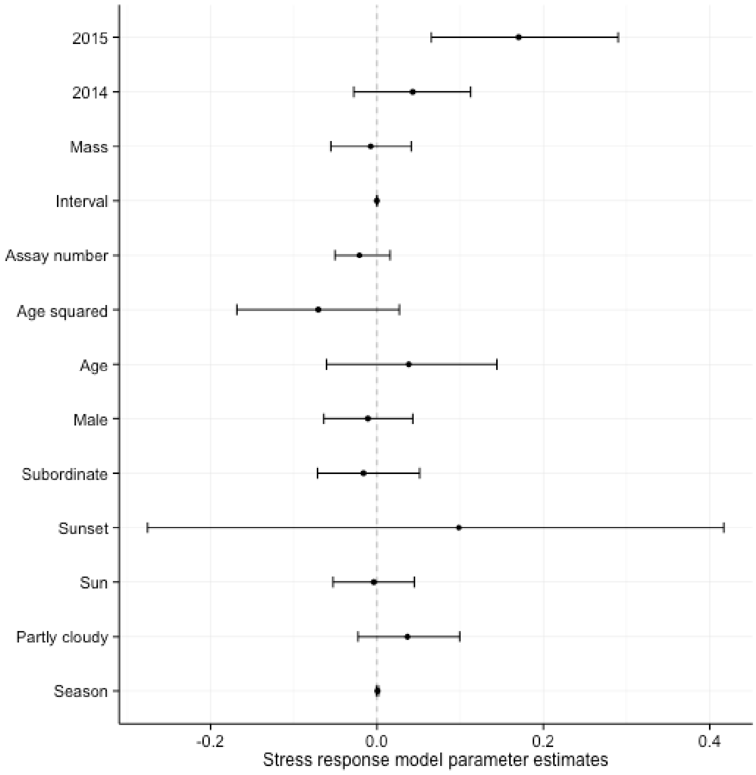
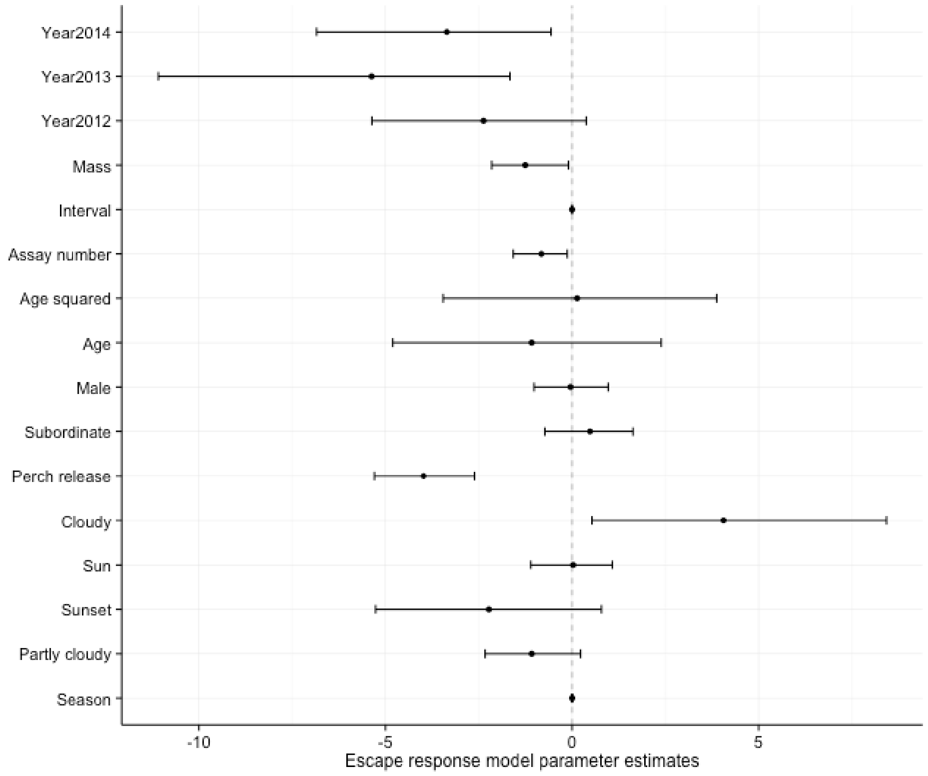


Figure S2.8: Estimates of the posterior mode distributions of the fixed effects in the escape response model: year* (2012 = 195, 2013 = 195, 2014 = 54; contrast = 2010), mass* (mean centred), interval (days between assay), assay number*, age (quadratic and linear terms), sex (male = 149, female = 151; contrast = female), social status (dominant = 284, subordinate = 223; contrast = dominant), method (hand = 259, perch = 248; contrast = hand), weather* (partly cloudy = 111, cloudy = 111, sun = 244, sunset = 8; contrast = rain) and season (number of days from the first of January or June). * indicates posterior modes whose 95% credible intervals do not overlap zero.

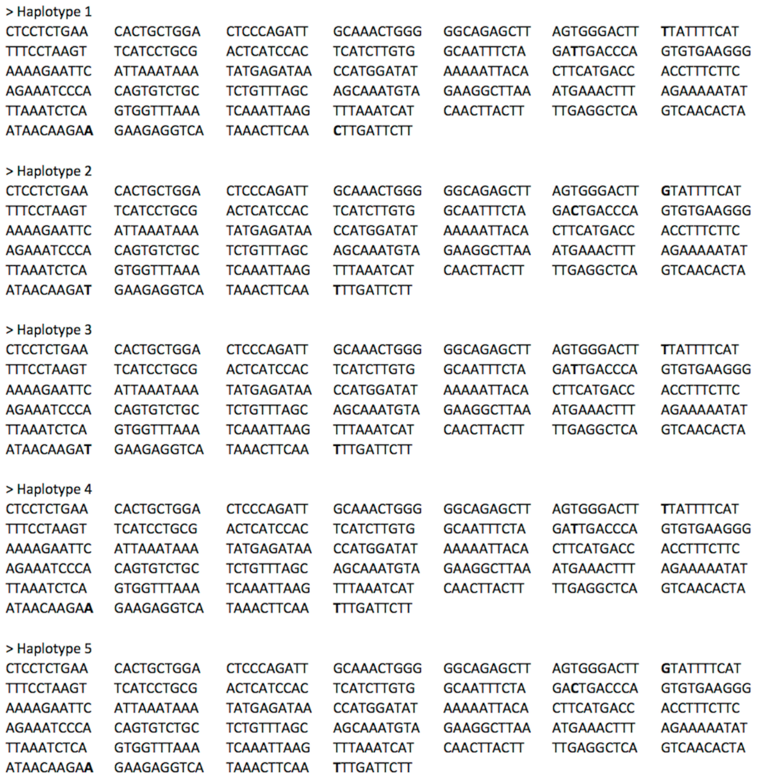


Chapter 3: No association between personality and candidate gene polymorphisms in a wild bird population

Table S3.1: Power analysis using R package pwr 1.1-2 (Cohen 1988) and the effect size from the Westerheide population (Korsten *et al.* 2010). U is the degrees of freedom in the numerator. The sample size (N) is calculated by adding the degrees of freedom in the denominator, U and one.

Model	**Effect size (V statistic= $\sqrt{(\chi^2/n)}$)**	**U**	**Significance level**	**Power of test**	**V (denominator degrees of freedom)**
Additive	0.24	3	0.05	0.8	49
Dominant	0.21	3	0.05	0.8	57

Table S3.2: The sequences of the five *SERT* haplotypes. Bold nucleotides indicate the SNPs.



\> Haplotype 1
CTCCTCTGAA CACTGCTGGA CTCCCAGATT GCAAACTGGG GGCAGAGCTT AGTGGGACTT **T**TATTTTCAT
TTTCCTAAGT TCATCCTGCG ACTCATCCAC TCATCTTGTG GCAATTTCTA GA**T**TGACCCA GTGTGAAGGG
AAAAGAATTC ATTAAATAAA TATGAGATAA CCATGGATAT AAAAATTACA CTTCATGACC ACCTTTCTTC
AGAAATCCCA CAGTGTCTGC TCTGTTTAGC AGCAAATGTA GAAGGCTTAA ATGAAACTTT AGAAAAATAT
TTAAATCTCA GTGGTTTAAA TCAAATTAAG TTTAAATCAT CAACTTACTT TTGAGGCTCA GTCAACACTA
ATAACAAGA**A** GAAGAGGTCA TAAACTTCAA **C**TTGATTCTT

\> Haplotype 2
CTCCTCTGAA CACTGCTGGA CTCCCAGATT GCAAACTGGG GGCAGAGCTT AGTGGGACTT **G**TATTTTCAT
TTTCCTAAGT TCATCCTGCG ACTCATCCAC TCATCTTGTG GCAATTTCTA GA**C**TGACCCA GTGTGAAGGG
AAAAGAATTC ATTAAATAAA TATGAGATAA CCATGGATAT AAAAATTACA CTTCATGACC ACCTTTCTTC
AGAAATCCCA CAGTGTCTGC TCTGTTTAGC AGCAAATGTA GAAGGCTTAA ATGAAACTTT AGAAAAATAT
TTAAATCTCA GTGGTTTAAA TCAAATTAAG TTTAAATCAT CAACTTACTT TTGAGGCTCA GTCAACACTA
ATAACAAGA**T** GAAGAGGTCA TAAACTTCAA **T**TTGATTCTT

\> Haplotype 3
CTCCTCTGAA CACTGCTGGA CTCCCAGATT GCAAACTGGG GGCAGAGCTT AGTGGGACTT **T**TATTTTCAT
TTTCCTAAGT TCATCCTGCG ACTCATCCAC TCATCTTGTG GCAATTTCTA GA**T**TGACCCA GTGTGAAGGG
AAAAGAATTC ATTAAATAAA TATGAGATAA CCATGGATAT AAAAATTACA CTTCATGACC ACCTTTCTTC
AGAAATCCCA CAGTGTCTGC TCTGTTTAGC AGCAAATGTA GAAGGCTTAA ATGAAACTTT AGAAAAATAT
TTAAATCTCA GTGGTTTAAA TCAAATTAAG TTTAAATCAT CAACTTACTT TTGAGGCTCA GTCAACACTA
ATAACAAGA**T** GAAGAGGTCA TAAACTTCAA **T**TTGATTCTT

\> Haplotype 4
CTCCTCTGAA CACTGCTGGA CTCCCAGATT GCAAACTGGG GGCAGAGCTT AGTGGGACTT **T**TATTTTCAT
TTTCCTAAGT TCATCCTGCG ACTCATCCAC TCATCTTGTG GCAATTTCTA GA**T**TGACCCA GTGTGAAGGG
AAAAGAATTC ATTAAATAAA TATGAGATAA CCATGGATAT AAAAATTACA CTTCATGACC ACCTTTCTTC
AGAAATCCCA CAGTGTCTGC TCTGTTTAGC AGCAAATGTA GAAGGCTTAA ATGAAACTTT AGAAAAATAT
TTAAATCTCA GTGGTTTAAA TCAAATTAAG TTTAAATCAT CAACTTACTT TTGAGGCTCA GTCAACACTA
ATAACAAGA**A** GAAGAGGTCA TAAACTTCAA **T**TTGATTCTT

\> Haplotype 5
CTCCTCTGAA CACTGCTGGA CTCCCAGATT GCAAACTGGG GGCAGAGCTT AGTGGGACTT **G**TATTTTCAT
TTTCCTAAGT TCATCCTGCG ACTCATCCAC TCATCTTGTG GCAATTTCTA GA**C**TGACCCA GTGTGAAGGG
AAAAGAATTC ATTAAATAAA TATGAGATAA CCATGGATAT AAAAATTACA CTTCATGACC ACCTTTCTTC
AGAAATCCCA CAGTGTCTGC TCTGTTTAGC AGCAAATGTA GAAGGCTTAA ATGAAACTTT AGAAAAATAT
TTAAATCTCA GTGGTTTAAA TCAAATTAAG TTTAAATCAT CAACTTACTT TTGAGGCTCA GTCAACACTA
ATAACAAGA**A** GAAGAGGTCA TAAACTTCAA **T**TTGATTCTT

Table S3.3: Number of Seychelles warblers with repeat assays for novel environment exploration and novel object exploration.

Assay number	**Novel environment exploration**	**Novel object exploration**
1	45	17
2	22	11
3	10	1
4	5	1
5	2	0
6	1	0

Table S3.4: Nucleotide change and p-values of Haldane's exact test for each *SERT* SNP***.***

Locus	**Major/Minor Allele**	**Weir's disequilibrium coefficient (D)**	**P value**
SNP147	T/G	0.14	1
SNP209	T/C	0.14	1
SNP446	T/A	-2.95	0.18
SNP467	T/C	2.56	0.33

Table S3.5: Likelihood ratio test results for novel environment exploration in the overdominant and additive models using SNPs and haplotypes. False Discovery Rate (FDR) p values control for running four SNP models and five haplotype models with alpha set at 0.05. d.f.=degrees of freedom.

Novel environment exploration model	**χ^2**	**d.f**	**P value**	**FDR P value**
Over-dominant 147	5.35	2	0.08	0.16
Over-dominant 209	5.35	2	0.08	0.16
Over-dominant 446	0.88	2	0.64	0.72
Over-dominant 467	0.75	2	0.72	0.72
Additive 147	3.86	1	0.06	0.12
Additive 209	3.86	1	0.06	0.12
Additive 446	0.81	1	0.37	0.49
Additive 467	0.03	1	0.93	0.93
Over-dominant Haplotype 1	1.04	2	0.61	0.67
Over-dominant Haplotype 2	4.46	2	0.13	0.33
Over-dominant Haplotype 3	0.87	2	0.67	0.67
Over-dominant Haplotype 4	4.78	2	0.1	0.33
Over-dominant Haplotype 5	0.83	1	0.31	0.52
Additive Haplotype 1	0.06	1	0.88	0.88
Additive Haplotype 2	3.93	1	0.06	0.3
Additive Haplotype 3	0.86	1	0.38	0.55
Additive Haplotype 4	0.73	1	0.44	0.55
Additive Haplotype 5	0.83	1	0.31	0.55

Table S3.6: Likelihood ratio test results for novel object exploration in the overdominant and additive models using SNPs and haplotypes. False Discovery Rate (FDR) p values control for running four SNP models and four haplotype models with alpha set at 0.05. d.f.=degrees of freedom.

Novel object exploration model	**χ^2**	**d.f**	**P value**	**FDR P value**
Over-dominant 147	0.59	1	0.44	0.59
Over-dominant 209	0.59	1	0.44	0.59
Over-dominant 446	0.66	2	0.72	0.72
Over-dominant 467	4.5	2	0.11	0.44
Additive 147	0.59	1	0.44	0.69
Additive 209	0.59	1	0.44	0.69
Additive 446	0.16	1	0.69	0.69
Additive 467	0.18	1	0.67	0.69
Over-dominant Haplotype 1	4.5	2	0.11	0.44
Over-dominant Haplotype 2	0.59	1	0.44	0.67
Over-dominant Haplotype 3	0.62	2	0.74	0.74
Over-dominant Haplotype 4	0.46	1	0.5	0.67
Additive Haplotype 1	0.18	1	0.67	0.67
Additive Haplotype 2	0.59	1	0.44	0.67
Additive Haplotype 3	0.4	1	0.53	0.67
Additive Haplotype 4	0.46	1	0.5	0.67

Figure S3.1: Neighbour-joining phylogenetic tree of avian *SERT* chromosome 19 sequences constructed in Mega 5.2 (Tamura *et al.* 2011). Numbers at branching points represent bootstrap values inferred from 5000 replicates. The horizontal scale bar indicates 0.1 nucleotide substitutions per site.

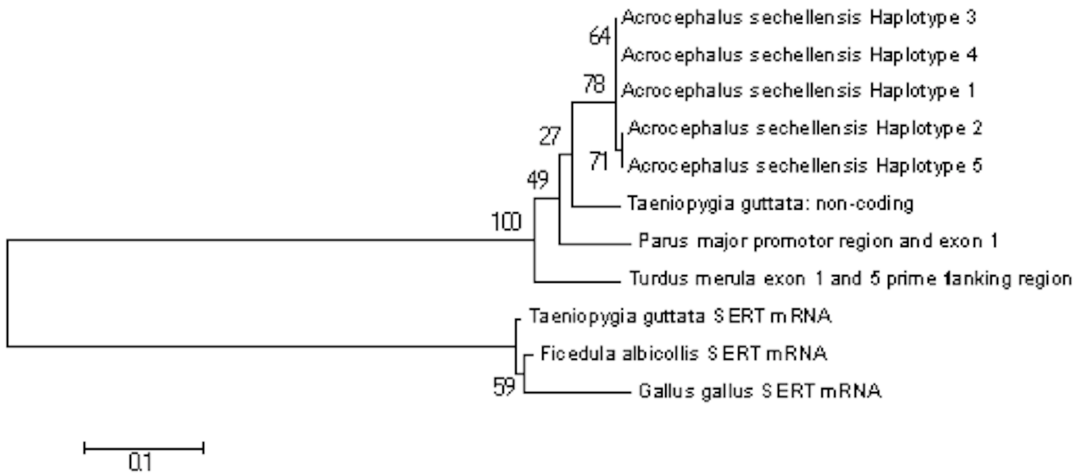


Figure S3.2: The coefficients and associated 95% confidence intervals (CI) of the overdominant SNP models for novel environment exploration. The models are relative to homozygotes with the G/C/A SNP, subordinate is relative to dominant, male is relative to female.

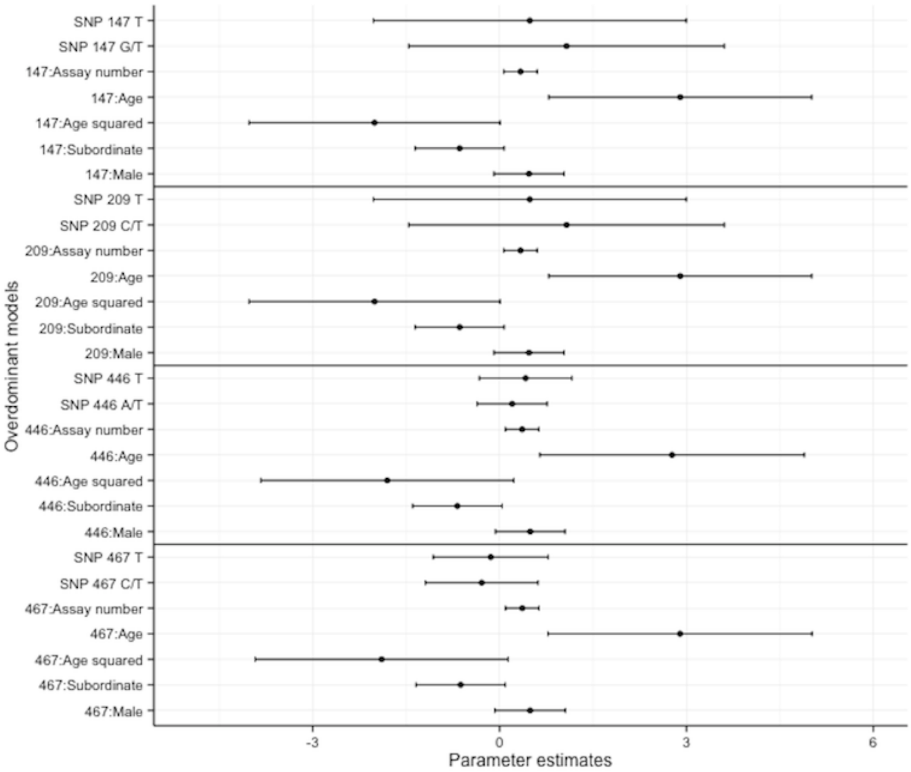


Figure S3.3: The coefficients and associated 95% confidence intervals (CI) of the overdominant SNP models for novel object exploration. The models are relative to homozygotes with the C/A SNP, subordinate is relative to dominant, male is relative to female.

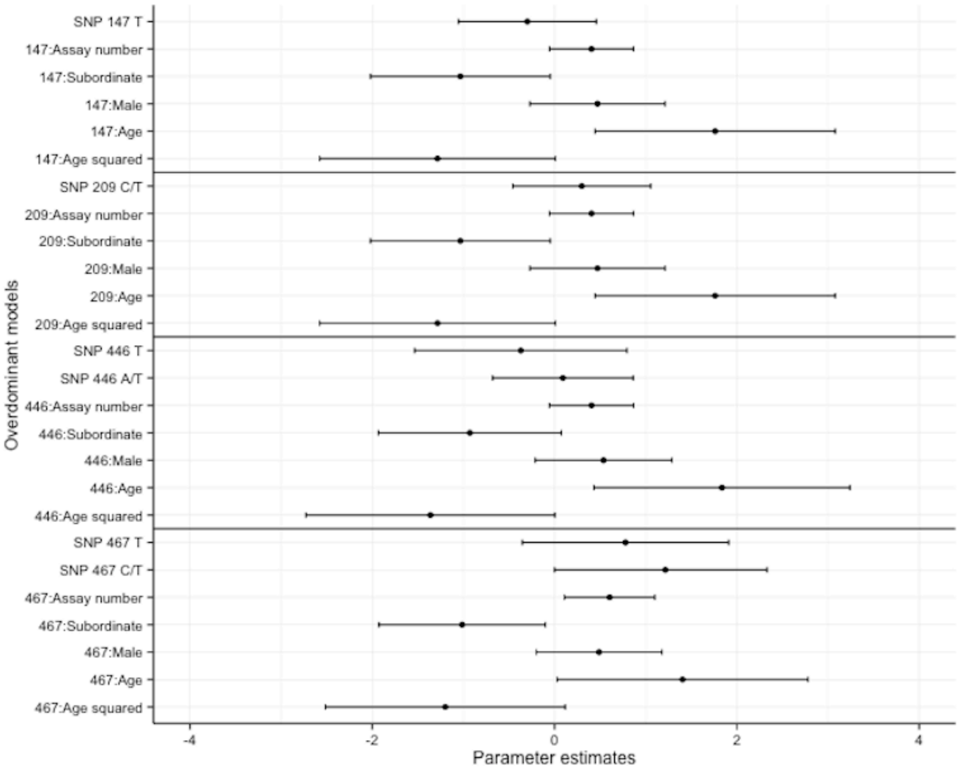


Figure S3.4: The coefficients and associated 95% confidence intervals (CI) of the additive SNP models for novel environment exploration. The models are relative to individuals with no copies of the SNP, subordinate is relative to dominant, male is relative to female.

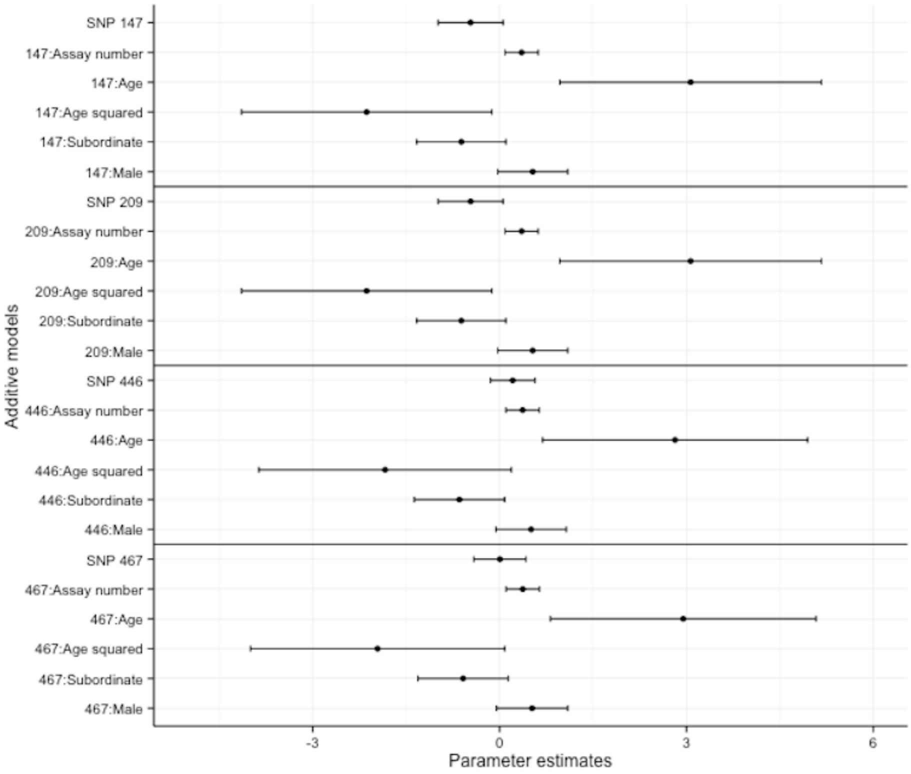
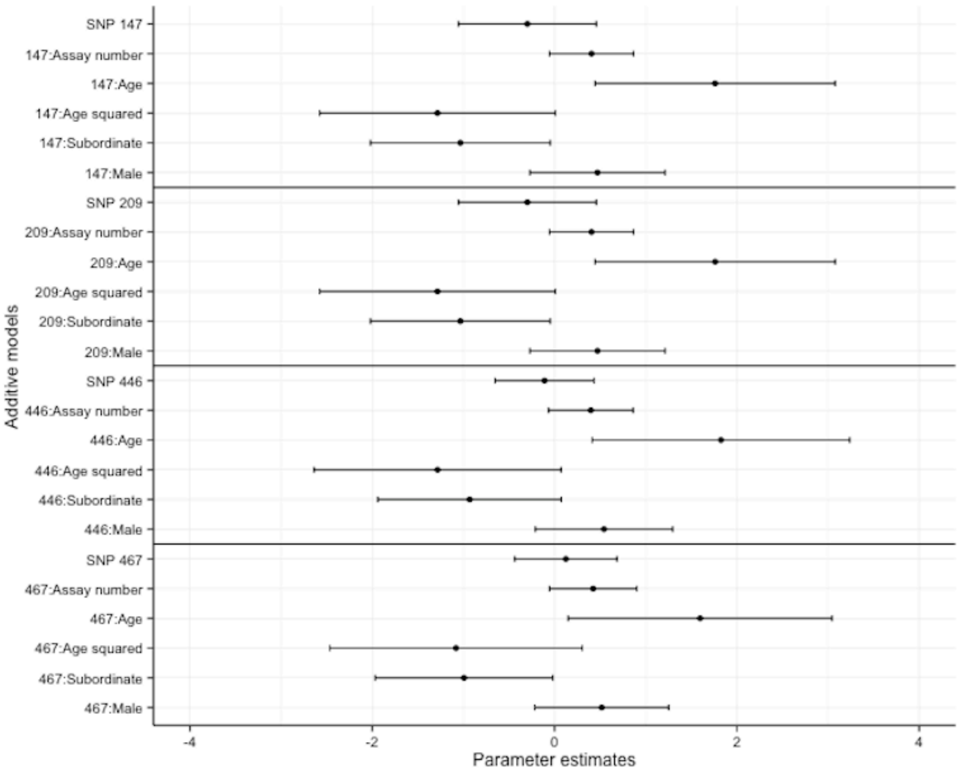


Figure S3.5: The coefficients and associated 95% confidence intervals (CI) of the additive SNP models for novel object exploration. The models are relative to individuals with no copies of the SNP, subordinate is relative to dominant, male is relative to female.



Chapter 4: Exploration is not social-state dependent in a wild cooperative breeder

Table S4.1: A simulation analysis using a standard Poisson HGLM with one random effect of three groups and a varying number of individuals in each group. Simulated datasets were created with an intercept of 0 and variance of 1 and a variance of 0.17. The R package HGLM 2.0-11 was used to assess the affect of sample size changes on the model intercept and variance estimates.

Intercept 0, variance 1	**Intercept mean**	**Variance mean**
3 groups, 11 observations per group	-0.07	1.18
3 groups, 35 observations per group	-0.01	0.97
3 groups, 50 observations per group	-0.09	1.25
3 groups, 100 observations per group	0.05	1.14
3 groups, 200 observations per group	0.04	1.12
3 groups, 500 observations per group	-0.04	1.11
Intercept 0, variance 0.17	**Intercept mean**	**Variance mean**
3 groups, 11 observations per group	-0.06	0.27

3 groups, 35 observations per group	-0.03	0.21
3 groups, 50 observations per group	-0.03	0.18
3 groups, 100 observations per group	-4e-3	0.19
3 groups, 200 observations per group	-0.03	0.19
3 groups, 500 observations per group	-0.04	0.16

Chapter 5: Extra-group parentage and personality in a wild cooperative breeder

Individual analysis novel environment exploration

Table S5.1: Estimates of the posterior distributions of the fixed effects in the Poisson model of the total number of offspring a focal individual was assigned parentage of per season: year of birth, social status (N: dominants = 147, subordinates = 8, sired offspring as subordinate and as a dominant = 16; contrast level = dominant), sex (N: males = 86, females = 85; contrast level = female), novel environment exploration score, tent colour (N: blue = 135, green = 36; contrast level = blue), age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season) and insect abundance (annual insect abundance). Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC***
Year of birth	-0.01	-0.04	0.02	0.68
Subordinate	-0.01	-0.43	0.30	0.95
Male	0.11	-0.09	0.30	0.26
Novel environment exploration score	-9e-4	-0.01	3e-3	0.69
Tent colour	-0.01	-0.25	0.21	0.96
Age	0.15	-0.53	0.87	0.70
Age squared	-0.16	-0.86	0.51	0.64
Helper variable	0.08	-0.11	0.28	0.42
Insect abundance	0.10	-0.07	0.25	0.27

*pMCMC=tests if the parameter is above or below zero

Table S5.2: Estimates of the posterior distributions of the fixed effects in the multinomial model of the ratio of extra-group offspring (EGO) to within-group offspring (WGO): year of birth, social status (N: dominants = 147, subordinates = 8, sired offspring as subordinate and as a dominant = 16; contrast level = dominant), sex (N: males = 86, females = 85; contrast level = female), novel environment exploration score, tent colour (N: blue = 135, green = 36; contrast level = blue), age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season) and insect abundance (annual insect abundance). Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Year of birth	**0.11**	**0.02**	**0.19**	**4e-3**
Subordinate	0.05	-0.88	0.92	0.90
Male	-0.06	-0.56	0.45	0.80
Novel environment exploration score	-2e-3	-0.02	0.01	0.66
Tent colour	-0.17	-0.79	0.49	0.62
Age	2.67	0.89	4.80	0.01
Age squared	-1.58	-3.45	0.33	0.09
Helper variable	0.43	-0.07	1.00	0.10
Insect abundance	-0.29	-0.74	0.13	0.16

Table S5.3: Estimates of the posterior distributions of the fixed effects in the categorical model for the likelihood to be assigned EGO (yes/no): year of birth, social status (N: dominants = 147, subordinates = 8, sired offspring as subordinate and as a dominant = 16; contrast level = dominant), sex (N: males = 86, females = 85; contrast level = female), novel environment exploration score, tent colour (N: blue = 135, green = 36; contrast level = blue), age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season) and insect abundance (annual insect abundance). Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Year of birth	**0.12**	**0.03**	**0.22**	**0.01**
Subordinate	0.11	-0.90	1.20	0.84
Male	0.16	-0.39	0.70	0.59
Novel environment exploration score	-0.01	-0.02	0.01	0.39
Tent colour	-0.06	-0.76	0.62	0.88
Age	**3.25**	**1.25**	**5.34**	**2e-3**
Age squared	-1.95	-3.92	-0.03	0.05
Helper variable	0.43	-0.11	1.05	0.14
Insect abundance	-0.15	-0.64	0.35	0.54

Individual analysis novel object exploration

Table S5.4: Estimates of the posterior distributions of the fixed effects in the Poisson model for total number of offspring: year of birth, social status (N: dominants = 82, subordinates = 5, sired offspring as subordinate and as a dominant = 6; contrast level = dominant), sex (N: males = 50, females = 43; contrast level = female), novel object exploration score, age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season) and insect abundance (annual insect abundance). Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Year of birth	0.01	-0.04	0.06	0.73
Subordinate	0.04	-0.45	0.56	0.87
Male	0.13	-0.14	0.38	0.32
Novel object exploration score	4e-4	-2e-3	3e-3	0.78
Age	0.24	-0.69	1.19	0.63
Age squared	-0.19	-1.07	0.70	0.72
Helper variable	0.09	-0.18	0.38	0.51
Insect abundance	0.15	-0.07	0.37	0.22

Table S5.5: Estimates of the posterior distributions of the fixed effects in the multinomial model of the variable of extra-group offspring (EGO) to within-group offspring (WGO): year of birth, social status (N: dominants = 82, subordinates = 5, sired offspring as subordinate and as a dominant = 6; contrast level = dominant), sex (N: males = 50, females = 43; contrast level = female), novel object exploration score, age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season) and insect abundance (annual insect abundance). Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Year of birth	**0.13**	**0.01**	**0.25**	**0.04**
Subordinate	0.08	-1.22	1.36	0.93
Male	0.10	-0.59	0.71	0.76
Novel object exploration score	0.01	0.00	0.01	0.21
Age	0.69	-1.72	3.42	0.58
Age squared	0.24	-2.14	2.68	0.84
Helper variable	0.23	-0.53	0.86	0.50
Insect abundance	-0.09	-0.64	0.52	0.74

Table S5.6: Estimates of the posterior distributions of the fixed effects in the categorical model for the likelihood to be assigned EGO (yes/no): year of birth, social status (N: dominants = 82, subordinates = 5, sired offspring as subordinate and as a dominant = 6; contrast level = dominant), sex (N: males = 50, females = 43; contrast level = female), novel object exploration score, age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season) and insect abundance (annual insect abundance). Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Year of birth	**0.15**	**0.01**	**0.28**	**0.03**
Subordinate	0.02	-1.40	1.41	0.97
Male	0.32	-0.35	1.15	0.40
Novel object exploration score	0.01	0.00	0.02	0.27
Age	0.73	-2.17	3.57	0.61
Age squared	0.61	-2.20	3.32	0.70
Helper variable	0.29	-0.41	1.16	0.46
Insect abundance	0.11	-0.55	0.78	0.75

Pair analysis novel environment exploration

Table S5.7: Estimates of the posterior distributions of the fixed effects in the Poisson model for total number of offspring sired by the female: female/male year of birth, female/male exploration score and their interaction, female/male age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season), insect abundance (annual insect abundance), season of offspring's birth, male tent colour (N: blue = 52, green = 24) and female tent colour (N: blue = 48, green = 28, contrast level = blue), and male/female assay number. Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Female year of birth	-0.01	-0.08	0.06	0.80
Male year of birth	0.01	-0.04	0.06	0.75
Female novel environment exploration score	-2e-4	-0.01	0.01	0.96
Male novel environment exploration score	0.01	-0.01	0.02	0.39
Female novel environment exploration score*Male novel environment exploration score	5e-3	-1e-1	1e-4	0.15
Male age	-0.24	-2.24	1.74	0.82
Female age	1.02	-1.00	2.95	0.32
Male age squared	0.41	-1.55	2.34	0.68
Female age squared	-1.00	-2.75	1.10	0.31
Helper variable	0.00	-0.48	0.50	0.97
Insect abundance	0.26	-0.09	0.65	0.19
Season of offspring's birth	0.04	-0.10	0.17	0.54
Male tent colour	-0.27	-0.90	0.43	0.42
Female tent colour	0.10	-0.44	0.65	0.69
Male assay number	-0.04	-0.30	0.22	0.74
Female assay number	0.24	-0.06	0.56	0.14

Table S5.8: Estimates of the posterior distributions of the fixed effects in the Poisson model for total number of offspring sired by the male: female/male year of birth, female/male exploration score and their interaction, female/male age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season), insect abundance (annual insect abundance), season of offspring's birth, male tent colour (N: blue = 52, green = 24), female tent colour (N: blue = 48, green = 28, contrast level = blue), and male/female assay number. Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Female year of birth	0.02	-0.04	0.09	0.49
Male year of birth	0.04	-0.01	0.09	0.09
Female novel environment exploration score	-0.01	-0.02	0.01	0.45
Male novel environment exploration score	-2e-3	-0.02	0.01	0.70
Female novel environment exploration score*Male novel environment exploration score	1e-4	-3e-4	5e-4	0.63
Male age	**2.56**	**0.45**	**4.70**	**0.01**
Female age	0.52	-1.40	2.40	0.57
Male age squared	**-2.18**	**-4.25**	**-0.18**	**0.04**
Female age squared	-0.47	-2.23	1.50	0.59
Helper variable	0.26	-0.21	0.74	0.30
Insect abundance	0.13	-0.22	0.51	0.50
Season of offspring's birth	-3e-3	-0.13	0.13	0.94
Male tent colour	0.06	-0.59	0.70	0.84
Female tent colour	-0.19	-0.80	0.36	0.52
Male assay number	0.07	-0.18	0.33	0.59
Female assay number	0.01	-0.39	0.36	0.95

Table S5.9: Estimates of the posterior distributions of the fixed effects in the categorical model for likelihood to be assigned EGO for the female: female/male year of birth, female/male exploration score and their interaction, female/male age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season), insect abundance (annual insect abundance), season of offspring's birth, male tent colour (N: blue = 52, green = 24) and female tent colour (N: blue = 48, green = 28, contrast level = blue), and male/female assay number. Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Female year of birth	-0.06	-0.36	0.25	0.65
Female novel environment exploration score	0.03	-0.03	0.10	0.24
Male novel environment exploration score	0.05	-0.01	0.13	0.07
Female novel environment exploration score*Male novel environment exploration score	**-4e-3**	**-0.01**	**-1e-3**	**1e-3**
Female age	0.43	-5.98	6.66	0.92
Female age squared	1.15	-4.10	7.08	0.69
Helper variable	-1.32	-2.90	0.42	0.11
Insect abundance	0.58	-0.74	1.83	0.38
Season of offspring's birth	0.02	-0.03	0.08	0.48
Male tent colour	-0.42	-3.49	2.34	0.75
Female tent colour	0.98	-1.49	3.49	0.42
Male assay number	0.41	-0.77	1.79	0.55
Female assay number	1.48	-0.07	3.46	0.05

Table S5.10: Estimates of the posterior distributions of the fixed effects in the categorical model for likelihood to be assigned EGO by the male: female/male year of birth, female/male exploration score and their interaction, female/male age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season), insect abundance (annual insect abundance), season of offspring's birth, male tent colour (N: blue = 52, green = 24) and female tent colour (N: blue = 48, green = 28, contrast level = blue), and male/female assay number. Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Male year of birth	0.13	-0.04	0.30	0.12
Female novel environment exploration score	0.01	-0.03	0.06	0.64
Male novel environment exploration score	-0.02	-0.05	0.02	0.38
Female novel environment exploration score*Male novel environment exploration score	6e-4	-7e-4	2e-3	0.31
Male age	4.43	-1.02	10.30	0.11
Male age squared	-3.40	-8.73	1.91	0.19
Helper variable	0.19	-1.11	1.56	0.79
Insect abundance	-0.70	-1.83	0.47	0.21
Season of offspring's birth	0.02	-0.33	0.36	0.87
Male tent colour	1.35	-0.74	3.35	0.17
Female tent colour	-1.31	-3.23	0.49	0.14
Male assay number	0.45	-0.47	1.41	0.30
Female assay number	**-1.40**	**-2.78**	**-2e-3**	**0.04**

Pair analysis novel object exploration

Table S5.11: Estimates of the posterior distributions of the fixed effects in the Poisson model for total number of offspring sired by the female: female/male year of birth, female/male novel object exploration score and their interaction, female/male age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season), insect abundance (annual insect abundance), season of offspring's birth, and male/female assay number. Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Female year of birth	-2.67	-6.22	0.77	0.11
Male year of birth	1.33	-0.77	4.32	0.19
Female novel object exploration score	0.01	-0.02	0.04	0.44
Male novel object exploration score	0.01	-0.02	0.04	0.53
Female novel object exploration score*Male novel object exploration score	-2e-4	-6e-5	2e-4	0.35
Male age	6.04	-10.19	26.22	0.36
Female age	-1.24	-43.16	9.53	0.2
Male age squared	3.37	-4.94	9.88	0.44
Female age squared	-4.84	-15.17	5.98	0.31
Helper variable	-0.61	-3.8	1.03	0.35
Insect abundance	0.59	-1.54	5.56	0.28
Season of offspring's birth	0.28	-0.08	0.63	0.11
Male assay number	0.16	-1.92	1.58	0.96
Female assay number	-0.29	-1.74	1.26	0.65

Table S5.12: Estimates of the posterior distributions of the fixed effects in the Poisson model for total number of offspring sired by the male: female/male year of birth, female/male novel object exploration score and their interaction, female/male age (quadratic and linear terms), helper variable (the number of helpers in an offspring's natal territory, summed over all offspring parentage was assigned to in a season, divided by the total number of offspring parentage was assigned to in a season), insect abundance (annual insect abundance), season of offspring's birth, and male/female assay number. Posterior modes and associated 95% credible intervals, bold text indicates effects for which the 95% credible interval does not overlap zero.

	Posterior mode	**Lower credible interval**	**Upper credible interval**	**pMCMC**
Female year of birth	-0.09	-0.22	0.06	0.23
Male year of birth	0.02	-0.12	0.18	0.72
Female novel object exploration score	0.01	-0.01	0.03	0.24
Male novel object exploration score	0.01	-0.01	0.02	0.50
Female novel object exploration score*Male novel object exploration score	-8e-5	-3e-4	2e-4	0.63
Male age	-1.49	-5.05	1.88	0.41
Female age	-0.91	-5.27	2.92	0.66
Male age squared	1.54	-1.80	4.71	0.34
Female age squared	0.25	-3.31	3.96	0.90
Helper variable	0.38	-0.42	1.24	0.37
Insect abundance	0.47	-0.45	1.35	0.33
Season of offspring's birth	0.15	-0.14	0.41	0.28
Male assay number	-0.13	-1.06	0.95	0.78
Female assay number	-0.10	-1.02	0.73	0.81