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Sexual selection and reproductive strategies in songbirds

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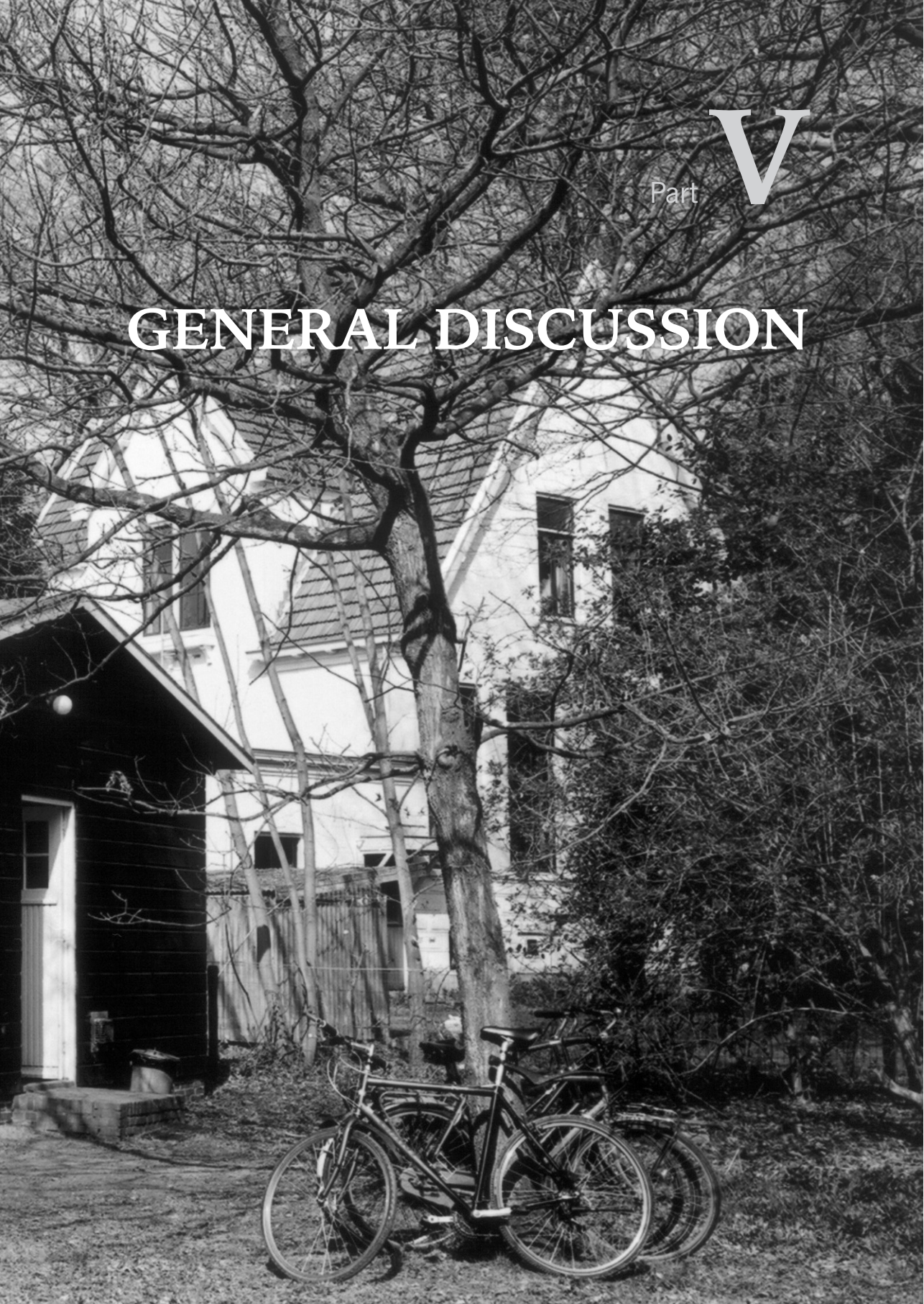
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Part

V

GENERAL DISCUSSION



Chapter **10**

**Sexual selection and reproductive strategies
in songbirds: general discussion**

Mathew L. Berg

INTRODUCTION

The basic components of reproduction studied in this thesis are territory defence, mate attraction and parental care of offspring (Fig. 10.1). The main premise is that not all individuals of the same sex should exhibit the same reproductive behaviour at all times, but that these should show facultative adjustment to the broader environment in which they find themselves. In particular, we tested the notion that habitat quality (broadly speaking, condition) and social circumstances (i.e. breeding status) will affect the way individuals go about maximising their success in reproductive ventures. We wanted to identify some of the key processes that affect reproductive behaviour in songbirds, and identify some of the potential for interplay between these processes at different stages of reproduction. Basic questions are: how does variation in condition and social circumstances affect the use of sexual signals?, to what extent is sexual signal use plastic and optimised to individual circumstances?, how do continued investment in territorial behaviour or mate attraction affect parental care?, and how does condition affect parental care? In this chapter I would like to summarize the major findings of this thesis, discuss some of the key issues in a more general framework (with a focus mostly on recent literature), touch upon some methodological issues, and suggest some directions for future research.

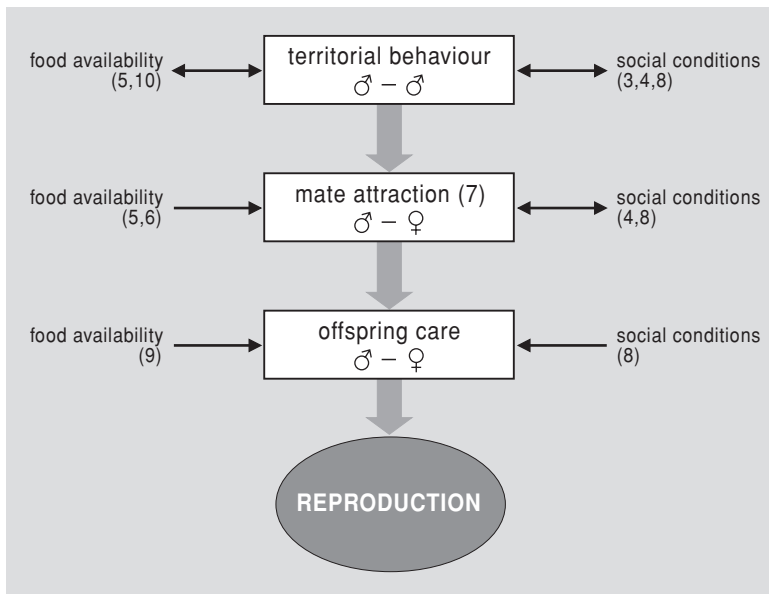


Figure 10.1. Simple flow diagram showing the main components of songbird reproductive behaviour studied in this thesis, and the hypothesised inter-relationships between these components, and variation in food availability (individual condition) and individual social conditions (e.g. pairing status). Numbers in parentheses indicate the chapters where these issues are addressed.

THE EVOLUTION OF TERRITORIAL BEHAVIOUR

Much of our knowledge about both inter- and intra-sexual selection processes comes from studies on birds. Competition and conflict between males for resources, whether for mates or territorial space or other resources, is central to sexual selection. There has been considerable interest over the last two to three decades in sexually selected traits, but most research attention has been focussed on the evolution of ornaments through female choice (Andersson 1994; Berglund *et al.* 1996). Nevertheless, it has always been acknowledged that intra-sexual selection, based on competition between males, can also have a substantial influence on the evolution of such traits as well (Andersson 1994; Berglund *et al.* 1996). Territorial interactions largely determine the breeding environment, and so have the potential to strongly influence the fitness and selection pressures that an individual experiences. Males of most species of songbirds compete to some extent for territorial space to control resources or their social arena.

Evolution of traits through intra-sexual selection can be driven by similar mechanisms to those that are thought to be involved in the evolution of most mate attraction ornaments (i.e. signalling the quality or condition of the bearer). However, it may also be strongly influenced by processes such as the need for individual recognition (Dale *et al.* 2001). This is a key distinction in the evolution of many signals (Dale *et al.* 2001), and of course, individual recognition may also be important from an inter-sexual perspective (e.g. O’Loghlen & Beecher 1999). In this thesis, I studied the role of song in two aspects of territorial behaviour in winter wrens (*Troglodytes troglodytes*). In the first place (chapter 3), I studied how individual recognition and previous experience affect territorial responses. In the second place (chapter 4 & Fig. 10.2), I studied the potential for song output during territorial interactions to act as a quality signal directed at fertile females. Table 10.1 summarizes the results of this thesis with regard to the variation found in male territorial behaviour.

Territorial behaviour and individual recognition

The evolution of conspicuous signals can be a means of resolving conflicts of interest in an economical manner. Instead of solving a conflict by costly fighting, competitors can assess each other through signals that honestly indicate the bearer’s quality or identity. Neighbour-stranger recognition and the ‘dear enemy’ effect (where territorial individuals display lower aggression towards familiar neighbours than unfamiliar individuals) is seen in many territorial animals (chapter 3) and represents a good example of the latter. Good fighters need to be recognizable and memorable to competitors, so signalling individual identity might be advantageous to competing males. The dear enemy phenomenon based on complex song in birds provides an excellent opportunity to study individual recognition, and the role of territory defence in the evolution and use of sexual signals.

Neighbour-stranger recognition based on song is very common in birds (chapter 3), and is likely to occur in both of the species studied in this thesis (the winter wren and the Australian reed warbler, *Acrocephalus australis*). However, at least two important aspects of dear enemy relationships remain poorly understood. First, the extent to which such rela-

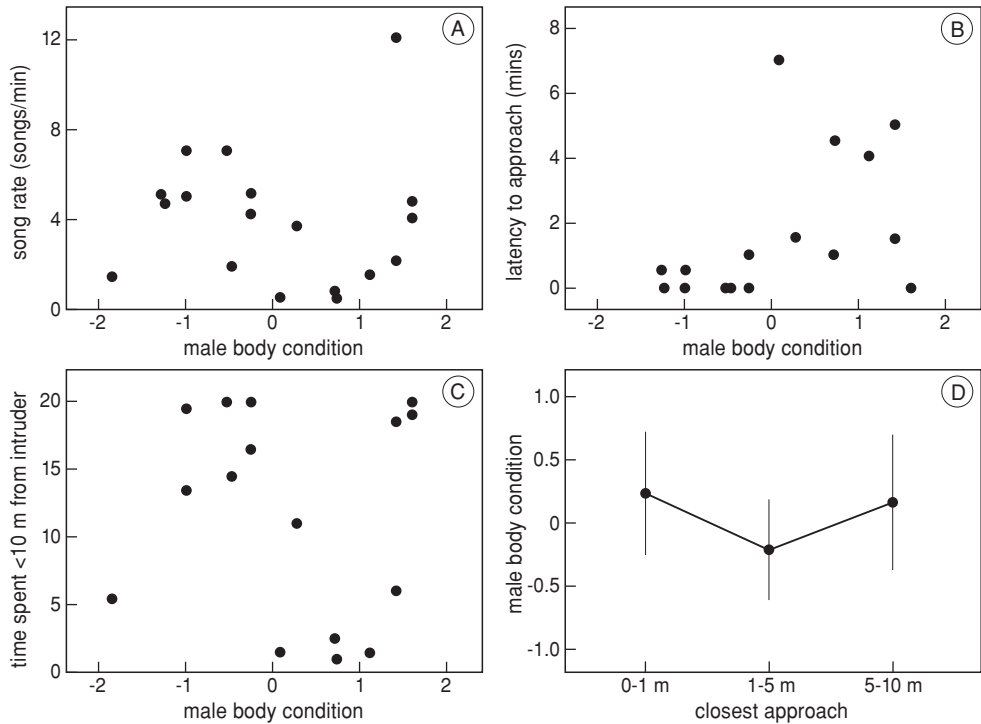


Figure 10.2. The relationship between male winter wren body condition and response to a 20 min simulated intrusion (model wren with song playback). The response variables are (A) song rate (songs per min; Spearman's rank correlation: $r_s = -0.187$, $p = 0.457$), (B) latency to approach <10m from intruder (min; $r_s = 0.192$, $p = 0.446$), (C) time spent within 10m of intruder (min; Pearson correlation: $r = -0.201$, $p = 0.424$), and (D) closest approach of intruder (<1m, 1-5m, 5-10m; logistic regression: $X^2 = 0.627$, $p = 0.731$; Nagelkerke pseudo $r^2 = 0.039$). Body condition is calculated as the studentized residuals of mass regressed over tarsus length ($t_{40} = 2.489$, $p = 0.017$, $B = 0.496 \pm 0.199$ SE) and capture date ($t_{40} = 3.026$, $p = 0.004$, $B = 0.018 \pm 0.006$ SE), based on a larger sample of individuals than that tested for territorial behaviour. See chapter 4 for further details of playback methodology.

Table 10.1. Summary of the results of this thesis regarding variation in male territorial behaviour. Numbers in parentheses indicate chapters where these issues are addressed.

Effect	Advertisement song rate	Song rate during contests	Territorial vigilance & approaching intruders
Condition dependent	Yes (5)	Fig. 10.2a	Fig. 10.2b-d
Female fertility	No (4)	Yes (4)	No (4)
Neighbour vs stranger	Not tested (3)	No (3)	Yes (3)
Previous experience with intruder	Not tested (3)	No (3)	Yes (3)
Intruder's repertoire	Not tested (3)	No (3)	Yes (3)

tionships are subject to proximate constraints on recognition, such as from song-type repertoire usage. Second, the role of a territory-holder's previous experience with known intruders in developing and maintaining such relationships. Using a series of playback experiments to simulate intrusions, we simultaneously tested these effects on male-male recognition and the dear enemy effect in winter wrens (chapter 3). Recognition of neighbouring males did not appear to be affected by song repertoire, because we did not detect an interaction between the ability of males to discriminate neighbours from strangers and song-types presented during trials. In contrast, even relatively small amounts of previous experience with competing males had a large effect on territorial aggression, with territorial males responding less aggressively (longer latency to respond) to males that they knew (and had 'defeated') based on a single recent intrusion. Furthermore, we found an intriguing relationship between latency to respond and the song-types presented by the playbacks (see also Box 10.1). In contrast to latency, no characteristics of intruder (neighbour or stranger, familiar or unfamiliar) affected song rate during experimental intrusions.

Territorial behaviour and sperm competition

Territorial behaviour and male-male competition in wrens was also investigated in relation to the breeding stage (from pre-fertilization through to post-breeding). This approach should provide insight into the interplay between inter-sexual selection processes and male-male competition, but has been often neglected by many previous researchers (perhaps due to the added complications of considering breeding parameters when studying male territorial behaviour). In songbirds, most species including winter wrens (chapter 7; Burn 1996) are genetically promiscuous to some extent (see Box 1.1). The fitness costs of cuckoldry to males in such species are likely to be considerable. Due to the decrease in certainty of paternity (Trivers 1972; Clutton-Brock 1991), one strategy for males in this case is to reduce their investment in offspring care (see discussion below). However, males may also attempt to minimise their loss of paternity by defending the mate or breeding territory against intruding males or, if females prefer to mate with high-quality males, advertising their own quality when their mate is fertile (e.g. through counter-singing performance, as discussed in chapter 4, or fertility announcement).

Møller's (1991a) 'fertility announcement' hypothesis has been the foremost contemporary hypothesis to explain a peak in male singing activity during their mate's fertile period. This hypothesis proposes that males sing more during the female fertile period because this will lead to greater paternity with females that prefer to mate with males that sing at a high rate. Our results did not specifically support this hypothesis in wrens (chapter 4), because males did not increase advertisement singing during the fertile period of their mate (when the risk of cuckoldry is highest). This is in accordance with many of the studies that have directly tested the predictions of this hypothesis (reviewed in Gil *et al.* 1999; Turner & Barber 2004; but see Forstmeier & Balsby 2002; Amrhein *et al.* 2004). However, our results did show that male wrens increase their investment in counter-singing during intrusions by competing males during their mate's fertile period. Since variation in song rate during intrusions was not reflected in other territorial behaviour

(measures of vigilance and approach behaviour), a discrepancy also found in chapter 3, this result is not consistent with the idea that males use territory or mate defence as paternity assurance. Instead, we argue that song rate in the context of male-male singing competitions, is actually driven at least partly by female choice. Specifically, if fertile females eavesdrop on competitions between males (i.e. gather information from such episodes without directly taking part), they may use the singing performance of males that they overhear during such contests as a basis for their subsequent choice of (extra-pair) mates. Primarily due to high nest predation rates in our study population, we could not determine whether the territorial performance of males was related to their actual mating success, but this effect has been shown previously in black-capped chickadees (*Poecile atricapilla*) and great tits (*Parus major*; reviewed in chapter 4).

As with traits used in mate attraction, the evolution of conspicuous signals used in competition within the sexes is thought to be a means through which animals can assess others in an effective and efficient manner. In many cases, these signals may have a dual function (e.g. Searcy & Andersson 1986; Catchpole & Slater 1995). They may operate in a similar way for both territorial and mate choice functions, by conveying honest information about certain qualities of the signaller. Our results in chapter 5 suggest that the use of flexible sexual signals such as song during male-male contests may also be influenced by the other side of the sexual selection coin, female choice. This may be a more widespread phenomenon than hitherto acknowledged. In order to avoid males that unreliably signal their quality, females might evolve to select mates from aggregations of males, where a trait is likely to be put to the test in male-male contests. Performance in such contests may be an effective way to enforce honesty (Berglund *et al.* 1996). If this is the case, females may often exploit intra-sexual signals, which contrasts with the idea that males exploit pre-existing female sensory biases (Berglund *et al.* 1996). More specifically, this may provide a framework for understanding why females may have evolved the habit of eavesdropping on singing contests between males in order to direct their choices of mates. It will be interesting to investigate further how males balance the potentially conflicting demands on dual-function signals of male-male competition and female attraction. Future research should look at variation in the composition of the songs used by male wrens during different breeding stages, in addition to the rate of singing, as this should help elucidate how intra- and inter-sexual processes together contribute to variation in song structure. Preliminary data indicates that male wrens employ tactics including song lengthening and song-type matching frequently during singing contests (Box 10.1).

In conclusion, I suggest that song complexity (e.g. song-type repertoire), a trait which is frequently linked to female attraction (Catchpole & Slater 1995), may be also strongly driven by its role in mediating territorial interactions among males. Conversely, recent evidence suggests that singing behaviour during male-male contests may be driven in part by selection imposed by female (extra-pair) mating behaviour and male efforts to compete for paternity, rather than just territorial space (Otter *et al.* 1999; Mennill *et al.* 2002; chapter 4). There is potentially a large amount of interplay between the way song functions in both inter-sexual and intra-sexual selection which is largely unrecognised, and how these

selective forces interact deserves greater attention in future studies. In the next section, I discuss more specifically how signals, including male song and the construction of display nests, can be honest indicators of male condition, and examine more directly how some male traits may function in reproduction in terms of female mate choice.

THE EVOLUTION OF MATE CHOICE

Honest signalling and the handicap principle

Females in many animal species have been shown to base their choice of mates on the degree of exaggeration of males' secondary sexual characters (Andersson 1994). These characters are thought to communicate information about a male's viability or attractiveness (Fisher 1930; Andersson 1994). An important prerequisite of such sexual advertisement is honesty. That is, the level of advertisement must reliably reveal the true qualities of the male (Grafan 1990), at least on average (Johnstone & Grafan 1993; Kokko 1993). The costliness of signals and the evolution of honesty gained renewed interest following Zahavi's landmark theoretical work, and has subsequently been the focus of much research in the field of sexual selection. Zahavi's handicap principle (Zahavi 1975, 1977) now provides the most commonly invoked and widely accepted mechanism to explain honesty in all natural signalling systems, particularly those involving mate choice. This principle proposes that traits that impose a cost on the signaller (i.e. are a 'handicap') may reliably advertise individual 'condition' (e.g. energetic or nutritional state; Nur & Hasson 1984). In essence, the idea put forward is that more viable males will be in better condition and better able to pay the costs of expression of costly signals, and thus express costly signals to a greater degree. Signal costliness should prevent cheating and thus ensure honesty (Grafan 1990).

Before discussing condition-dependent signalling further, two additional points should be considered. First, condition is rather loosely defined in this context (Nur & Hasson 1984). Males might be considered to be in better condition if they have better nutritional histories, better social status, are better foragers or occupy higher quality territories. Females would be expected to benefit from choosing males in better condition if that aspect of condition has a heritable genetic basis (Andersson 1994), in terms of good genes. However, even if that is not the case, females might also benefit from choosing males in better condition if males provide direct benefits such as territorial food resources or parental care. In this way, the handicap principle encompasses not only the good genes (indirect benefits) models of mate choice, but also direct benefits. In short, females that discriminate amongst males on the basis of condition dependent signals may get a high-quality territory and/or a mate with good genes. Second, it should be pointed out that the direct relationship between signal expression and physical condition need not always be positive, as the most viable males also express the most costly signals and these may 'cancel out' (e.g. Nur & Hasson 1984). Nevertheless, a positive relationship is the pattern that has been most commonly reported in previous studies on this topic (reviewed in Andersson 1994).

With these points in mind, one way to study the expression of condition dependent signals in a natural setting is to offer supplementary food as a means to artificially manipulate male condition. This experimental approach does not rely on direct assessment of the physical condition of males, but instead operates on the assumption that an increase in territorial food availability will result in an increase in male condition as defined above. As territorial food availability cannot be considered a heritable trait of males, this approach is only appropriate in systems where males typically provide direct benefits such as parental care, which is the case in most songbirds including Australian reed warblers. In this thesis, we used this approach to study two potential male mate attraction signals in Australian reed warblers, from the viewpoint of signal costliness and the handicap principle. The two signals studied were: 1) male song, a well-known sexually selected signal in birds, and 2) the construction of display nests, An unusual behavioural trait in the same species.

Condition-dependent signals

Bird song is often considered to be a good example of a reliable handicap signal due to the costs potentially associated with song production. In addition to costs related to social competition, as touched upon in part II of this thesis, other costs are thought to arise from the energetic demands of song production (e.g. Lambrechts & Dhondt 1988, Eberhardt 1994, Chappel *et al.* 1995, Oberweger & Goller 2001), the increased risk of depredation (Krams 2001) or the temporal constraints imposed on other activities such as foraging (e.g. Reid 1987; Galeotti *et al.* 1997, Saino *et al.* 1997, Lucas *et al.* 1999). These costs of production are particularly relevant to the handicap principle, however there is surprisingly little empirical data on the potential costs of bird song. While variation in song rate should clearly involve a change in energy expenditure, recent work (e.g. Ward *et al.* 2003, 2004) measuring the energetic demands (oxygen consumption) of singing directly in captive canaries (*Serinus canaria*) and pied flycatchers (*Ficedula hypoleuca*) has suggested that singing is not particularly energetically costly itself when compared to other normal activities, such as perching. Both of these studies indicated that the metabolic costs of singing, while 2.2-2.7 times higher than the basal metabolic rate, were in fact only marginally higher (1.05-1.12 times) than simply perching or standing. Thus, the actual extent and nature of the costs associated with singing in free-living birds remains a subject of current debate.

We found that male reed warblers that were provided with supplementary food on their breeding territories sang at a higher rate than controls (chapter 5). Thus, our data supports the notion that song production is constrained by food availability. In light of the aforementioned studies on the energetics of singing, this suggests that costs of song production may arise principally through constraints imposed on foraging (temporal constraints or reduced foraging efficiency). In line with this, song production did not appear to be affected by several weather variables including ambient temperature, which might be expected of a signal constrained by the energetic costs of production (e.g. Gottlander 1987, Reid 1987, Thomas 1999; Godfrey & Bryant 2000). Therefore, male song rate may be a good cue to territory quality, and the main benefit accruing to females choosing males with a high song rate could therefore be not just a high quality male (able to

acquire and defend a high quality territory) but also direct benefits arising from a good breeding territory (e.g. Yasukawa 1981; Alatalo *et al.* 1990a).

In chapter 6, we employed the same supplementary feeding experiment reported in chapter 5 to study the functional significance of multiple nest building. Australian reed warblers build two types of nest structures: ‘type I’ nests, which are used for eggs and nestlings, and ‘type II’ nests, also sometimes referred to as ‘display’ nests or ‘cock’ nests, which are structurally distinct from type I nests, never support eggs, nestlings or adults, and are not essential for successful breeding. Interestingly, more type II nests were built on territories supplied with supplementary food, indicating that constructing such nests, like singing, is constrained by food availability. It may therefore function as a ‘non-bodily’ condition-dependent signal. Although several bird species build more than one nest (see studies reviewed in chapter 6), the function of most multiple nest building remains unclear. Notably, this interesting system differs from other similar ones in that the additional (type II) nests can only function for display, i.e. they are distinct from breeding nests, and breeding can take place with only a type I nest.

Taken together, the results in chapters 5 and 6 suggest that food availability may be a primary mediating factor in avian handicap signals (Berglund *et al.* 1996). This would make territoriality integral to the process in species that gain most or all of their food from their defended territory. We also examined the potential for condition-dependence in song output and other measures of territorial aggression during experimental intrusions in the wren (Fig. 10.2). We compared the strength of male responses with an estimate of male body condition (mass controlled for body size). In this case, we found no effects of body condition on any of these behavioural traits. However, such non-experimental data should be interpreted with caution. This is particularly true as the optimum body condition may not be the heaviest mass for a given body size, but lower (e.g. to reduce flight or predation costs; Witter & Cuthill 1993). Further experimental work will need to be carried out to investigate the potential condition dependence of sexual signalling in the context of territory defence.

The existence of multiple condition-dependent signals (e.g. singing and nest-building) may also limit dishonest signalling by males (Møller & Pomiankowski 1993), even if the information content of the signals is usually similar. For example, multiple condition dependent ornaments may allow more reliable signalling and assessment of male condition, especially when environmental circumstances (e.g. high predation or abnormal weather) make one signal less suitable at a given time. Although there is a rapidly growing body of theoretical and empirical research into the evolution of multiple ornaments, few studies have examined both physical and non-bodily ornamentation simultaneously; although studies on Australian bowerbirds (family Ptilinorhynchidae) being one notable exception (e.g. Endler *et al.* 2005). Australian reed warblers would now seem to provide another useful system in which this could be further studied.

The handicap principle revisited: the importance of flexibility

From an individual optimisation viewpoint, behavioural signals such as singing and nest building should be viewed as ‘strategic choice’ handicap signals (Grafen 1990). This is

where each signaller (male) chooses the level of handicap they advertise in a way that maximises their fitness, given their individual circumstances. Our results indicate that both song and building type II nests are constrained by food availability in Australian reed warblers. This suggests that these activities are costly in some way, which is a key feature for all handicap signals. However, two additional features are required in order for signals to function as effective strategic handicap signals (Zahavi 1977; Nur & Hasson 1984; Grafen 1990), which I will now briefly discuss.

First, the handicap must be phenotypically plastic, i.e. predictable variation in the level of handicap displayed by an individual (Zahavi 1977; Nur & Hasson 1984). This is an important feature that means the signal in question can be adjusted in response to variation in the environment. This will allow the signal to reliably and accurately convey the *current* condition of the bearer. Clearly, such flexibility will be important when there is temporal or spatial variation in the costs of a trait, by allowing the trait to be maintained at the optimum level of expression (Brooke *et al.* 1998). Plasticity will itself have a genetic basis (Nur & Hasson 1984), and in many cases flexibility in the expression of costly signals may be under selection, and more important to fitness than the actual value for a particular signal (Jordan & Snell 2002). In showing predictable effects from randomly supplying supplementary food to experimental groups, we already demonstrated some degree of behavioural flexibility. Additionally, our experimental design (providing supplementary food only every other day) allowed us to determine whether male reed warblers are able to adjust their level of song production according to food availability on a daily basis. This shows that this signal can be highly flexible (chapter 5). Meaningful data on the plasticity of nest-building behaviour were not available. This is because although type II nests appear to be built very rapidly (in the order of hours rather than days), males accumulate relatively few nests (compared to the number of songs sung) and finding them is very labour intensive. This means that day-to-day changes in the rate of nest building are hard to record.

The other essential component of strategic handicap signals is that the costs of signalling must increase differentially for individuals of different quality (Grafen 1990). Consider the case of a signal where individuals are able to choose their level of signalling strategically, and where it is always advantageous to signal the highest condition or viability possible. All males would then do best by signalling the highest possible condition, irrespective of their actual state. Therefore, in order for strategic handicap signals to be honest, not only must the signal be costly, but the marginal cost of the signal must be higher for an individual of low viability than for one of high viability. We are not aware of how the costs or constraints related to song production or nest construction differed among individuals in our studies, and unfortunately this point has not been adequately investigated in most other studies on these topics. It would be most feasible to test these ideas by manipulating sexual signals in a species where information about the intrinsic quality of males is available (e.g. age, spring arrival timing or extra-pair mating success).

Condition-dependent signals and female choice

So far, I have discussed male advertisement purely in terms of the costs of signalling, as this is what is thought to maintain essential honesty. However, a full understanding of

any signalling system requires knowledge not only of the costs but also the benefits of producing the signal. The function and benefits of singing has not been directly tested in the Australian reed warbler, although it is clear from playback trials that song is used extensively in male-male competition by this species (J. Welbergen & M. Berg, unpubl. data). In other species, a positive association between male song rate and pairing or mating success has been reported in several field studies (Radesäter *et al.* 1987; Alatalo *et al.* 1990a; Buchanan & Catchpole 1997; Nyström 1997; Møller 1998), but not in others (e.g. Greig-Smith 1982; Davies & Lundberg 1984). Playback of higher song rates has been positively related to female display rates (e.g. copulation solicitation) in several studies (Wasserman & Cigliano 1991; Collins *et al.* 1994). Furthermore, song rate has been associated with female fertility (Møller 1991a; Pinxten & Eens 1998; Forstmeier & Balsby 2002; Amrhein *et al.* 2004; chapter 5; but see Gil *et al.* 1999).

On the whole, these results suggest that the relationship between male song rate and female choice may be far from simple or universal. In some cases, particularly those relating mating success to natural variation in song rates, this effect is potentially confounded by female preferences for high quality territories rather than song rate itself (Nyström 1997). Model systems in which females are attracted to unoccupied nests on the basis of song playback, such as pied flycatchers (*Ficedula hypoleuca*; Eriksson & Wallin 1986) and house wrens (*Troglodytes aedon*; Johnson & Searcy 1996) provide an excellent opportunity to gain further insight into the direct effect of song rate on female choice.

The multiple nests of Australian reed warblers have not been described in detail previously. The function of multiple-nest construction remains unclear in this and other species where it has been studied (reviewed in chapter 6). As with song rate (chapter 5), unpaired male reed warblers that built more type II nests as a result of supplementary feeding did not appear to be more successful at becoming paired. We also conducted an experiment involving the addition of artificial type II nests to the breeding territories of unpaired males, to investigate the effect of the number of type II nests on pairing success in the absence of the potentially confounding effect of food availability. This experiment did not reveal a change in pairing success in relation to display nests either. One possibility to explain these results is that our study population was relatively successful and that almost all males were able to pair at some point during the season. Perhaps more detailed data on pairing date would reveal a female preference for certain males. Unfortunately, our experiment was only conducted late in the breeding season and detailed information on pairing dates was not collected due to time constraints. Alternatively, female preferences for male signalling performance may be revealed by their behaviour subsequent to pairing, such as extra-pair mate choice (Mennill *et al.* 2002), or their investment in clutch size or incubation attendance (see discussion about chapter 9 below) or offspring feeding. For example, several studies indicate that females lay larger clutches when paired with high quality males (e.g. Slagsvold *et al.* 1988, 1990). As male offspring care seems to increase with food availability in Australian reed warblers (M. Berg & J. Welbergen, unpubl. data), signals that reliably communicate food availability might be a useful cue for females to how much offspring care they can expect from a male. This might in turn lead them to produce a larger clutch in anticipation of a higher level of male care (Slagsvold & Lifjeld 1988; Davies & Hatchwell 1992).

Genetic diversity and mating success

A leading explanation put forward to explain female promiscuity in birds is to increase the genetic quality of their offspring, and several hypotheses based on the genetic benefits of extra-pair mate choice have been put forward (Box 10.2). One process through which this may occur is for females to prefer to copulate with the most heterozygous or genetically diverse males. In this way, promiscuous females may avoid the costs of inbreeding. It was from this perspective that we set out to investigate the occurrence of extra-pair paternity, and the relationship between cuckoldry and male genetic diversity, in wrens (chapter 7). We used a panel of five microsatellite markers identified using efficient cross-species amplification approach (chapter 2). Genetic diversity was calculated using three commonly used indices of individual heterozygosity or allelic diversity (see Box 10.3). We detected extra-pair young (EPY) at eleven of the 29 nests analysed (38%), accounting for 25 of 134 typed offspring (19%). Surprisingly, we found that more heterozygous males (estimated by the proportion of heterozygous microsatellite loci) were more likely to have EPY in their brood but, once cuckolded, had fewer EPY than less heterozygous males (Fig. 10.3). In addition, more genetically diverse males (measured by the distance between alleles at microsatellite loci) had higher body condition (body mass and mass controlled for body size), but we found no associations between male morphology and paternity loss. Due to high nest failure rates, the relationships between male or offspring heterozygosity and other measures of reproductive success could not be established in this study.

Unfortunately, our current data is not sufficient to offer a satisfactory explanation for these unexpected results. In order to obtain a better picture of whether females seek males with high heterozygosity for extra-pair mates, we need to examine the heterozygosity of successful extra-pair sires relative to the heterozygosity of the males they cuckold. Since our current results indicate most strongly that male heterozygosity is related to male success at obtaining paternity in their own nest, another useful step might be to examine how heterozygosity of males relates to traits that might be involved in preventing cuckoldry. This is because males with low heterozygosity might adopt a strategy of paternity assurance, while more heterozygous males may instead adopt a strategy geared towards seeking additional mating opportunities even at the expense of losing some paternity in their own nests. This may be particularly true for polygynous species such as wrens (but see Burn 1996). In songbirds traits that might prevent cuckoldry could include advertisements of quality such as singing at a high rate (Møller 1991a; chapter 4) or nest building (Evans 1997a, 1997b; chapter 6), territory size and territorial behaviour (Møller 1990; Langmore 1996; Eason & Hannon 2003) and mate guarding (Møller & Birkhead 1991; Komdeur *et al.* 1999). However, for such a mechanism to give rise to the patterns described in chapter 7, the male paternity assurance strategies adopted by males with low heterozygosity would have to be rather effective. Furthermore, we found no evidence that females select more heterozygous males as extra-pair mates (although a direct comparison of the heterozygosity of within-pair and extra-pair males would be required to confirm this).

On the whole, a compelling picture seems to be emerging from recent work, at least in birds, regarding the important role of heterozygosity (genetic compatibility, genetic diver-

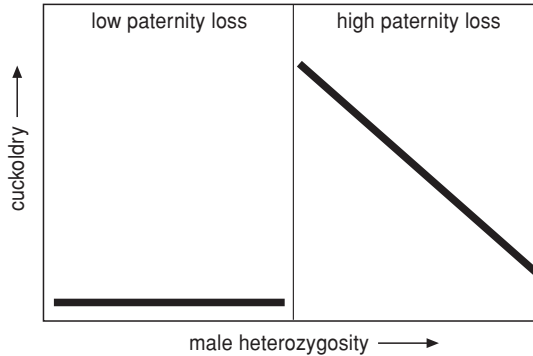


Figure 10.3. Schematic diagram representing the effect of male heterozygosity on the probability of being cuckolded observed in chapter 7. Note that heterozygosity data in chapter 7 is plotted as internal relatedness, which is strongly negatively related to simple measures of heterozygosity as represented here.

sity and rare alleles) in (extra-pair) mate choice. There is great scope for future research into the potential role of heterozygosity in mating behaviour in general. More specifically, future research in this field may shed considerable light on why female birds seek extra-pair fertilizations. This avenue of research differs fundamentally from previous ‘good genes’ and Fisherian runaway models of mate choice, which generally assume that there are universally ‘high quality’ and ‘low quality’ genotypes for which females are selecting (Fisher 1930; Hamilton & Zuk 1982; Andersson 1994; Kokko *et al.* 2003). In contrast, heterozygosity concerns non-additive genetic benefits: heterozygosity itself is not heritable, and the heterozygosity of offspring is a property not only of the male’s genotype but also the female’s genotype. Obviously, issues surrounding mate choice for heterozygosity are intricately linked with genetic compatibility and inbreeding avoidance.

Signalling genetic diversity

If females do actively base their mate choice on male genetic diversity, how could females select this male quality? There are two main possibilities. One is that selection for sperm from more or less genetically diverse males, or sperm carrying particular alleles, may occur within the female reproductive tract (cryptic female choice). This possibility has been supported by a study by Olsson *et al.* (1996), which provided evidence that female Swedish sand lizards (*Lacerta agilis*) selected sperm that reduced inbreeding. In birds, females are known to select sperm based on male dominance (Pizzari & Birkhead 2000) and age (Wagner *et al.* 2004), and not all copulations with (extra-pair) males will necessarily lead to fertilization (Fossøy *et al.* 2006). In the future it will be interesting to examine such aspects as sperm volume and the timing of copulations when relating male genetic diversity to parentage, as these factors are known to influence the outcome of sperm competition (Birkhead & Møller 1993).

The second possibility could be that females choose to mate with males with greater genetic diversity (direct female choice). If this diversity is associated with the health or

condition of males, this could be expressed by condition dependent cues that females could use for assessment of potential mates, such as plumage ornaments or song. There is mounting evidence for this in songbirds. In blue tits, heterozygosity was positively related to ultraviolet-blue crown coloration, which is a sexually selected trait used by females in this species (Foerster *et al.* 2003). Two studies have reported a relationship between acoustic features of male song and a measure of genetic diversity. One of these studies found a relationship using only d^2 in sedge warblers (*Acrocephalus schoenobaenus*; Marshall *et al.* 2003), while the other, studying subdesert mesites (*Monias benschii*; Seddon *et al.* 2004), found a relationship with individual heterozygosity and IR but not with d^2 . A third study has reported that the song repertoire of male song sparrows (*Melospiza melodia*) declined with increasing inbreeding, using a true measure of inbreeding obtained from pedigrees (Reid *et al.* 2005). So far, the role of male heterozygosity in female choice has not been reported directly in any of these species, although extra-pair or extra-group paternity is known to occur in all four species (e.g. Gullberg *et al.* 1992; Langefors *et al.* 1998; Buchanan & Catchpole 2000; Major & Barber 2004; Seddon *et al.* 2005). Despite this early progress, further studies are needed to examine natural variation in potential cues, such as song, morphological characteristics and territorial performance, in order to determine reliable connections between genetic diversity and assessable traits. If direct female choice for male genetic diversity occurs in winter wrens, vocal traits are probably the most like means of signalling of this quality as males possess complex songs but no obvious plumage ornaments. Indeed, wrens provide considerable scope for studying this issues, as they have a reasonably high rate of extra-pair paternity, relation between male heterozygosity and female promiscuity, and a high degree of structural song complexity (Box 10.1).

THE EVOLUTION OF PARENTAL CARE

Trade-offs and the level of male care

Care of offspring, together with somatic activities and mating success, are the major components of successful reproduction in many animal taxa. The costs and benefits of parental care has a large influence on mating systems, so a good understanding of the proximate mechanisms that determine the pattern of parental care is crucial to our understanding of mating system evolution. Some degree of parental care is ubiquitous in songbirds (e.g. Lack 1968; Oring 1982; Clutton-Brock 1991; Davies 1991), and most species display bi-parental care. This care may take several forms, but the most commonly encountered in birds are incubating the eggs and providing food to the offspring. Providing this sort of care to offspring is likely to be a time-consuming and energetically costly activity, so caring for current offspring will probably somehow reduce the ability to produce future offspring (Lessels 1991; Stearns 1992; Daan & Tinbergen 1997). Individuals can be expected to optimize the level of care they provide during each breeding event. Parents with dependent offspring and other concurrent responsibilities such as maintaining a territory or seeking further mating opportunities, must decide how to divide resources amongst these competing pursuits.

Although male care is common in songbirds, it is usually not obligatory for the survival of the offspring (Bart & Tornes 1989; Dunn & Hannon 1992; Dunn & Robertson 1992). This is true for Australian reed warblers, where nestling starvation is rare regardless of how little care the male parent provides (M. Berg & J. Welbergen unpubl. data). Therefore, males have the opportunity to balance their investment in caring for current offspring (parental effort, PE) with competing activities that are important for future reproduction or survival, in order to achieve the best fitness. Typically, these competing activities will include pursuing further (extra-pair) fertilizations (mating effort, ME) or maintaining their territory (territorial effort, TE). Trade-offs between these activities can be expected when time and energy are limited, and this conflict will usually be greater for males and in highly territorial species where extra-pair fertilizations are common throughout the offspring-rearing period (Magrath & Elgar 1997; Magrath & Komdeur 2003). The extent to which a male should invest in PE, ME and TE when these activities compete for attention could depend on several factors: (i) variation in female competence to provision a brood without male help (e.g. Whittingham 1989; Beletsky & Orians 1990; Dunn & Robertson 1992), (ii) the value of the brood (e.g. Westneat *et al.* 1990; Magrath & Elgar 1997; Komdeur *et al.* 2002), (iii) stochastic factors that may affect the chance of successful reproduction, such as nest predation (e.g. Komdeur & Kats 1999; Veiga *et al.* 2003), and (iv) the opportunity for males to gain additional matings (e.g. Smith 1995; Komdeur *et al.* 2002), such as the experience or quality of the male (e.g. Morton & Derrickson 1990), the social organisation of the population (e.g. Mulder *et al.* 1994), or the temporal (e.g. Westneat 1988; Magrath & Elgar 1997) or spatial availability of potential mates (Westneat & Sherman 1997). Surprisingly, many of these issues have only infrequently been tested empirically in birds.

A recent comparative study linked avian families with male-only care to low population density (considered an index of remating opportunities for both sexes) and vice versa (Owens 2002). Importantly, this pattern should not necessarily be restricted to systems featuring uni-parental care (Owens & Bennett 1997). This study suggests that variation in density might also mediate relative parental care of the sexes in species with bi-parental care due to remating opportunities. In chapter 8, we show that male PE (nestling feeding rate) was much lower in territories with many neighbours than in territories with few neighbours, while female PE remained unchanged. Of all the influences on male PE outlined above, we believe that the spatial availability of neighbouring territories is the main variable differing between the groups analysed in this study. We analysed the number of neighbouring territories independently of actual density or territory size, by comparing territories with a (mostly artificially maintained) linear spatial arrangement to those with a clumped spatial arrangement (in general, higher densities can be expected to lead to more neighbours for each territory). Furthermore, brood sizes were not related to the spatial arrangement of territories, and there was no clear differences in habitat quality between groups of territories with a high or low number of neighbours. Accordingly, we interpret this result as suggesting that behavioural trade-offs for males lead to a reduction in paternal care when the chance of encountering conspecifics near the breeding territory is greater.

In order to confirm the potential role of such a mechanism, and examine the potential differences in TE (aggression towards male intruders) or ME (attention to female intruders) in this system, we conducted an experiment. We experimentally introduced caged reed warblers of known sex to territories with nestlings, and compared the resulting changes in PE with that elicited from a control empty cage. While male reed warblers vigorously defend their territories throughout the breeding season against intruding males, the presence of females on the territory may be interpreted by males as an extra-pair mating opportunity. Females in this and other species (Double & Cockburn 2000 and references therein) frequently undertake extra-territorial forays. The results further confirmed that male PE is strongly reduced by the presence of conspecifics of either sex. The results further indicated that trade-offs with TE and ME have similar effects male PE. Male feeding rates were lower in the experimental presence of either a female or a male conspecific, while female behaviour was not affected. Females did not appear to respond to presence of conspecifics of either sex at all. In several cases, females were observed continue brooding nestlings apparently unperturbed by the experimental presence of a conspecific only a few metres from the nest (and the ensuing commotion involving their mate). Thus, females do not appear to assist with territory defence (at least during the nestling-rearing period), nor do they seem to do anything to repel intruding females which compete for the attention of the male. Neither sex reduced feeding significantly in response to a caged greenfinch. Our results suggest that both extra-pair mating and territory defence, mediated by local breeding density, could be important factors in the evolution of paternal care and mating systems. This suggests that both the requirements of territorial and mating activities could substantially affect the expression of male care, and thus can be expected to influence the evolution of parental care and mating systems. In many natural systems, these factors are likely to be largely mediated by breeding density, synchrony and local food availability. Ultimately, trade-offs such as those between PE, ME and/or TE might be resolved over evolutionary time such that responses become fixed rather than facultative to some extent (Westneat & Sherman 1993; Wright 1998; Whittingham & Dunn 2001). This may explain some sex differences in parental care in songbirds. For example, trade-offs facing males between PE and ME are likely to be greater during the early stages of reproduction in reasonably synchronously breeding populations, which explain why males in many species with biparental care of some sort do not contribute to incubation (only 30% of the 56 Passeriforme families for which data is available display male incubation; Deeming 2001).

Female compensation: energetic ceiling or adaptive response?

Interestingly, there was no evidence from either the observational (chronic reduction in male care) or experimental (acute reduction in male care) components of our study that females compensated for reduced male care by increasing their own level of care. Because females did not compensate for reductions in male PE, the level of male food provisioning was associated with overall reductions in food delivered to the offspring. Such variation in total food delivery is likely to be one of the most important influences on the success of reproduction (Nagy & Holmes 2004). We provide evidence that this leads to considerably

lower nestling growth in territories with many neighbours, where males are caring less. Although nestling starvation is rare in Australian reed warblers, it is likely that such markedly lower growth would result in lower post-fledging survival or future reproductive success, particularly in long-distance migratory species such as reed warblers. Several studies in passerine birds have shown that survival or future reproduction of young after leaving the nest is closely related to their body mass or body condition (mass corrected for body size) prior to fledging (e.g. Perrins 1965; Garnett 1981; Nur 1984b; Gustafsson & Sutherland 1988; van Noordwijk *et al.* 1988; Alatalo *et al.* 1990b; Tinbergen & Boerlijst 1990; Hochachka & Smith 1991; Haywood & Perrins 1992; Linden *et al.* 1992; Verboven & Visser 1998; Merilä *et al.* 1999).

An incomplete or completely absent degree of female compensation is common in studies where male care has been experimentally reduced or removed (e.g. Davies & Hatchwell 1992; Lozano & Lemon 1996; Markman *et al.* 1996). This may be due to a lack of female knowledge about the workload of the male, an 'energetic ceiling' to female parental care (Drent & Daan 1980; Tinbergen & Verhulst 2000), or an adaptive response on the part of females (Houston & Davies 1985; Winkler 1987; Wright & Cuthill 1989). A lack of female knowledge about male care seems unlikely as chick hunger and begging behaviour should provide an effective cue to relative level of care being provided by the male. An energetic ceiling on female brood care has been shown experimentally by Tinbergen & Verhulst (2000). However, several studies have shown that both male and female passerines can increase their parental investment in response to experimentally increased brood demand (e.g. Sanz & Tinbergen 1999; Wright *et al.* 1998), although this may not always be the case (Moreno *et al.* 1995) or may be population or species specific. To investigate this issue, it may be possible to increase the workload of care-giving parents by enlarging brood sizes or handicapping parents, and observing the subsequent changes in parental effort. If females are subject to an energetic ceiling which constrains parental care, one would expect males but not females to increase food provisioning with brood enlargement. On the other hand, one would expect females but not males to decrease food provisioning when both parents are handicapped. Finally, lack of female compensation may be an adaptive response, because complete compensation would mean selection would favour males that provided less care (Houston & Davies 1985; Winkler 1987; Wright & Cuthill 1989). Usually, reduced male care has been reported in conjunction with some decline in fitness components, such as a decline in nestling growth as we present in chapter 8. Males that do not maintain their contributions to offspring care will suffer reduced offspring growth and the associated negative effects on their fitness. However, this might be overcome by the fitness gains of the other activities concerned: males should choose the option that leads to the greatest reproductive success (Davies & Hatchwell 1992). The problem for females is that compensating for a lack of male care can be expected to lead to a reduction in her fitness in other respects, such as reduced survival or ability to invest in subsequent breeding attempts.

Certainty of paternity

If the rate of extra-pair paternity does increase with breeding density (Birkhead & Møller

1992a; Westneat & Sherman 1997; Møller & Ninni 1998; Griffith *et al.* 2002; Charmantier & Perret 2004; Mougeot 2004), then an interesting alternative explanation for a reduction in male PE with increasing breeding density could be a change in the male's certainty of paternity. Certainty of paternity is likely to be a major factor influencing the optimal strategy for care-giving males (Ligon 1999), because uncertain parentage is likely to reduce the potential fitness gains of males from parental care to a level lower than that of females (Houston 1995; Queller 1997; Wade & Shuster 2002; Kokko & Jennions 2003). Paternal care should be less common in species where the rate of extra-pair paternity is high. This is particularly interesting in most songbirds given the widespread occurrence of extra-pair fertilizations in this group (see Box 1.1). Several previous studies have provided evidence that males adjust care to their degree of relatedness to the brood (e.g. Møller 1988; Davies *et al.* 1992; Wright 1992; 1998; Westneat & Sherman 1993), but the idea remains contentious even within the same species (Sheldon 2002). For example, Dixon *et al.* (1994) found this to be the case in reed buntings (*Emberiza schoeniclus*), but the same pattern was not found when the study was replicated (with a larger sample size) in a Dutch population of reed buntings (Bouwman *et al.* 2005). We cannot rule out a role for certainty of paternity in controlling the level of male care in Australian reed warblers. However, our preliminary analysis of microsatellite parentage data and feeding observations indicates that male reed warblers do not reduce their level of care in response to cuckoldry. Furthermore, our experimental approach provides evidence that the pursuit of other activities (TE and ME) by males, irrespective of their certainty of paternity, could be sufficient to explain most of the variation in male PE we observed in relation to the spatial presence of conspecifics.

In the future, it will be interesting to investigate whether males also vary their level of PE in relation to *temporal* variation in the availability of additional mates (i.e. local breeding synchrony). We could do this by comparing male feeding rates to the number of fertile females in surrounding territories, as has been done to investigate male investment in incubation in fairy martins (*Cecropis ariel*; Magrath & Elgar 1997). This may allow a clearer interpretation than studying spatial variation. This is because if an effect similar to that described in chapter 8 is shown, it is much more likely to have to do with the mating opportunities provided by the presence of fertile females than territorial defence or other unmeasured differences among territories. Future studies may even be able to test this issue experimentally by manipulating local breeding synchrony through careful disruptions to the early nesting cycle.

Incubation strategies: a female perspective

While both sexes share parental responsibilities to at least some extent in most songbirds, such as feeding offspring and guarding the nest, there can also be considerable division of labour between the sexes. One of the best examples for this is incubation. Although males of some songbirds contribute substantially to incubation, it is often a female-only duty in this group (e.g. Lack 1968; Clutton-Brock 1991). Often, only the female develops a brood patch. It appears that incubation has become such a specialized activity in many species that males have lost the ability to do it altogether. Variation in

incubation can be crucial to breeding success, and incubating the eggs properly is a vital component of reproduction for all altricial birds because the embryo is very vulnerable to perturbations in the developmental conditions (Lundy 1969; Drent 1975). The effects of variation in incubation capacity on life-histories can be wide-ranging, with likely effects on traits such as clutch size (Reid *et al.* 2001).

In chapter 9, we used video observations and automatic temperature loggers inside the nests of Australian reed warblers to examine incubation behaviour. In this species, only the female contributes to incubation (chapter 9). One of the main factors that is often thought to affect the level of incubation is the energetic constraints of warming the eggs. Accordingly, we tested the hypothesis that the level of incubation is constrained by food availability. If food is abundant, the energetic constraints may be reduced and the time spent incubating (incubation attendance) may increase. Moreover, the onset of incubation in relation to clutch completion may be advanced, resulting in a higher degree of hatching asynchrony. We tested these ideas using a supplementary feeding experiment. Supplementary food was provided to individual nests during the incubation period, and incubation attendance was measured with temperature loggers at nests receiving supplementary food and control nests. Hatching asynchrony was inferred from mass and size differences between siblings shortly after hatching. As in chapters 5 and 6, supplementary food was provided only every other day, which allowed us to infer the degree of flexibility in incubation regimes and provided an internal control. Our results showed that incubation attendance did increase with food availability. In addition, hatching asynchrony was greater in nests with supplementary food, suggesting that the onset of incubation was advanced in these nests. The increase in hatching asynchrony with higher food availability may simply be the incidental result of lifting constraints on the amount of time a bird can spend on the nest, particularly before clutch completion when she is still producing eggs. However, we speculate that the increased hatching asynchrony may be an adaptive response to higher food availability. Hatching asynchrony may be advantageous in allowing the first laid eggs to hatch sooner and fledge sooner, thereby reducing the risk from nest predators. However, birds may only be able to support an asynchronous brood, where the smaller nestlings will be at a competitive disadvantage to their larger siblings, under conditions of high food availability.

While our results do appear to support the view that energetic costs limit incubation, this may not necessarily be the case. It is possible that the costs of many common activities away from the nest actually exceed those of sitting on the nest (see Cresswell *et al.* 2004, although this study was performed in an arctic breeding bird). One possibility is of course that the costs really stem from temporal constraints on foraging, as might be quite likely in the case of singing (chapter 5). Alternatively, there is some evidence for a fitness cost to incubation in terms of parental survival or reduced success of subsequent broods (de Heij *et al.* 2006). It is possible that these costs of incubation may be ameliorated by higher food availability.

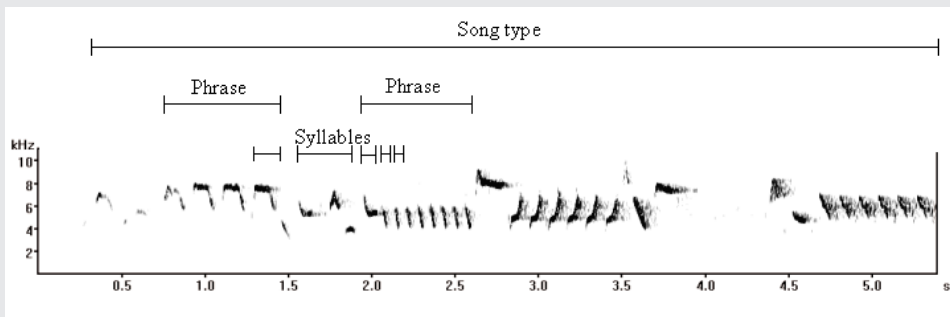
Another possible explanation for our results that cannot be discounted from the current data is that of differential allocation (Burley 1986; Sheldon 2002). Recently, Gorman *et al.* (2005) have found support for this idea in zebra finches (*Taeniopygia guttata*). They

manipulated the physical attractiveness of males with differently coloured leg rings, and found that females increased their share of incubation when mated to more attractive males. As both sexes regularly contribute to incubation in zebra finches, the authors therefore also point out that this could be due to a reduction in male care and female compensation. In our case, males on territories with high food availability are likely to signal their higher condition, for example through singing (chapter 5) or nest building (chapter 6). Therefore, females may respond by increasing their investment in the male's offspring, resulting in an increase in female incubation attendance independently of the constraints imposed on incubation by food availability. It would be difficult to disentangle the effects, because of the potentially confounding effects of food availability on both the cues to male quality and incubation attendance. A potential solution might be an experiment that positively manipulates food availability while simultaneously depressing female condition or increasing her work load, particularly in relation to incubation. The latter might be achieved by cooling the nest, enlarging the clutch, attaching small weights or selectively removing flight feathers.

Future work will be required to examine the fitness outcomes of such changes in incubation attendance and hatching asynchrony. In particular, more accurate data on the timing of fledging and nest predation would be useful to establish the benefits of increased hatching asynchrony. At this stage, we do not know how food supplementation or hatching asynchrony affected fledging time in reed warblers. Unfortunately, reed warblers have a poorly defined fledging process, whereby they gradually venture further from the nest amongst the dense reeds over a period of days. This characteristic probably helps them escape nest predators but makes it very difficult to establish the timing of fledging. A solution to this problem might be to develop some sort of measure of escape abilities, such as an estimate of variation nestling mobility through a standardized reed bed "maze" in relation to age and size.

BOX 10.1. TERRITORIAL INTERACTIONS AND THE USE OF SONG REPERTOIRES

The dual function of song is well recognised (e.g. Catchpole & Slater 1995). However, most studies on the evolution and maintenance of song complexity have focussed on its role in female choice. Currently, there is growing awareness of the role of male-male competition in shaping song complexity (e.g. Leitaó *et al.* 2006). Birds are known to use many strategies involving variation in song structure and song-type usage during intra-sexual interactions as a means of communication, such as overlapping, song-lengthening and song-type matching (reviewed in Vehrencamp 2000; Collins 2004). It is thought that these aspects of singing behaviour may contain considerable signal value. More recently, intriguing effects have been found involving variation in the acoustic structure of the song itself (Illes *et al.* 2006). The figure below shows a spectrogram of a typical male winter wren song, and the labels identify the components that make up the song.



Most of the studies in this thesis were primarily concerned with variation in the absolute amount of song output, without regard to the intricacies of the structure of the songs themselves (although results in chapter 3 provide a hint to the possible importance of song-type to territorial interactions in wrens). However, the highly variable song structure and ease of empirical study make the winter wren an attractive and tractable study system for these questions. Kroodsma (1980) has described the winter wren song as “the pinnacle of song complexity”. Wrens achieve such extraordinary complexity by combining numerous syllables (up to 26 in our studies) in a varying sequence to form their songs. Many syllables are repeated consecutively several times during a song at rapid succession (“trills”), a pattern that is thought to be related to aggression and dominance (e.g. Leitaó & Riebel 2003). In the winter wren populations studied in this thesis, individuals possessed up to 6 song types, which is typical for this species in Europe (see also Kroodsma & Momose 1991; Catchpole & Rowell 1993). Each song type comprised a mean of 18.7 ± 3.7 SD syllables, while average song length was $5.3 \text{ s} \pm 0.9$ SD (range 3.9–8.4 s). Throughout the population, we identified 66 distinct syllable variants.

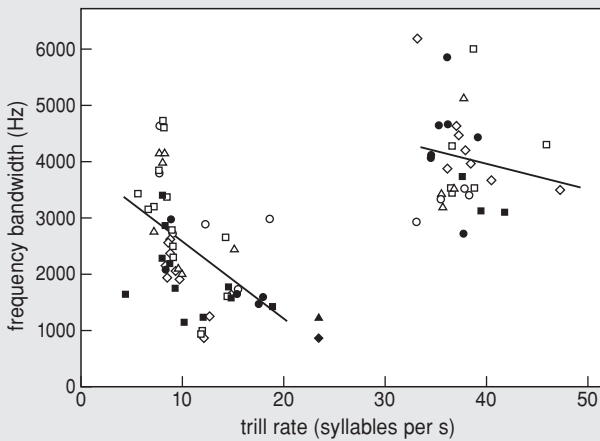
These values apply to advertisement songs, i.e. songs recorded during normal singing bouts, when males were not in direct singing contests with intruders (see chapter 3). During simulated singing contests (song playback), wrens often responded with much longer songs (songs up to 25 s in length were observed), even when the intruder (playback) was not using unusually long songs. These longer songs were typically produced by matching song types of the intruder, and repeating one of the phrases or sequences of phrases several more times than in the original song-type. Thus, song lengthening and song-type matching appear to be important components of territorial signalling in wrens, as has been found in several other species. We did not quantify variation in song overlapping during these studies.

Another interesting component of song structure that may have considerable signal value in wrens is variation in the relationship between trill rate and frequency bandwidth across different syllable types. The idea is that there will be a performance trade-off between how fast a bird can repeat trilled syllables and the frequency range of those syllables. High performance syllables (those close the limit of trill rate vs frequency bandwidth) are thought to be more difficult to produce (Podos 1996). Such a pattern has been demonstrated in the songs of several species (Podos 1997). It is thought that how well individuals balance this trade-off could be an important aspect of vocal performance with important signal value in either mate attraction (Draganoiu *et al.* 2002; Ballantine *et al.* 2004) or intra-sexual competition (Illes *et al.* 2006). There is evidence for this effect on the syllables of wren songs too (see figure below). However the wrens studied in this thesis frequently produced syllables that were of considerably higher trill rate (and to a lesser extent frequency bandwidth) than the most commonly produced syllables and, intriguingly, these syllables showed no evidence of the performance constraint between trill rate and bandwidth. The figure below shows the relationship between frequency bandwidth and trill rate for syllables recorded from advertisement songs of six male wrens (4.0 ± 1.5 SD (range 2-6) song types per male with 4.0 ± 1.2 SD (range 1-6) trilled syllables per song type). Note the two distinct groups of syllables, which differ in the relationship between trill rate and frequency bandwidth. All the syllables presented in this figure constituted phrases that were part of advertisement songs (i.e. no 'rattle' or alarm calls are included), and syllables from both groups occurred in all except two songs. The different symbols represent individual males, and the lines show least squares regression lines for each group of syllables separately.

Future work will be required to determine the function and signal value of this unusual variation in syllable structure and vocal performance. This could be done by examining context-dependent variation in the frequency of use of these different syllable types, and the relationship between syllable types and other male traits. In addition, these sorts of studies would benefit from examining the components of song production itself within a 'multiple signals' framework (see e.g. Møller &

Pomiankowski 1993). Such investigations should yield useful insight into the evolution of the structurally complex wren song.

The figure below shows the relationship between frequency bandwidth and trill rate for syllables recorded from advertisement songs of six male wrens (4.0 ± 1.5 SD (range 2-6) song types per male with 4.0 ± 1.2 SD (range 1-6) trilled syllables per song type). Note the two distinct groups of syllables, which differ in the relationship between trill rate and frequency bandwidth. All the syllables presented in this figure constituted phrases that were part of advertisement songs (i.e. no 'rattle' or alarm calls are included). The different symbols represent different males, and the lines show least squares regression lines for each group of syllables separately.



BOX 10.2. THE GENETIC QUALITY HYPOTHESIS (AND EXTRA-PAIR MATING IN BIRDS)

It is easy to understand the benefits to males of extra-pair fertilizations (EPFs). But in most birds, females seem to play an active role in gaining EPFs. What are the benefits that drive females to pursue EPFs? Extra-pair males rarely contribute to the care of their extra-pair offspring directly, and few studies have identified direct benefits to females from extra-pair mating. Therefore, most research has focused on genetic explanations for the benefits female accrue from EPFs (reviews in Birkhead 1998; Petrie & Kempenaers 1998; Jennions & Petrie 2000; Tregenza & Wedell 2000); Griffith *et al.* 2002). These explanations come in four main flavours: females may seek EPFs to (1) increase fertility, (2) acquire good genes for their offspring (e.g. genes for quality or attractiveness), (3) mate with more genetically compatible males, and (4) mate with males with greater genetic diversity. Female choice for good genes is an ongoing and important area of research. However, when considering these hypotheses, it should also be remembered that females may derive non-genetic benefits from social partners with desirable genes. For example, if high genetic quality or less inbred males are healthier or more viable, they may provide better resources, for example through a better territory or more paternal care of the offspring. It is unlikely that good genes models are the sole explanation for female pursuit of EPFs.

BOX 10.3. MARKER-BASED MEASURES OF GENETIC DIVERSITY

What do the currently used marker-based measures of genetic diversity reveal about the genetic history of the individual, and why do they frequently yield different conclusions? In our study (chapter 7), the conclusions arising from the analysis of simple heterozygosity (i.e. standardized heterozygosity, H_S , and internal relatedness, IR) differed markedly from those arising from the similarity between alleles (d^2). Specifically, the relationships between genetic diversity and paternity were found with H_S and IR but not d^2 , while the relationships with mass and condition were found with d^2 but not H_S or IR. There seem to be two plausible explanations for these discrepancies. First, these two classes of measurement probably represent different forms of inbreeding. Recent theoretical work has suggested that the simple measures of heterozygosity relate primarily to recent inbreeding events, while d^2 may be more closely related to inbreeding deeper in an individual's ancestry (Tsitroni *et al.* 2001). Accordingly, a positive correla-



tion between heterozygosity and an estimate of fitness or condition is thought to indicate shallow inbreeding depression (e.g. dissimilarity between parental genotypes) and/or heterosis (i.e. superior hybrid vigour). In contrast, a positive correlation with d^2 , such as we found with male body mass and condition, would suggest that individuals with the most dissimilar alleles (indicating a deeper history of outbreeding) have greater fitness (Coltman & Slate 2003). Second, d^2 is thought to require a larger sample size than the other measures of heterozygosity, due to differences in the typical effect sizes encountered (Coltman & Slate 2003) and the greater variance of d^2 (Tsitrone *et al.* 2001). In general, d^2 is thought to be less powerful at detecting inbreeding depression (Coltman & Slate 2003), or may be effective under only certain restricted circumstances (Tsitrone *et al.* 2001). When comparing results it is difficult to take this into account, because the effect sizes relating to all measures of genetic diversity, as well as the relationships between them, can differ markedly among populations, depending on such aspects as inbreeding history, environmental variation and differences in genetic architecture. The effect sizes we report in chapter 7 are very large in relation to those reviewed in Coltman & Slate (2003). The figure below shows a schematic representation of the main marker-based methods used in this thesis to estimate genetic diversity.

 d^2

Allele 1 
Allele 2 

↑
d = 10bp
↓


LOW GENETIC DIVERSITY
- alleles close together
- $d^2 = 100$

↑
d = 50bp
↓

HIGH GENETIC DIVERSITY
- alleles further apart
- $d^2 = 250$


Heterozygosity & internal relatedness (IR)

Loci: 1 2 3 4


LOW HETEROZYGOSITY
- 1 out of 4 loci heterozygous
- $H = 0.25$
- $IR > 1$



MODERATE HETEROZYGOSITY
- 2 out of 4 loci heterozygous
- $H = 0.50$
- $IR \approx 0$



HIGH HETEROZYGOSITY
- 4 out of 4 loci heterozygous
- $H = 1.00$
- $IR < 1$

