Inbreeding and its Avoidance in a Wild Bird Population

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Abstract

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Inbreeding occurs when relatives mate and have offspring. Inbreeding depression is hypothesized to have influenced the evolution of mating systems and behavioural mechanisms of inbreeding avoidance in the animal kingdom. Inbreeding in the wild is difficult to measure, as in order to build a pedigree allowing us to identify matings between relatives, the identity of as many as possible members of a population needs to be known. For a long time, the main source of knowledge about inbreeding depression was based on laboratory and agricultural studies, which did not reflect the array of environmental pressures wild populations have to cope with. In consequence, the deleterious consequences of inbreeding have often been underestimated. This is problematic because accurate estimates of the effect size of inbreeding depression are needed to study the strength of selection on inbreeding avoidance mechanisms, and are also of importance to conservation genetics.

The aim of this thesis was to use pedigree data to infer the occurrence and effects of inbreeding using over forty years of breeding events of the great tit *Parus major* from Wytham Woods, Oxfordshire. The effects of inbreeding on fitness were investigated across a life-history continuum, and across environments. I found that close inbreeding (f=0.25) resulted in pronounced inbreeding depression, which acted independently on hatching success, fledging success, and recruitment success, and reduced the number of fledged grand-offspring by 55%. My results therefore suggest that estimates of fitness costs of inbreeding must focus on the entire life cycle. I also show that the variation in the strength of inbreeding depression varies across environments, particularly so the more the environmental variable considered is linked to fitness. These results emphasise the need of using relevant environmental contrasts when investigating inbreeding by environment interactions.

I further asked whether individuals involved in matings with relatives differed relative to individuals mating with unrelated partners. I did not find any evidence for clear predictors of inbreeding, and I show that inbreeding depression in our population is entirely independent of any tendency for low quality parental genotypes, or phenotypes, to inbreed. Neither did I find any evidence for active inbreeding avoidance: great tits did not mate less often with kin than expected based on several scenarios of random mating, nor did I find increased rates of extra-pair paternity among birds breeding with relatives. In fact, I observed quite the contrary, as birds mating with kin exhibited a higher than average rate of close inbreeding relative to all scenarios of random mating investigated, showed lower rates of extra-pair paternity and divorce than birds mated to unrelated partners. I hypothesise that cases of occasional inbreeding in this population may result from mis-imprinting or a related process whereby some birds develop particularly strong bonds that are at odds with all predictions of avoiding inbreeding. Finally, I asked to what extent natal dispersal, a behaviour that is often hypothesized to play an important role in avoiding inbreeding, indeed reduces the likelihood of inbreeding. I found that male and female individuals breeding with a relative dispersed over several-fold shorter distances than those outbreeding. This led to a 3.4 fold increase (2.3-5, 95% CI) in the likelihood of close inbreeding relative to the population average when individuals dispersed less than 200m.

This thesis demonstrates that inbreeding has deleterious effects on a wild population of birds, occurring throughout an individual's life, and is of varying strength across environments. My findings strongly support the theory that natal dispersal should be considered as a mechanism of prime importance for inbreeding avoidance.

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I dedicate this thesis to my family.

Dla mojej rodziny.

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(Research articles on unrelated subjects published while a DPhil student)

Chapter 1

Introduction

Introduction

Most of the characteristics studied by animal ecologists relate to the features of individuals measured in study populations such as morphology, life-history and behaviour, which are the very characteristics upon which natural selection operates (Merilä & Sheldon, 2001). One of the central issues in evolutionary biology is to identify the causes and consequences of ***variability*** between individuals in a population (Fowler & Whitlock, 1999); ***heritable variation***, combined with the occurrence of natural selection, will allow evolutionary change to act upon a population. Although ***inbreeding*** is not heritable *per se* (but see Reid, Arcese & Keller, 2006), it is central to many issues in evolutionary biology, as by increasing genomewide homozygosity, it increases the likelihood of expression of deleterious recessive alleles, causing ***inbreeding depression*** (see below for more definitions). The latter is likely to be an important selective force responsible for the evolution of various aspects of mating systems in plants and animals (Charlesworth & Charlesworth, 1999; Lynch & Walsh, 1998), and of various behavioural mechanisms for avoiding mating with close relatives in animals (Lynch & Walsh 1998; Pusey & Wolf, 1996; Pusey, 1987).

Individual differences in offspring survival are one of the most important components of variation in lifetime reproductive success (Clutton-Brock, 1988), which broadly reflects fitness (Graffen, 1988); it is therefore important to understand the extent to which the relative contribution of environmental and genetic sources of variation can influence offspring survival. One of the main aims of this thesis was to ask about the impact of inbreeding, a non-additive component of genetic variance on parental reproductive success, thereby favouring the selection of particular [parental] genotypes which in turn might have favoured the evolution of inbreeding avoidance. I explain below key concepts revolving around inbreeding and inbreeding avoidance, and the evolutionary importance of studying such effects in a natural setting. I then introduce my study population, the great tit *Parus major* from Wytham Woods, and summarise the observed variability of their main life-history characteristics. Finally, I present the key questions related to inbreeding and inbreeding avoidance that I aim to answer to in the following chapters.

Inbreeding - definitions

Two individuals X and Y are related if, among the ancestors of one individual, we find one or several ancestors of the other, or the other himself. Whatever the complexity and the nature of this kinship, the genetic implication of this relationship lies in the possibility that the genes possessed by X and Y are copies of the same gene possessed by one of their common ancestors, that is, the possibility of the genes being "identical by descent". (Jacquard, 1975)

I believe that the two sentences above most accurately define what inbreeding is. The need for a *coefficient of inbreeding* to express the degree to which individuals in a pedigree are inbred was first recognized by Pearl (1913), but it was Wright (1922) who first formalised that the most appropriate coefficient of inbreeding is one that gives the probability of individual homozygosity for a given system of mating (Boyce, 1983). As emphasised by Jacquard (1975) in his seminal work "Inbreeding: one word, several meanings", inbreeding is used in relation to at least five concepts: relationship between relatives, genetic drift, departure from panmixia in mating behaviour, subdivision of a population into several isolated groups, and divergence between the actual genotypic structure of a population and the reference state of the Hardy-Weinberg Equilibrium. Several authors have also raised the issue that there are several alternative definitions of relatedness and inbreeding in the literature (e.g. Jacquard, 1975; e.g. Rousset, 2002), and it is therefore important to be explicit about which definition one is referring to (Keller & Waller, 2002). **Pedigree inbreeding** uses pedigree information to determine inbreeding coefficients relative to known founders of the population. The term *f* measures the inbreeding contributed by that pedigree, which is always relative to a particular ancestral generation (Keller *et al.*, 2002b). Pedigree inbreeding is usually obtained from a record of matings between individuals, which is generally based on intentional crossings of specific males and females in stock animals (as in Wiener, Lee & Woolliams, 1992a) or in captive breeding (e.g. Kalinowski, Hedrick & Miller, 2000; Laikre *et al.*, 1996). **Inbreeding as non-random mating**, typically measured by F_{is}, is the deviation of an individual's observed heterozygosity relative to the heterozygosity expected under random mating at Hardy-Weinberg equilibrium (i.e. in a large population with no mutation, selection and migration). Finally, **inbreeding due to population subdivision**, equal to Wright's F_{st}, can occur even under random mating; inbreeding may here result solely from the fact that population size is restricted and genetic drift results (Keller & Waller, 2002). Inbreeding as non-random mating and inbreeding due to population subdivision can be estimated from pedigree data (Keller & Arcese, 1998; Wright, 1956), but they are most often inferred from selectively neutral molecular markers. In this thesis, I will be focussing solely on inbreeding inferred from pedigree information.

When using pedigrees, the standard measure of an individual's degree of inbreeding is the coefficient of inbreeding f (Charlesworth *et al.*, 1999; Wright, 1922). Originally described as a correlation between uniting gametes (Wright, 1922), it is now interpreted as the probability of identity by descent of two alleles at a locus in an individual (Boyce, 1983; Crow & Kimura, 1970; Jacquard, 1975; Malécot, 1948). Thus, an individual with inbreeding coefficient f has a probability f that two alleles at a particular locus are identical by descent, and a probability of $1-f$ that they are not (Keller & Waller, 2002). The general formula for the inbreeding coefficient of an individual, originally derived by Wright (1922) is as follows:

$$f_X = \Sigma\, [(1\,/\,2)^n\,(1 + f_A)] \qquad \text{(Crow \& Kimura, 1970; Falconer \& Mackay, 1996),}$$

where n is the number of individuals in any path of relationship counting the parents of X, the common ancestor A, and all individuals in the path connecting parents to the common ancestor; f_A is the inbreeding coefficient of the common ancestor at the apex of the path; summation is over all paths of the relationship. Thus, a brother-sister or mother-son mating would yield offspring with f=0.25. Offspring sired in grandparent-grandoffspring mating will have an inbreeding coefficient of f=0.125.

The genetic basis of inbreeding depression

The direct genetic consequence of inbreeding is increased homozygosity, as inbred individuals will more frequently inherit the same allele from each parent (Crow & Kimura, 1970). This has different implications for the phenotype depending on the type of gene action involved. Because each autosomal locus in a diploid organism carries two alleles, they can behave in either additive or dominant fashion. In the case of two alleles acting in an additive manner, the resulting phenotype will be the exact additive expression of the two alleles. Alternatively, one allele can exhibit some degree of dominance over the other, and the resulting phenotype will be dependent on the degree of dominance of one allele over the other. In a situation where dominance effects occur, one allele is usually defined as “dominant”, and the other allele whose phenotypic expression is being suppressed is referred to as “recessive”. One observable effect of inbreeding is that deleterious recessive genes, previously hidden by heterozygosity with dominant alleles, will be expressed in homozygous individuals, causing inbreeding depression (Lynch & Walsh, 1998).

While inbreeding depression is most commonly explained by dominance (or partial dominance) effects (Carr & Dudash, 2003; Kristensen *et al.*, 2005; Lynch & Walsh, 1998; Roff, 2002), the genetic basis for inbreeding depression has been challenged by another mechanism, the overdominance hypothesis (Lynch & Walsh, 1998). The overdominance hypothesis argues that there is something special about the heterozygous state which causes increased vigour relative to both homozygotes (Lynch & Walsh, 1998). Independently on whether it is dominance or overdominance that is responsible for the existence of inbreeding depression, in both cases mean fitness will decline linearly with increasing f (Keller & Waller, 2002; Lynch & Walsh 1998). The evolutionary implications of the two mechanisms differ: for the dominance hypothesis, inbreeding depression is a consequence of recurrent deleterious mutations at the genomic level, and while selection removes some of these alleles each generation, mutation replaces them again. In contrast, for the overdominance hypothesis, genetic variation is retained by selection favouring the heterozygous state at multiple loci, and variation is therefore maintained even in the absence of mutation pressure (Lynch & Walsh, 1998).

As stated by Roff (2002), in terms of the relative importance of the two mechanisms in inbreeding depression, historically the pendulum has swung between the two hypotheses; importantly however, there is a worthy difference between the predictions of the two models with respect to the mean trait value of a population undergoing inbreeding, elegantly demonstrated by Barrett & Charlesworth (1991) using the water hyacinth *Eichhornia paniculata* as a study system. Indeed, it is possible to identify which mechanism is responsible for inbreeding depression in a particular study system by creating a number of separate inbred lines, and then constructing all possible crosses among the lines. Under the overdominance hypothesis, the mean trait value of the among-line crosses will be equal to that of the outbred population; under the dominance hypothesis, the mean trait value of the among-line crosses will exceed that of the outbred population because of the purging of deleterious alleles. Purging occurring in inbred lines will evict some of the deleterious recessive alleles, resulting in the overall mean of crosses between the inbred lines to be larger than the means of outbred lines. The major role of dominance effects (rather than overdominance) in inbreeding depression has since been confirmed by numerous studies using both plant and animal species as study system (Barret & Charlesworth 1991; Charlesworth & Charesworth 1999; Carr & Dudash 2003; Roff, 2002 to name only a few), although due to the nature of these experiments, we are not aware of studies disentangling the two effects in a natural system. It must also be remembered that the two hypotheses do not have to be mutually exclusive, as overdominance may exist at some loci, and dominance at other loci (Kristensen *et al.*, 2005).

Genetic variance (V_G) among individuals is the main focus of many studies investigating natural selection (Falconer & Mackay, 1996; Lynch & Walsh, 1998), and together with environmental variance they constitute the entire population phenotypic variance. V_G can be partitioned into additive genetic variance (V_A) and non-additive genetic variance components, such as dominance (V_D - interaction within loci), and interaction variance (V_I - interaction between loci) (Merilä & Sheldon, 2001). Thus,

$$V_G=V_A + V_D + V_I \qquad \text{(Falconer \& Mackay, 1996)}$$

In order to observe inbreeding depression as a consequence of dominance effects, it is important to note that quantitative traits are the product of multiple loci. It is indeed possible that considerable cancelling occurs among the effects at different loci exhibiting dominance variance, leading to negligible inbreeding depression; thus, in order to observe a decline in the mean of a trait with inbreeding, directional dominance is required (Lynch & Walsh, 1998). Unfortunately, non-additive genetic components of variation (V_D and V_I) are to a large extent neglected in studies of quantitative traits and their response to selection in a natural setting

(Charmantier & Garant, 2005; Merilä & Sheldon, 2001; Roff & Emerson, 2006). This occurs despite the fact that some studies have shown that non-additive genetic effects influence heritability estimates (Kristensen *et al.*, 2005; Meffert, Hicks & Regan, 2002) and patterns of genetic associations among traits (Phillips, Whitlock & Fowler, 2001). Overlooking dominance effects in quantitative genetic analyses is an unfortunate limitation of field studies - the estimation of non-additive genetic components in the wild is often not possible as complex breeding designs are needed (Merilä & Sheldon, 2001), which are not applicable in a setting where unrestricted mate choice occurs.

Variation in the level of genetic variance differs depending on the type of trait investigated; in particular, such difference can be observed when comparing morphological and life-history traits, the former showing mostly additive genetic variance, while the latter, being more closely related to fitness, exhibit greater levels of dominance genetic variance (Crnokrak & Roff, 1995; Lynch & Walsh 1998). The reason for such dichotomy is elegantly explained by Lynch & Walsh (1998) using selection theory: alleles with favourable effects on fitness should move rapidly into fixation (regardless of their degree of dominance), while dominant alleles with deleterious effects will be eliminated (purged) rapidly. However, deleterious recessive alleles will be maintained at low frequencies in the population due to mutation pressure. Hence, in characters weakly related to fitness (or under stabilizing selection), directional dominance (i.e. where a quantitative trait will change in mean as a result of inbreeding) will be less pronounced since mutations that cause a shift in the mean in either direction will be selectively equivalent. Under directional selection, the response to selection acts primarily on the additive component of the genetic variance, resulting in an overall reduced additive component of variance. In contrast, the non-additive component of genetic variance is not affected by directional selection. As a consequence, life-history traits (with often depleted additive genetic variance) are found to display a higher proportion of non-additive genetic variance, while morphological traits (often showing little or no directional selection) have lower levels of dominance effects, and therefore are less prone to inbreeding depression (Crnokrak & Roff 1995; DeRose & Roff 1999; Roff & Emerson, 2006).

Interestingly, inbreeding depression is a direct visualisation of directional dominance effects acting with increasing strength along the inbreeding gradient. Hence, the study of inbreeding provides an important opportunity to have an estimate of the effect size of directional dominance gene action on fitness. I believe that records of the impact of non-additive genetic effects on fitness are currently particularly needed, since recent work by Reid (Reid, Arcese & Keller, 2006; Reid, 2007;) re-emphasises the degree to which dominance gene action can influence with a large array of evolutionary important questions. For example, it suggests that dominance effects can truly interfere with our estimates of the genetic architecture of quantitative traits (Reid, Arcese & Keller, 2006; Szulkin & Sheldon, 2006), and that non-additive genetic benefits can be the target for directional female preference, suggesting that some assumptions of genetic models of sexual selection may need to be reconsidered in the case of structured populations where the variance of inbreeding coefficients is high (Reid, 2007).

Investigating inbreeding depression in a simplified world

The ubiquitous existence of inbreeding depression bears on many basic issues in evolutionary biology, as well as on a number of issues in agriculture and conservation biology. As early as in 1876, Darwin suggested that the adaptations of many plants aimed to ensure outcrossing could be understood in terms of the selective advantage of avoiding inbreeding. Deleterious consequences of self-fertilization are likely to be the selective forces responsible for the evolution of various aspects of mating systems in plants (Charlesworth & Charlesworth, 1987), and of behavioural mechanisms for avoiding mating between close relatives in animals (Pusey, 1987; Pusey & Wolf, 1996). The genetic mechanisms of inbreeding have been well studied in laboratory conditions on *Drosophila* sp. (Bijlsma, Bundgaard & Van Putten, 1999; Charlesworth & Charlesworth, 1999; Dahlgaard & Hoffmann, 2000; Dahlgaard & Loeschcke, 1997; Kristensen, Dahlgaard & Loeschcke, 2003; Kristensen *et al.*, 2005; Miller, 1994; Phillips *et al.*, 2001; Reed *et al.*, 2003), and those studies provide valuable insight into the fitness costs of inbreeding at particular life history stages, the interactions of inbreeding with environmental quality, or the effects of dominance caused by inbreeding on quantitative genetic measures of different traits in the fruit fly. However, it is difficult to predict whether the costs of inbreeding will be similar in vertebrates, and to what extent the effect size of inbreeding depression, measured in controlled laboratory conditions, will be equivalent in a natural setting.

Similarly, animal and plant breeders have long recognized the deleterious effects of inbreeding, which have been documented in domestic and livestock animals such as poultry (Abplanalp, 1990), dairy cattle (Smith, Cassell & Pearson, 1998), sheep (Wiener *et al.*, 1992a; Wiener, Lee & Woolliams, 1992b, 1992c), or salmon (Wang, Hard & Utter, 2001). In contrast to laboratory studies investigating the genetic basis of inbreeding, research on inbreeding in farm animals focuses on economically important traits. Thus, Smith et al. (1998) noted that "*inbreeding decreased […] the lifetime production of milk, fat and protein by 177, 6.0, 5.5 kg, respectively, per 1% increase in inbreeding*". Those traits, however, are not necessarily of major importance in a natural setting, especially given that they are measured in a highly selected population. Captive-bred and zoo-kept animals also provide some insight into patterns of inbreeding depression in vertebrate animals (e.g. Cassinello, 2005; Kalinowski, Hedrick & Miller, 2000; Laikre *et al.*, 1996), and deleterious effects of inbreeding are often recorded. However, the extent to which the living conditions experienced by captive bred animals may mask or exacerbate the effects of inbreeding relative to what would be expressed in the wild is yet again unknown. It is thus difficult to predict the potential strength of selection against inbreeding on the basis of these types of study. The effects of inbreeding on individual fitness in a natural setting, where environmental variance interacts with the expression of deleterious recessive alleles, may be different than what would be expected based on farm or laboratory studies (Crnokrak & Roff, 1999), and can therefore be of great interest.

Inbreeding in the wild

Endler (1986) emphasised the importance of studying animals in the wild, as it is only in such context that one can infer the importance of particular processes in natural selection. Asking about many of the evolutionary properties of wild populations requires individual-based long term monitoring, inherently based on many years of field data. Thanks to the development of molecular and quantitative genetic tools adapted to the population properties of species living in the wild (Garant & Kruuk, 2005; Kruuk, 2004), we have recently witnessed a growing interest in the evolutionary potential of such populations, allowing us to understand the expression of genetic variation in different environmental conditions (Charmantier & Garant, 2005). A summary of some of the best known long-term studies of wild populations is presented in Table 1. It must be noted however that many long-term studies of vertebrates, historically recorded for purposes other than those relevant to evolutionary biologists, may still await to be discovered.

Table 1. An outline of some well-known long term, individual-based, non-primate population studies (more information on some of them can also be found on http://wildevolution.biology.ed.ac.uk/study_systems.html). "Insular" refers to island-type populations with limited immigration levels. The "further reference" section emphasise inbreeding-related studies, if investigated.

Species	**Country**	**Start date**	**Insular?**	**Inbreeding investigated?**	**Further reference**
Great tit *Parus major*	U.K. - Wytham	1947	no	yes	(Bulmer, 1973; Greenwood, Harvey & Perrins, 1978; Szulkin *et al.*, 2007); also see Table 2.
Great tit *Parus major*	Netherlands - Hoge Veluwe and Vlieland	~ 1950	no (Veluwe), yes (Vlieland)	yes	(van Noordwijk & Scharloo, 1981; van Tienderen & van Noordwijk, 1988)
Marsh tit *Parus palustris*	Poland - Bialowieza	1979	no	no	(Wesolowski, 1998)
Great reed warbler *Acrocephalus arundinaceus*	Sweden - Kvismaren	1983	no	yes	(Hansson *et al.*, 2007)
Collared flycatcher *Ficedula albilicollis*	Sweden – Gotland	1980	not really	yes	(Kruuk, Sheldon & Merila, 2002)
Song sparrow *Medlospiza melodia*	Canada - Mandarte	1974	yes	yes	(Keller, 1998; Reid, Arcese & Keller, 2003; Smith *et al.*, 2006)
Mexican jay *Aphelocoma ultramarina*	USA - Chiricahua mountains, Arizona	1969	no	yes	(Brown & Brown, 1998)
Medium ground finch *Geospiza fortis*	Galapagos – Isla Daphne major	1976	yes	yes	(Gibbs & Grant, 1989)
Red cockaded woodpecker *Picoides borealis*	USA – Sandhills, North Carolina	1980	no	yes	(Daniels & Walters, 2000; Schiegg *et al.*, 2006)
Mute swan *Cygnus olor*	UK - Abbotsbury	1976 (for banding)	no	no	(Charmantier *et al.*, 2006; McCleery *et al.*, 2002)
Snow goose *Anser caerulescens*	Canada, Manitoba	1968-1990's	no	no	(Cooch *et al.*, 1991; Finney & Cooke, 1978)
European badger *Meles meles*	UK - Wytham	1987	no	yes	(Dugdale, DPhil thesis; Macdonald & Newman, 2002)
Soay sheep *Ovis aries*	UK - St Kilda	1985	yes	yes	(Coltman *et al.*, 1999; Overall *et al.*, 2005)
Red deer *Cervus elaphus*	UK - Isle of Rum	1971	yes	yes	(Slate *et al.*, 2000)
Bighorn sheep *Ovis canadiensis*	Canada - Ram Mountain	1971	yes	no	(Coltman *et al.*, 2003)

Studies of inbreeding in vertebrates living in the wild are often carried out on geographically isolated locations such as islands (see studies of Coltman *et al.* (1999), Daniels & Walters (2000), Jamieson *et al.* (2003), Keller (1998), Keller *et al.* (2002), van Nordwijk & Scharloo (1981), Overall et al. (2005), Reid, Arcese & Keller (2003); see table 1) . Importantly, immigration levels are often limited on islands, hence reducing gene flow and often increasing inbreeding due to limited population size. In these circumstances, population bottlenecks may be important phenomena allowing for the purging of deleterious recessives (Keller *et al.*, 1994), with a potential to reduce the strength of inbreeding depression in such population, provided that immigration is hardly occurring.

The magnitude of inbreeding depression while measured in a natural setting varies considerably from one study to another. Environmental conditions affected the magnitude of inbreeding depression in some studies (Jamieson, Roy & Lettink, 2003; Keller *et al.*, 2002; Marr *et al.*, 2006), but not in others (Kruuk *et al.*, 2002). Similarly, life history stages affected by inbreeding have also differed between species and studies published (compare the effects of inbreeding on recruitment in the studies of Kruuk *et al.*, 2002 and van Noordwijk & Scharloo, 1981). While the genetic load (deleterious recessive alleles in the genome) is held responsible for the severity of inbreeding depression, it is difficult to say to what extent reports on limited inbreeding depression in the wild suffer due to limited statistical power, or indeed low number of recessive alleles in some of the populations inferred. Moreover, because it is difficult to measure inbreeding in the wild at all stages of the life-cycle, many authors have suggested that inbreeding depression is often underestimated (Charlesworth & Charlesworth, 1987; Hoglund *et al.*, 2002). More studies of inbreeding based on populations with large effective size, taking into consideration as many variables affecting fitness and fitness traits themselves – such as environmental quality, parental quality, early and late life-history stages and individual immunity

- are therefore needed. However, building pedigrees in unmanaged and non-isolated populations is a much more difficult task, as (i) large levels of immigration often limit pedigree completeness, and (ii) renders the monitoring of mammal herds difficult. To my knowledge, relatively few such populations have been investigated relative to inbreeding (see table 1).

Birds dominate studies of inbreeding in the wild; indeed, some of the longest running studies of bird population in the wild focused on passerine species (order: Passeriformes), such as the great tit *Parus major* study in Oranje in the Netherlands (started by H. Wolda in 1912, and further developed by H. N. Kluijver in Vlieland and Veluwe (Gosler & Perrins, in press), or the great tit study in Wytham, Oxfordshire, U.K. (see table 1). An important reason for studying those species is their relative abundance, but most importantly, it is their readiness to accept nestboxes instead of nesting in natural cavities. Hence, because it is possible to induce most pairs of great tits in a given area to accept nesting in boxes (Perrins, 1979), which are easily found by human fieldworkers, the survey of a large breeding population becomes logistically possible.

Inbreeding avoidance

A natural consequence of investigating the fitness costs of inbreeding is to ask about the selective pressures that might have favoured the evolution of inbreeding avoidance. It is important to realise that inbreeding avoidance may not be selected for if the costs of inbreeding are lower than the costs of avoiding it. Kokko and Ots (2006) developed various models showing that inbreeding tolerance may be expected to be context-dependent. For example, in the case of egalitarian parental care, both parents should avoid inbreeding when the fitness costs to offspring exceed 10%. In other scenarios of mate choice described by the authors however, tolerance of inbreeding can occur even when the fitness costs are decreased by 2/3.

Independently of the estimates presented above, it is clear that mechanisms of inbreeding avoidance have been recorded in a large array of species (Pusey, 1987, Pusey & Wolf, 1996). In order to have as complete a picture as possible of the costs of inbreeding, and their effects on the evolution of inbreeding avoidance, I aimed to test for the presence, or absence, of all the most often acknowledged means of inbreeding avoidance. Identifying and understanding the relative importance of different means of inbreeding avoidance occurring in my study system, either mediated by active kin recognition where non-random mating, divorce, or increased rates of extra-pair paternity occur, or by passive dispersal, are detailed below.

Passive inbreeding avoidance - dispersal

It is often argued that dispersal is an important means of inbreeding avoidance. Causes of the evolution of dispersal are much debated, and the relative importance of competition between relatives (Hamilton & May, 1977), the temporal variability of the environment (Gandon & Michalakis, 1999; Olivieri, Michalakis & Gouyon, 1995), or inbreeding avoidance (Greenwood, Harvey & Perrins, 1978; Moore & Ali, 1984) in the evolution of dispersal is unknown. However, the number of theoretical studies on the evolution of dispersal (reviewed in Clobert *et al.*, 2001; Gandon & Michalakis, 2001; Greenwood & Harvey, 1982; Handley & Perrin, 2007; Johnson & Gaines, 1990; Lambin, Aars & Piertney, 2001) clearly contrasts substantially with few empirical studies where inbreeding and dispersal are contrasted (but see Greenwood, Harvey & Perrins (1978), Schiegg *et al.* (2006)). More empirical evidence testing the relationship between inbreeding and dispersal are therefore needed, and this was a goal of this study.

Active means of inbreeding avoidance – non-random mating, extra-pair copulation and divorce.

As stated by Reid (2007), animals are often "suggested" to recognize kin, and avoid mating with close relatives. It is unknown, however, to what extent active kin recognition truly occurs in the majority of bird species. While active kin recognition has been found among cooperatively breeding passerines (Komdeur, Richardson & Burke, 2004; Russell & Hatchwell, 2001), evidence for avoiding mating with kin is poor in non-cooperatively breeding species (Gibbs & Grant, 1989; Hansson *et al.*, 2007; Keller & Arcese, 1998; van Tienderen & van Noordwijk, 1988). Confirming, or ruling out, a pattern where kin are disfavoured as mates is therefore important when one aims to identify possible means of avoiding inbreeding.

Recently, the use of molecular methods to infer inbreeding has shed light on the occurrence of inbreeding and means of inbreeding avoidance. Although there is still a major controversy over the relationship between pedigree inbreeding and multi-locus heterozygosity inferred from molecular markers (Balloux, Amos & Coulson, 2004; Pemberton, 2004; Slate *et al.*, 2004), one can not ignore the large amount of studies using molecular tools to answer inbreeding related questions (as in Spottiswoode & Moller (2004), Stow & Sunnucks (2004), Coltman *et al.* (1998), Bensch, Hasselquist & von Schantz (1994), and many others). In terms of inbreeding avoidance and heterozygosity, recent studies suggest that extra-pair paternity may be a means of inbreeding avoidance if the social partners have a high level of genetic similarity, and are therefore more likely to sire more homozygous offspring (Blomqvist *et al.*, 2002; Eimes *et al.*, 2005).

Debates on the relationship between inbreeding and molecular-based estimates of heterozygosity (Balloux, Amos & Coulson, 2004; Slate *et al.*, 2004) and the biological significance of extra-pair paternity in the context of parental genetic similarity (Griffith &

Montgomerie, 2003) are ongoing. In this context, studies of whether inbreeding birds have elevated or lowered rates of extra-pair paternity are very relevant. While it is not clear when social pairs are formed in great tits (Gosler, 1993), it is believed that most birds are paired by the end of February, before the breeding territory is clearly established. Hence, while the choice of a social mate occurs most likely in late winter, it is theoretically possible that, in the light of cues suggesting that the social mate was not an optimal choice, means for improving reproductive success can still be made, either by mating with extra-pair males, or by divorcing with the current social partner, and choosing another one at the start of the following breeding season. Divorcing as a means of inbreeding avoidance does not necessarily require active kin recognition; instead, cues related to reproductive success, such as hatching failure (Kempenaers, Adriaensen & Dhondt, 1998) or reduced reproductive success might be used to assess the quality of a particular partner. Asking whether divorce is a response to true inbreeding depression, and not to a surrogate such as genetic similarity or hatching failure, however, has not been formally tested.

Often means of inbreeding avoidance are tested in relation to surrogates of inbreeding, and not true inbreeding (i.e. by using genetic similarity or hatching failure as evidence to inbreeding). We are aware that such criticism simply targets the limitations of particular study systems where pedigreed relatedness between individuals is not available. An important aim of this thesis was therefore to validate, or refute, previous findings of inbreeding avoidance tested under the assumption, but not proof of, inbreeding.

Study site and study species

Study site

Wytham Woods is a mixed deciduous woodland of c. 388 ha near Wytham village, Oxfordshire, U.K. (1°20'W, 51°46'N). It came into University ownership in 1942 , and the long-term population study of great tits was started by David Lack in 1947, shortly after he became Director of the Edward Grey Institute (EGI) in 1945 (Gosler & Perrins, in press). The first 100 nestboxes were erected in the winter of 1946 by John Gibb in Marley Wood, one of the central parts of Wytham, and by 1963, just over 1000 nestboxes were erected throughout Wytham at variable densities under the initiative of Chris Perrins and David Lack (Gosler & Perrins, in press). All current nestboxes were mapped using GPS technology by Teddy Wilkin in the winter of 2005 (Wilkin, 2006). An aerial representation of Wytham Woods, with the location of individual sites is presented on Figure 1.

The predominant tree species in Wytham are oak (*Quercus robur*), ash (*Fraxinus excelsior*), sycamore (*Acer pseudoplatanus*) and beech (*Fagus sylvatica*); the understory is mainly composed of elder (*Sambucus nigra*), bramble (*Rubus fruticosa*), hawthorn (*Crataegus monogyna*), blackthorn (P*runus spinosa*), bracken (*Pteridium aquilinum*) and field maple (*Acer campestre*) (Wilkin, 2006). Typical habitats from the study area, photographed during the past three breeding seasons, are presented in Figure 2.

Figure 2. Wytham Woods in spring time.
(Photo credit: top right: Joanna Łoś, all others: Przemysław Żelazowski)

Study species: the great tit, **Parus major**

The great tit *Parus major* is a representative of the Paridae family (Passeriformes) of the sub-order Oscines, or song birds (Perrins, 1979). As mentioned previously, birds nest in natural cavities, most often in hollow trees in stumps. But they also readily accept, and in fact prefer, artificial nestboxes, to the extent that in Wytham virtually all the great tit breeding population is now breeding in nestboxes. The life-history characteristics of Wytham great tits have been well described since 1950, and are summarised in Table 2, together with further references related to particular features of great tit biology.

British great tits do not migrate (Perrins, 1979), and winter in the same grounds throughout the year. In fact, once settled as recruits, great tits move very short distances between successive breeding events (median distance between successive breeding events: 50-143m. Harvey, Greenwood & Perrins, 1979). Nestlings are ringed on day 15 after hatching (hatching = day 1), and leave the nest any time between day 16 and up to 21 days after hatching. An overview of offspring developmental stages is presented in Figure 3.

Table 2. Life-history traits of great tits from Wytham Woods. Life-history estimates are derived from a dataset of breeding events spanning from 1964 to 2004 where both parents were known, and inbreeding coefficients estimated (e.g. where at least one offspring survived till ringing date on day 15). In the case of fitness data, broods subject to experiments / manipulations were excluded. Divorce rates are based on a dataset ranging from 1964 to 2006 (manipulations excluded), and dispersal data on a dataset ranging from 1964-2007.

Trait	**Average (+ variability estimate)**	**Example References**
Population size	279 breeding events yearly (SD=93, n=11419)	Chapter 5, (Wilkin *et al.*, 2006)
Proportion of identified individuals	61% of males and 74% of females (note that parental identity is unknown if the brood failed before trapping attempts are made); more birds were identified post 1975	Chapter 5
Immigration rate	43% and 49% of identified males and females in each year are born outside Wytham	(McCleery *et al.*, 2004). (Verhulst, Perrins & Riddington, 1997)
Lay date of first egg	27th of April (9.20 SD, n=5455)	(McCleery & Perrins, 1998; Perrins, 1991; van Noordwijk, McCleery & Perrins, 1995)
Clutch size	8.7 eggs (1.88 SD, n=5628)	(Boyce & Perrins, 1987; Lack, 1947; Liou *et al.*, 1993; Perrins & McCleery, 1989; Pettifor, Perrins & McCleery, 1988)
Egg mass	1.7 g (0.13 SD, n=4768)	(Wilkin *et al.*, 2006)
Brood size	8.2 (1.88 SD, n=5608)	(Lack, 1957)
Nr of fledged individuals	7.6 (2.12 SD, n=5632)	(Szulkin *et al.*, 2007)
Fledgling mass	18.6 g (12.2 SD, n=5496)	(Garant *et al.*, 2005)
Recruitment	0.81 recruit per brood(0-1 IQR, n=5635) note: highly variable between years, ranging from 0.27 to 1.57 (SD=0.34, n=41)	(McCleery *et al.*, 2004)
Natal dispersal	528 m. for males (298-931 m. IQR, n=2772) and 778 m. for females (456-1338 m. IQR, n=2517) within Wytham.	(Garant *et al.*, 2005; Greenwood *et al.*, 1978; Szulkin & Sheldon, 2008)
Parental age structure and survival	c.54% of birds breeding in Wytham are first year birds, 25% are 2nd year, 12% are 3rd year birds; n=10449. Birds breed on average 1.48 times in their lifetime.	(Bulmer & Perrins, 1973; Clobert *et al.*, 1988)
Divorce	Occurring 32% of the time (n=1018)	Chapter 4 (Perrins & McCleery, 1985)

Figure 3. The great tit breeding cycle. (a) a typical great tit clutch. (b) 1 day old pulli. (c) growing feathers. (d) a 15 day old nestling (e) adult great tit. (Photo credit: top left: Przemysław Żelazowski, bottom picture: Samuel Cotton. All other pictures: Joanna Łoś)

Most breeders have only a single brood during a season (Perrins, 1979), and the nestling period is timed to coincide with a good food supply for the young (Perrins, 1991), mainly based on caterpillars such as the winter moth (*Operophtera brumata*) and the green tortrix (*Tortrix viridiana*) (Gosler & Perrins, in press), mostly found on oaks scattered across the woodland (Figure 4).

Figure 4. Food for the young: caterpillar of the winter moth (Photo credit: Przemysław Żelazowski)

Thesis Overview

In this thesis, I investigated inbreeding and inbreeding avoidance in the great tit population in Wytham, Oxfordshire. By looking at the causes and consequences of inbreeding and means of inbreeding avoidance, this study characterises the nature of inbreeding from several angles; this approach thus allows some insight in the importance of inbreeding in an evolutionary context. By investigating the environmental dependence of inbreeding depression, or the life-history stages most affected by inbreeding depression in a vertebrate species, the results presented in this thesis may also be relevant to the fields of conservation genetics and management of fragmented populations.

This thesis consists of 5 stand-alone data chapters; 3 chapters are published or currently in press, one chapter is submitted, and a final chapter is formatted for future submission. In addition to the introduction (Chapter 1), five data chapters (Chapter 2-6) and discussion (Chapter 7), "*Intermezzos*" are aimed to present some thoughts and ideas, either developed by other authors (*Intermezzo A*), or by me (*Intermezzo B*), but where the results are presented in the context of a pilot study only. They are not intended to be considered as fully-fledged chapters; they simply aim to add additional perspectives to the data presented in Chapters 2-6, and to the discussion in Chapter 7.

I aimed to answer the following questions:

Inbreeding and inbreeding depression

- What are the patterns of inbreeding and inbreeding depression in a wild bird population? (Chapter 2)
- Does the magnitude of inbreeding depression vary depending on environmental quality? (Chapter 3)
- What are the characteristics of birds involved in inbred matings? Can they enhance estimates of offspring inbreeding depression with other aspects of phenotypic quality? (Chapter 4)

Inbreeding avoidance

- Do great tits actively avoid mating with kin when compared to different scenarios of random mating? (Chapter 5)
- Is extra-pair paternity used as a mean of inbreeding avoidance? (*Intermezzo B*)
- Does dispersal function as an efficient mean of inbreeding avoidance? (Chapter 6)

Author Contributions

Main contributors

Marta Szulkin. Collected three years of field data, conceived, analysed & wrote first drafts of each manuscript, corrected subsequent drafts and submitted manuscripts to journals.

Ben C. Sheldon. Overall guidance in research techniques, advice on statistical approaches, analysis methods and writing. Gave detailed comments on all drafts.

Additional contributors

Joanne Chapman (co-author of *Intermezzo* B) - optimised microsatellite markers used in *Intermezzo* B, provided guidance in the lab and information on molecular marker properties, as well as comments *for Intermezzo B.*

Robin McCleery (co-author in Chapter I) - participated in pedigree construction, corrected subsequent drafts of Chapter I. Participated in generating output files for divorce data analysis (Chapter IV).

Dany Garant (co-author in Chapter I) - participated in the first stages of pedigree construction and analysis, corrected subsequent drafts of Chapter I.

George Nicholson (co-author in Chapter V) - participated in generating simulations of different scenarios of random mating required for Chapter V.

Sam Patrick (co-author in *Intermezzo* B) - provided comments for *Intermezzo B*, guidance in the lab and supplied microsatellite data for two broods analysed for extra-pair paternity.

Teddy A. Wilkin - provided Wytham Woods nestbox coordinates and data on Wytham Woods' environmental heterogeneity.

Przemysław Żelazowski (co-author in Chapter V) - participated in generating simulations of different scenarios of random mating required for Chapter V, wrote a script for nearest-neighbour analysis (Chapter VI).

The EGI – collected great tit life-history data over the past 60 years.

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