Resolving sexual conflict

Behavioural mechanisms underlying parental coordination

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Table of contents

Chapter 1

General introduction

1.1 Sexual conflict over parental care

Raising a family is not free of conflicts for parents because, as Trivers was the first to point out, an evolutionary conflict of interest exists between parents over care (Trivers, 1972). This conflict, known as sexual conflict, arises because parents are usually unrelated individuals and are each selected to maximize their own fitness when caring for shared offspring (Trivers, 1972). Furthermore, each parent pays the cost of its own parental investment but benefits from care provided by either parent. The consequence of this conflict is that each parent is selected to exploit its mate's parental investment, leaving the latter to carry out a larger portion of care (Lessells, 2006; Wedell et al., 2006).

The existence of sexual conflict can be graphically illustrated by plotting the benefit and cost functions in relation to the parental investment of an individual (for example parental investment by the female in Figure 1.1). When one parent (in this case, female) decides the amount of care to provide, its optimal level of investment (that gives it the highest fitness benefit for the offspring) occurs when the difference between costs and benefits is the largest (female symbol in Figure 1.1). In contrast, the optimum level of parental investment for its mate (male in this case) is obtained when the investment of the carer reaches the highest benefit as it does not pay any costs (male symbol in Figure 1.1). This difference between the optimal levels of parental investment by the two parents as shown in figure 1.1 is the sexual conflict over parental investment, in which each parent is

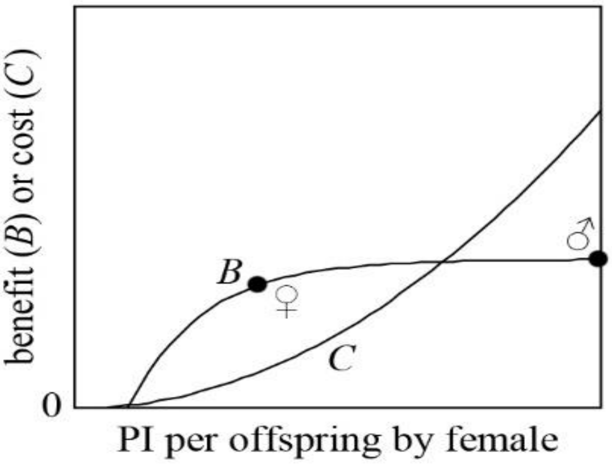

Figure 1.1. Levels of parental investment (PI) of the female that maximizes female and male fitness for an offspring given the benefit and cost functions. The benefits of PI are assumed to increase as the amount of PI increases at a decelerating rate (curve B) until the curve reaches an asymptote. The costs of PI are assumed to increase at an accelerating rate (curve C). The optimal level of PI for the female (care provider in this figure) is reached when the difference between benefits and costs is maximized (female symbol) as she directly pays costs for this care. In contrast, the optimal level of PI of the female which maximize male fitness for that offspring (male symbol) occurs when the benefit curve reaches the asymptote (from Lessells 2006).

selected to manipulate the parental investment of its mate towards its own optimum while maintaining its own optimum level of investment (Lessells, 2006).

Sexual conflict causes an antagonistic selection acting on morphological and behavioural traits directly involved in parental investment. Evidence that one sex directly manipulates the parental investment of the other comes from different species and can involve the exploitation of signalling systems (Chapman et al., 2003; Müller et al., 2007), coercive behaviours (Balshine-Earn and Earn, 1998; Hansson et al., 1997) and deceit of the partner (Valera et al., 1997). For instance, this latter mechanism is present in the penduline tit, *Remit pendulinus*, which performs uniparental care because one parent always deserts the breeding attempt before the eggs have hatched (van DijK et al., 2007). Females increase their chances of being able to desert before the male by hiding the eggs in the nest material. By deceiving the partner on the state of the breeding attempt, females can manipulate the parental investment of the male (Valera et al., 1997).

1.2 The evolutionary outcome of sexual conflict

In most cases, sexual conflict over parental care seems not to involve a direct manipulation of the investment of the other sex, instead each parent only controls its own investment (Lessells, 2012). However, the fitness of a parent depends not only on its own investment but also that of its mate, so when no manipulation occurs, parental care of both sexes coevolves. Game theoretical approaches have been used to investigate the outcome of sexual conflict when no manipulation occurs, as game theory considers multi-player situations in which the fitness of an individual depends on the decisions made by itself but also by others (Maynard Smith, 1982). This theoretical framework models parental care in the form of evolutionarily stable strategies (ESS) that identify the levels of male and female effort, which provides them the highest fitness benefit (Houston and Davies, 1985; McNamara et al., 1999).

The first theoretical model investigating the outcome of sexual conflict over parental investment was proposed by Houston and Davies (1985). The ESS predicted in this model specified, in terms of the best response curve, the level of care that each parent should provide to maximize its own fitness given the effort adopted by its mate (Houston and Davies, 1985). This model assumes that each parent makes a single decision ("sealed bid") about its parental investment, and that these decisions are independent from the investment adopted by their mate. This results in a lower overall parental investment than a purely cooperative situation in which each parent acts to maximize the sum of its own and its partner's reproductive success (McNamara et al., 2003). The best response curve of Houston and Davies' model has been, in later studies, mistakenly considered as the best behavioural response with which parents are expected to respond to the investment of its mate during a breeding attempt (Hatchwell and Davies, 1990; Markman et al., 1995; Wright and Cuthill, 1990). These studies on parental investment observed that parents were highly responsive to each other and adjusted their provisioning behaviour in response to an experimental manipulation of the partner's provisioning behaviour. This behavioural response of one parent to a change of parental investment of the mate is known as negotiation.

These empirical findings triggered new theoretical models that considered the parental investment as the result of a negotiation during which parents behaviourally respond to the investment of their mate over time (Johnstone and Hinde, 2006; Johnstone et al., 2014; Lessells and McNamara, 2012; McNamara et al., 1999). For this reason, McNamara et al. (1999) developed a newer model, in which the final level of parental investment is the result of an interactive process in which parents, according to their negotiation rules, make repeated bouts of investment in response to each other on a behavioural timescale. They found that when individuals negotiate their efforts by responding to one another, each parent expends less overall effort than in the Houston and Davies' solution, showing that sexual conflict and negotiation reduce parental care and parent and offspring fitness (McNamara et al., 1999). Lessells and McNamara (2012) further extended the concept of negotiation over parental care into another model, in which the negotiation and investment phase are not separated, and parents make a series of bouts of investment as in the model of McNamara et al. (1999). In Lessells and McNamara's model, each parent assesses the amount of care that offspring have already received via offspring state (indicated by their current mass) at the start of each bout and decides its investment in response to it (Lessells and McNamara, 2012). This negotiation rule results in a lower amount of care and parents and offspring fitness when parents care together than when parents care singularly for half the amount of offspring.

Despite the different assumptions adopted by these models, they all predict that sexual conflict reduces parental care, and the parents' and offspring's fitness. This raised the question whether there might be cases in which young will be better cared for by a single parent than by the pair. Further theoretical explorations of McNamara et al 1999's model indeed show that, in specific negotiated solutions, young are better off with one parent than with two (McNamara et al., 2003). This outcome also finds empirical support in the zebra finch, *Taeniopygia guttata*, in which offspring received greater *per capita* parental investment from single females rearing two chicks than from both parents working together to rear four (Royle et al., 2002).

In addition to an overall decrease in parenting, the above-mentioned models also predict that, in biparental species, a reduction or increase in parental care by one parent should be partially compensated by the other. This prediction has been intensively studied empirically by using different types of experimental manipulations, ranging from mate removal (Smiseth et al., 2005) to handicapping (Griggio et al., 2005) and selective playback of begging signals (Hinde, 2006; Hinde and Kilner, 2007). The results of these experiments are in line with the theoretical prediction of partial compensation, as suggested by a meta-analysis of 54 studies in birds (Harrison et al., 2009). However, there was substantial variation across studies and species in the direction and magnitude of the response: in addition to partial compensation (Wright and Cuthill, 1989), in some studies parents were observed to match the response of the partner (Hinde, 2006; Hinde and Kilner, 2007), in others there was no response (Rauter and Moore, 2004; Smiseth et al., 2005; Suzuki and Nagano, 2009), complete (Paredes et al., 2005; Tomotani et al., 2018), or even over-compensation (Griggio and Pilastro, 2007). Such behavioural variation was thought to occur when parents use the negotiation process to gain information about the current need of their offspring (Johnstone and Hinde, 2006). This implies that negotiation is a complex phase in determining parental investment, in which parents gather information from multiple family members. Parents may indirectly asses partner's contributions via offspring condition (Lessells and McNamara, 2012) or may directly respond to each other's behaviour (Hinde, 2006; Johnstone and Hinde, 2006; Johnstone et al., 2014). In birds for instance, there is evidence that parents negotiate via the offspring, by positively responding to an increase of begging calls from the young (Hinde and Kilner, 2007; Ottosson et al., 1997), and via the observation of their partner's behaviour

(Hinde, 2006). There is also evidence that parental investment is simultaneously affected by both of these stimuli (Hinde and Kilner, 2007).

1.3 Turn-taking as negotiation rule

In the theoretical models described so far, the resolution of sexual conflict leads to an overall reduction of parental care. These models implicitly assume that parents cannot make a 'binding agreement' about how much each parent should invest in the offspring, with the consequence that each individual is expected to withhold part of their potential investment to avoid being exploited by the mate (Lessells and McNamara, 2012).

Intriguingly, Johnstone et al. (2014) proposed that simple forms of conditional cooperation while provisioning offspring may be able to create this 'binding agreement.' They modelled a new negotiation mechanism where parents take turns investing (e.g. provisioning the offspring) over time (Johnstone et al., 2014). Specifically, this negotiation rule was modelled with parents visiting the brood to provide care at two different rates, depending on whether they were, or were not, the last to visit the nest. The evolutionary stable outcome of this strategy is a turn-taking rule where each parent does not provision when it was the last to feed, but only after a visit by the mate, leading to a strict alternation of the nest visits. This negotiation rule ensures an equal provisioning rate by the parents and results in the most efficient overall level of care as parents work at their highest rate (Johnstone et al., 2014). Implicitly, this turn-taking rule creates this 'binding agreement' between the parents because it guarantees that each investment bout by one parent can no longer be exploited by the partner but will instead be reciprocated.

Following the outcome of their model, Johnstone et al. (2014) investigated whether great tit (*Parus major)* parents alternate their nest visits, and whether a turn-taking negotiation rule might be responsible for this effect. They found that alternation was greater than expected by chance, but also realised that 'alternation' could be due to other biological mechanisms instead of, or in addition to, turn-taking. In particular, the presence of a refractory period, the lack of short intervisit intervals (possibly caused by the impossibility for parents of returning immediately to the nest with a new food item) could create some degree of alternation without needing any form of behavioural interaction between the parents.

However, the observed alternation was still greater than expected while controlling for the presence of the refractory period and was considered as evidence of turn-taking (Johnstone et al., 2014, 2016a).

Johnstone et al.’s (2014) theoretical model and empirical results triggered new studies showing that, in several avian species, parents tended to alternate their visits at the nest more than expected by chance (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Johnstone et al., 2014; Koenig and Walters, 2016; Savage et al., 2017). Only a few of them, however, have investigated the biological processes generating the higher-than-random alternation of the nest visits (Bebbington and Hatchwell, 2016; Savage et al., 2017; Schlicht et al., 2016) and whether parents actively take turns feeding the young. In this regard, Schlicht et al. (2016) argued that significant amount of alternation, even higher than observed by Johnstone et al.’s (2014), can be produced by processes other than turn-taking and refractory periods, such as variation in the provisioning rate throughout the sequence of visits (perhaps due to variation in weather conditions, offspring begging, etc). Hence, there is still uncertainty as to whether the observed alternation of nest visits is the result of parental interaction via a turn-taking rule or if other mechanisms are responsible for this visit pattern.

1.4 Aim and outline of the thesis

Understanding how parents negotiate over parental care is a major goal of evolutionary biology, as the negotiation rules crucially affect the outcome of sexual conflict and thus reproductive success (Lessells and McNamara, 2012). Previous theoretical studies suggest that parents and offspring, as result of this conflict, suffer a reduction in fitness due to decreased parental care, but recent work suggests that a turn-taking rule increases the level of care (Johnstone et al., 2014). However, most of the work after this theoretical paper investigated the alternation of the nest visits but did not directly address whether a turn-taking rule is responsible for the observed pattern. Therefore, my thesis is an in-depth examination of the behaviour mechanisms underlying alternation of the nest visits (Figure 1.2).

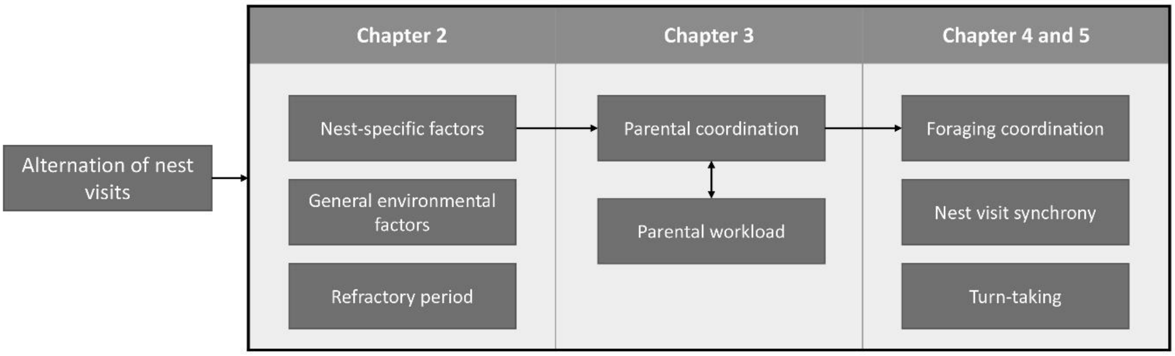

Figure 1.2. Schematic depiction of the factors that can contribute to the observed alternation of nest visits as outlined by the chapters of the thesis.

To do this, I employed a state-of-the-art radio-tracking system (Encounternet) that can remotely monitor the location of provisioning pairs of great tits (Figure 4.1 in chapter 4). The great tit is a common biparental European songbird that readily nests in nest boxes and has been used as a model species to study the evolution of parental care. For **chapter two**, I explored the contribution of different processes expected to generate alternation (Table 2.1). To do so, I used provisioning sequences to investigate whether nest-specific (perhaps due to turn-taking or offspring begging) or general environmental (perhaps due to weather conditions) factors are responsible for the observed alternation of the nest visits. In **chapter three,** I looked into whether one nest-specific factor, parental workload, affects alternation. I used a brood size manipulation to test the effect of workload on alternation and whether this effect could be attributed to negotiation rules. For both **chapters four** and **five,** I used Encounternet to collect detailed spatial movement and temporal data to investigate parental coordination. In particular, for chapter three, I examined whether parents coordinate their foraging activity and whether this relates to alternation of the nest visits. For chapter four, I scrutinized single foraging trips to test whether the pattern of nest visits could be attributed to parental reactions to each other, including possibly turn-taking.

Chapter 2

Determinants of alternation of nest visits in a bird with biparental care

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In preparation

Abstract

The amount of parental care provided to offspring is affected by sexual conflict and the negotiation rules that parents adopt. Recently, ’turn-taking’ in provisioning visits has been proposed as a negotiation rule by which parents respond to their partner’s behaviour and previous empirical studies indicate that in several bird species parents do indeed alternate their nest-visits more than expected by chance. However, this alternation could be explained by processes other than ‘turn taking’, such as correlated temporal heterogeneity in the nest-visit rates of the two parents and the presence of a refractory period between two consecutive visits by the same parent. In this study, we collected data from 17 wild great tit (*Parus major*) pairs during chick provisioning and partitioned the observed amount of alternation of the nest visits into these processes. We found that great tit parents significantly alternate their visits more than expected by chance and that there was correlated temporal heterogeneity in their provisioning rates. We then investigated whether nest-specific (perhaps due to e.g. turn-taking or offspring begging) or general environmental (perhaps due to e.g. weather conditions) factors are responsible for the observed alternation and correlated temporal heterogeneity of parental provisioning. We created ‘pseudo-pairs’ in which provisioning data from one parent were paired with data from the opposite-sexed parent feeding over the same time period at a nearby nest and found that alternation is mostly generated by nest-specific and, to a smaller extent, by general environmental conditions affecting groups of nearby nests. Lastly, we carried out a randomization test on the ‘pseudo-pairs’ to explore the contribution of the refractory period between visits on the observed alternation. We found that alternation in the randomized pseudo pairs did not differ from chance, indicating that the refractory period has little effect on the observed amount of alternation. Even though we cannot discriminate whether alternation is produced by turn-taking or only by biological processes responsible for within-pair correlation in provisioning rates, these results indicate that biological processes occurring at the nest level contribute for approximately 80% of the observed alternation, whereas general environmental factors are responsible for the remaining 20%, with no significant contribution of the refractory period.

2.1 Introduction

Parents caring for dependent young have to decide how much to invest in their offspring (Royle et al., 2012; Trivers, 1972). In species with biparental care, however, this decision does not only depend on a trade-off between the production of current and future offspring (Roff, 2002; Stearns, 1992) but also on sexual conflict, an evolutionary conflict of interest between the two sexes (Lessells, 2006; Trivers, 1972). This conflict stems from the fact that parents caring for common offspring share the benefit of their joint investment but only pay the cost of their own care (Lessells, 2006; Wedell et al., 2006), with the consequence that each parent is selected to exploit its mate’s parental investment and, possibly, to do a smaller share of the care.

Theoretical work has extensively investigated how sexual conflict affects the amount of care that parents devote to offspring. Parental care was initially modelled with each parent making a single independent bout of investment, which could change only over an evolutionary time scale (Houston and Davies, 1985), whereas later models allowed parents to behaviourally respond to each other, so to negotiate their investment in response to that of their mate over multiple investment bouts (Johnstone and Hinde, 2006; Lessells and McNamara, 2012; McNamara et al., 1999; McNamara et al., 2003). In both cases, however, sexual conflict and negotiation are predicted to lower overall parental investment and fitness compared to a situation in which each parent maximizes the sum of its own and its partner’s fitness (McNamara et al., 2003). The theoretical prediction that sexual conflict lowers the overall parental investment seems also to find empirical support in a zebra finch *Taeniopygia guttata* study, in which offspring experimentally reared by single females received greater *per capita* parental investment than offspring raised by both parents (Royle et al., 2002).

In addition to an overall decrease in care, all the above mentioned negotiation models also predict that, in biparental species, a reduction in parental care by one parent should be only partially compensated by the other, to prevent exploitation by the latter. This theoretical prediction has been intensively studied and overall the empirical studies are in line with the prediction of partial compensation, as suggested by a meta-analysis in birds species (Harrison et al., 2009). Nevertheless there was substantial variation between studies and species, with responses including matching (Hinde, 2006; Hinde and Kilner, 2007), no (Rauter and Moore, 2004; Smiseth et al., 2005; Suzuki and Nagano, 2009), complete (Paredes et al., 2005;

Tomotani et al., 2018), or even over-compensation (Griggio and Pilastro, 2007). This variation in the behavioural responses was further investigated theoretically by Johnstone and Hinde (2006) showing that different responses can occur when parents use the negotiation process to gain information about the current need of their offspring. This implies that parents may negotiate their investment by gathering information from multiple family members. Parents may indirectly asses partner's contributions via offspring condition (Lessells and McNamara, 2012) or may directly respond to each other's behaviour (Hinde, 2006; Johnstone and Hinde, 2006; Johnstone et al., 2014). In birds for instance, parents are seen to negotiate via the offspring, by positively responding to an increase of begging calls by the young (Hinde and Kilner, 2007; Ottosson et al., 1997), and via the observation of their partner's behaviour (Hinde, 2006) with also evidence that parental investment is simultaneously affected by both of these stimuli (Hinde and Kilner, 2007).

More importantly, the adopted negotiation mechanism critically affects the evolutionary outcome of sexual conflict (Johnstone et al., 2014; Lessells and McNamara, 2012). Contrary to the predictions of the early theoretical models (Houston and Davies, 1985; Lessells and McNamara, 2012; McNamara et al., 1999; McNamara et al., 2003), Johnstone et al. (2014) proposed a new negotiation mechanism, based on a form of conditional cooperation between the parents while provisioning offspring, which can reduce the potential costs of sexual conflict and support a more efficient level of care. This negotiation rule was modelled with parents visiting the brood to provide care at two different rates, depending on whether they were, or were not, the last to visit the nest. The evolutionary stable outcome of this strategy is a turn-taking rule where each parent does not provision when it was the last to feed, but only after a visit by the mate, leading to a strict alternation of the nest visits. The same authors investigated great tit *Parus major* provisioning data and found that great tit parents tend to alternate their visits at the nest more than expected by chance (Johnstone et al., 2014).

Although the higher than expected alternation is in line with turn-taking in provisioning activity between the parents, Johnstone et al. (2014) pointed out that above-random alternation could also potentially be accounted for by the presence of a refractory period. This refractory period represents a lack of short intervals in the distribution of the inter-visit intervals (the time difference between two consecutive visits by the same individual), which could be biologically explained by the fact that parents cannot easily return to the nest in a short time with a new food item. This latency can *per se* produce some degree of alternation in the sequence of visits because, after a visit by one parent, it increases the likelihood that the next visit is made by its mate. However, to explore whether the refractory period could solely account for the higher than expected alternation, Johnstone et al. (2014) created randomized sequences from the original provisioning data, in which the order of the inter-visit intervals was randomized over the entire period in both male and female visit sequences. These randomized sequences maintain the same distribution of inter-visit intervals as the original sequences, thus preserving the effect of a refractory period, but do not incorporate any form of turn-taking between the parents. The comparison between the observed sequences and the randomized sequences showed that alternation was still significantly higher in the original data and this difference was interpreted as evidence for turn-taking (Johnstone et al., 2014, 2016a). These results have triggered further studies on parental behaviour in avian species (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Khwaja et al., 2017; Koenig and Walters, 2016), showing that alternation of the nest visits occurs also in other species (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Koenig and Walters, 2016).

However, it is still uncertain whether this alternation occurs because parents are indeed taking turns in feeding, or if other mechanisms are responsible for this pattern of visits. In this regard, Schlicht et al. (2016) argue that the Johnstone et al. (2014) randomization of the intervals over the entire sequence not only eliminates any effect of turn-taking by the parents but also some other mechanisms that might generate alternation. Specifically Schlicht et al. (2016) focused on a situation in which both male and female provisioning rates concurrently changed over a nest visit sequence and showed that this property can also generate alternation of nest visits. They performed an alternative randomization on simulated sequences in which the inter-visit intervals in both sexes were arranged so that their length on average increased or decreased along the sequence to simulate a situation in which parents simultaneously varied their provisioning rates. The inclusion of this correlated temporal variation in provisioning showed similar or higher level of alternation achieved in Johnstone et al. (2014)'s data, without any turn-taking occurring between the parents

(Schlicht et al., 2016). Although Johnstone and collegues did not find evidence for monodirectional changes in the parental provisioning rates in their dataset (Johnstone et al., 2016b), in general, correlated variation (henceforward also called correlated temporal heterogeneity) of the provisioning rate can produce alternation (Schlicht et al., 2016). Turn-taking in provisioning as described in Johnstone et al. (2014)'s theoretical framework, does not produce heterogeneity in parental provisioning rates, because the rate at which parents feed when the partner was the last to feed is constant over time.

In turn however, correlated temporal heterogeneity in provisioning rates may occur in response to variation in biological and environmental factors that affect provisioning activity such as offspring begging (Hinde, 2006; Hinde and Kilner, 2007; Ottosson et al., 1997), partner's behaviour (Hinde and Kilner, 2007), predation risk (Fontaine and Martin, 2006; Ghalambor et al., 2013), food availability (Naef-Daenzer and Keller, 1999; Tremblay et al., 2005) and weather conditions (Radford et al., 2001; Wiley and Ridley, 2016). For the purpose of this study, the above-mentioned factors can be grouped into nest-specific (offspring begging, partner's behaviour, food availability) or general factors (predation risk, weather conditions) on the basis that they influence the behaviour of a single breeding pair or of multiple breeding pairs.

In this study, we used provisioning data from great tits to investigate which of these processes are responsible for alternation of the nest visits. Firstly, we explored whether great tit parents alternated their visits more than expected by chance. Secondly, we investigated and partitioned the contributions of nest-specific factors (including turn-taking), general factors and the refractory period on the observed amount of nest-visit alternation.

2.2 Methods

Data collection

We collected provisioning data from a great tit population at the Hoge Veluwe National Park (The Netherlands; 52°02′07″ N, 5°51′32″ E) in 2014. This area contains around 400 nest-boxes that were checked weekly from the beginning of April to determine the onset of egg laying and incubation. Active nests were then checked daily from the day before predicted hatching to determine the exact hatch date (day 0). Parental nest visit data were collected on 17 nests (brood size (mean ± SE): 7.76 ± 0.29) at day 10 of chick age using a small video camera mounted in the roof of the nest-box and connected to an external video recorder at the foot of the tree. The camera was placed on day 9, and the recordings (720 x 576 pixels of resolution) started before 0930h of the following day. Recorders were synchronized (to the nearest second) to a digital watch daily before each recording began. Four hours of video (1000h – 1400h) were scored for each nest, and the time the bird entered the nest-box (to the nearest second) and the sex of the provisioning parent (determined from the blackness of the crown feathers) were identified from the video recordings.

Sequence manipulation

To address our research questions, we manipulated the provisioning sequences collected in the field (henceforward called real pairs) to create artificial sequences that preserve or loose specific factors that we expected to generate alternation (Table 2.1).

Firstly, to explore whether great tit parents alternate their provisioning visits more than expected by chance, from the real pairs we created randomized sequences (henceforward called randomized real pairs). These randomized sequences were formed by randomly rearranging the order of the inter-visit intervals of each parental time series (henceforward we refer to a time series as the provisioning sequence made by a single parent) and then aligning the two newly created time series. Furthermore, to explore whether alternation is determined by nest-specific or general sources of correlated temporal heterogeneity in provisioning rates we created two more sequences, defined as pseudo-pairs and randomized pseudo-pairs. Pseudo-pairs were created by matching the time series of one individual with the time series of an individual of opposite sex recorded at a different nest on the same day. By doing so, we expected that alternation in these sequences cannot be caused by a possible turn-taking behavioural response between the parents and by nest-specific factors responsible for correlated temporal heterogeneity in parental provisioning rates, as each parental sequence belonged to different nests, but could still be produced by correlated temporal heterogeneity due to general factors and by a refractory period.

Table 2.1. Biological and ecological processes expected to generate alternation in the studied sequences. Processes that might be present in that specific sequence are marked with ticks, while crosses indicate their absence. Processes generating correlated heterogeneity in provisioning rates are divided in two categories (nest-specific and general) to differentiate the spatial scale of their influence.

	Turn-taking	Correlated heterogeneity in provisioning rates		Refractory period
		Nest-specific	General	
Real pairs	✓	✓	✓	✓
Pseudo-pairs	✗	✗	✓	✓
Randomized pseudo-pairs	✗	✗	✗	✓
Randomized real pairs	✗	✗	✗	✓

Our 17 real pair sequences were recorded on four days (recorded nests per day: 3, 4, 5, 5) so that we generated 58 possible pseudo pairs (pseudo pairs per day: 6, 12, 20, 20). Randomized pseudo-pairs were instead created from the pseudo-pairs by applying the same randomization procedure of the randomized real pairs. In these sequences, we expected that the randomization of the intervals within each parental time series would also remove any effect of general factors but not of the refractory period on alternation of the nest visits.

Calculation of alternation of the nest visits and correlated temporal heterogeneity in provisioning rates

For each type of sequence, we calculated alternation of the nest visits and correlated temporal heterogeneity in parental provisioning rate. Alternated nest visits were defined as visits of one individual that followed a visit of its mate. For each sequence of nest visits, we scored the status (alternated or non-alternated) of all the visits within the four-hour sampling period except for the first visit. Different amounts of alternation can arise by chance in a sequence of visits depending on the proportion of visits by the two parents. In a situation where, for instance, one parent makes either all or none of the visits in a sequence, no alternated visits can occur. Conversely, when parents feed the offspring at similar rates, the proportion of alternated visits expected by chance increases. To account for this effect, we calculated and used in the statistical analysis an alternation score (see supplementary material 2.S1 for calculation of alternation score) that represents the deviation of the observed amount of alternation from that expected given the proportion of visits by each of the two parents. This score for alternation assumes that visits by each sex occur at a constant rate over time, so that inter-visit intervals follow a negative exponential distribution.

Correlated temporal heterogeneity in provisioning rates between two parental time series was estimated as the Kendall correlation coefficient between the length of male and female inter-visit intervals selected at 20 time points. By doing so, we expected that a correlated temporal variation in provisioning rate by the parents would result in a correlation coefficient greater than zero because short intervals by one individual should be associated by short intervals by the mate and viceversa. The twenty selected time points were equally distributed over the four-hour period (one interval every 11.43 minutes) to obtain a sample of twenty intervals for each sex. In this dataset we chose 20 time points so to reduce that long intervals were selected twice, given that the lowest provisioning rate of a single parent in our data was 9.25 visits/hr). In the cases where pairs of long intervals were selected in more than one time point, duplicates were removed. We used a non-parametric measure of correlation as the selected intervals were not normally distributed.

Statistical analysis

All the statistical analyses were performed in R environment (version 3.2.3). To investigate whether alternation score and correlated temporal heterogeneity in provisioning rates in real pairs are significantly higher than expected by chance, for each real pair we generated 10000 randomized sequences and calculated the two variables of interest for each randomized sequence. By comparing the observed value of the variable with the distribution of randomized values we obtained a p-value for each one-tailed real pair – randomized real pair comparison. We defined an observed value to be significantly higher from random if it fell within the top 5% of the random distribution. We then used Fisher’s method (Rosenthal, 1978) to combine the p-values from each comparison using the sumlog function implemented in the R package *metap* (Dewey, 2017). Furthermore, we carried out a pairwise comparison between real pairs and pseudo-pairs. Here we investigated whether alternation and correlated temporal heterogeneity in provisioning was higher when a parental time series was matched with its own partner's time series (real pair) or with time series of other individuals of the opposite sex (pseudo-pair). This approach created a pseudoreplication issue since the same time series was present multiple times in the dataset. To resolve this problem, this analysis was performed using linear mixed models with alternation score or correlated temporal heterogeneity as response variables, "pair type" as fixed effect and "time series ID" as random factor. We ran this comparison twice, one with "male time series ID" and one with "female time series ID" as random factor to test whether the difference between pair type depended on the sex of the time series. To explore differences in alternation score and correlated temporal heterogeneity between pseudo-pairs and randomized pseudo-pairs, we used the same procedure describe above for the comparison real pairs – randomized real pairs. Lastly, to investigate the contribution of the refractory period on the observed amount of alternation, we tested whether alternation score in the randomized pseudo-pairs was significantly different from zero. For the 58 random distributions of the randomized pseudo-pairs we extracted the median value of alternation score and performed a (two-tailed) one sample t-test.

All mixed models were performed with the *lme4* package (Bates et al., 2015) and the significance of the main effect was calculated with the Kenward-Roger approximation implemented in the *pbkrtest* package (Halekoh and Hojsgaard, 2014). We report means and standard errors of the estimates as mean ± SE.

2.3 Results

Alternation score and correlated temporal heterogeneity in provisioning rates were significantly higher in real pairs than in the randomized real pairs (Alternation score real pairs: 0.34 ± 0.05; Alternation score randomized real pairs: 0.02 ± 0.05; Fisher's combined probability test for alternation score: $\chi2 = 132.58$, df = 34, $P < 0.001$, Figure 2.1A; Correlated temporal heterogeneity real pairs: 0.20 ± 0.05; Correlated temporal heterogeneity randomized real pairs: -0.01 ± 0.01; Fisher's combined probability test for correlated temporal heterogeneity: $\chi2 = 71.72$, df = 34, $P < 0.001$, Figure 2.1B). Furthermore, we found that

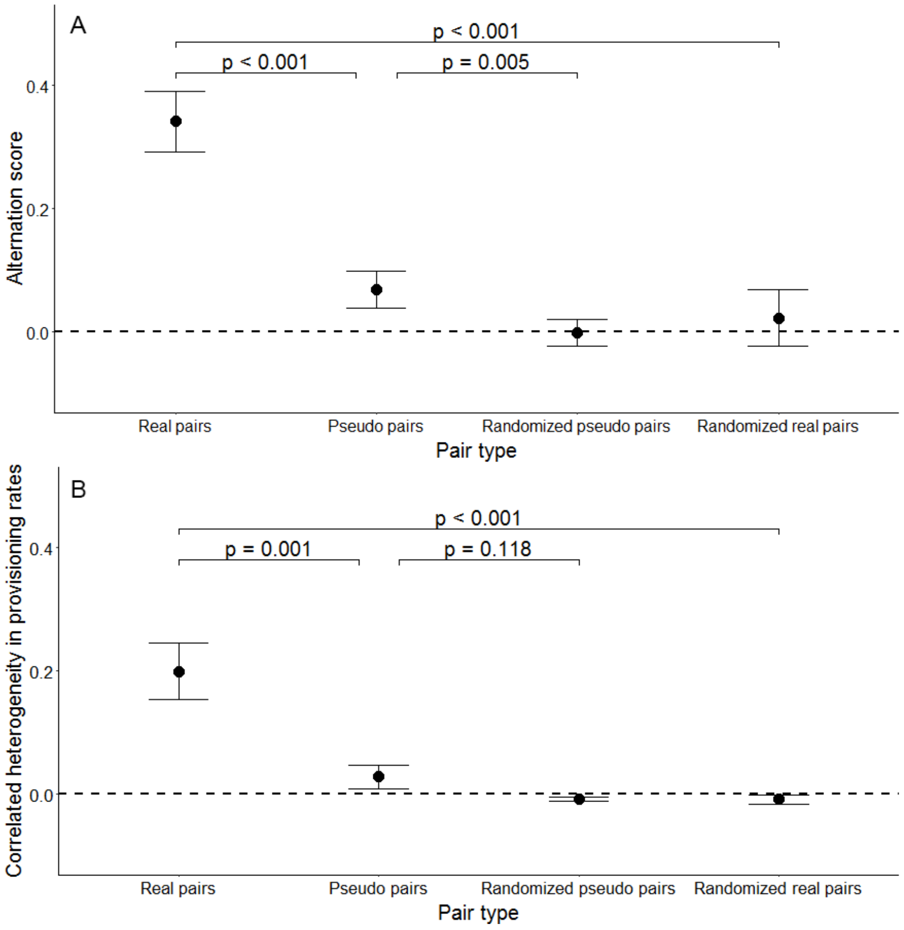

Figure 2.1. Alternation score (A) and correlated heterogeneity in provisioning rates (B) in real, pseudo, randomized pseudo, pairs and randomized real pairs. Mean ± SE are given for real and pseudo pairs. For randomized pseudo pairs and randomized real pairs, mean ± SE are calculated from the median values of the randomized distributions. A - An alternation score of zero (dashed line) represents the amount of alternation expected by chance, assuming that the probability of a nest visit by a parent is constant with respect to time.

alternation score differed between real pairs and pseudo-pairs (Alternation score real pairs: 0.34 ± 0.05; Alternation score pseudo pairs: 0.07 ± 0.03; F1,16 = 31.83, P < 0.001 when “female time series ID” was used as random factor; F1,16 = 25.03, P < 0.001 when “male time series ID” was used as random factor, Figure 2.1A), and between pseudo-pairs and randomized pseudo-pairs (Alternation score pseudo pairs: 0.07 ± 0.03; Alternation score randomized pseudo pairs: 0.00 ± 0.02; Fisher’s combined probability test: χ2 = 159.02, df = 116, P = 0.005, Figure 2.1A), indicating that both nest-specific and general factors are responsible for alternation of the nest visits. A similar pattern was found for correlated temporal heterogeneity in provisioning rates which significantly differs between real and pseudo-pairs (Correlated temporal heterogeneity real pairs: 0.20 ± 0.05; Correlated temporal heterogeneity pseudo pairs: 0.02 ± 0.02; $F_{1,16} = 15.20$, $P = 0.001$ when “female time series ID” was used as random factor; $F_{1,16} = 17.43$, $P < 0.001$ when “male time series ID” was used as random factor, Figure 2.1B) but not between pseudo and randomized pseudo-pairs (Correlated temporal heterogeneity pseudo pairs: 0.02 ± 0.02; Correlated temporal heterogeneity randomized pseudo pairs: -0.02 ± 0.01; Fisher’s combined probability test: $\chi^2 = 134.21$, df = 116, $P = 0.118$, Figure 2.1B), indicating that a decrease in alternation of provisioning was associated with a decrease in correlated temporal heterogeneity in provisioning rates. Lastly alternation score in randomized pseudo-pairs did not differ from zero ($t_{16} = -0.03$, $P = 0.97$) suggesting that the refractory period has no effect on generating alternation.

2.4 Discussion

In this study we investigated whether great tit parents alternate their nest visits more than expected by chance and explored which mechanisms are responsible for this difference in alternation. We found that nest-specific factors are the major contributors to alternation of nest visits and correlated temporal heterogeneity in parental provisioning, explaining approximately 80% of the observed amount of alternation. At the same time general factors affecting the provisioning of multiple breeding pairs in the same area account for the remaining observed alternation with no significant contribution of the refractory period.

The question is then whether the alternation we observed can be attributed to turn-taking between the parents or solely to processes producing correlated temporal heterogeneity in provisioning rates. By creating pseudo-pairs, we removed any effects of turn-taking and nest-specific biological factors creating correlated temporal heterogeneity in parental provisioning rates and this produced a significant decrease in both alternation and correlated heterogeneity. This result is in line with Schlicht et al. (2016) indicating that some alternation is generated by within nest sources of correlated variation in provisioning. However, our manipulation does not enable us to discriminate the potential contribution of turn-taking over correlated temporal heterogeneity in parental provisioning rates and therefore we cannot conclude whether alternation is at least partially generated by turn-taking.

Johnstone et al. (2014)'s theoretical framework assumes that both parental provisioning rates only depend on the identity of the last visiting parent, and that these rates are constant over time. However, provisioning activity is affected by several behavioural and environmental factors (Ghalambor et al., 2013; Hinde, 2006; Hinde and Kilner, 2007; Naef-Daenzer and Keller, 1999; Tremblay et al., 2005; Wiley and Ridley, 2016) so that the observed pattern of visits is likely to be the result of multiple factors acting simultaneously. Isolating the effect of a turn-taking rule in observational data, for instance by performing local randomization of the intervals, is difficult to achieve without also undermining the correlated temporal heterogeneity of the provisioning intervals. Experimental manipulations of the provisioning of one parent, by selective playback at the nest for instance, and investigating whether the other individual responds to maintain an equal provisioning and alternation of the nest visits may be reasonable approaches to investigate the existence of turn-taking.

We showed that nest-specific sources of correlated temporal heterogeneity in provisioning are generating a significant amount of alternation. Interestingly, some of these could be behavioural interactions between the family members. Correlated temporal variation in provisioning may for instance be mediated by offspring begging, to which parents are highly responsive (Hinde, 2006; Hinde and Kilner, 2007; Kölliker et al., 2000; Ottosson et al., 1997). If so, in a scenario where parents indirectly respond to each other via offspring begging or status (Lessells and McNamara, 2012), then alternation would still be the result of a negotiation mechanism different than turn-taking. Similarly, correlated changes in provisioning rate could also occur because parents are directly responding to each other by using other behavioural mechanisms that creates a matching change in provisioning rate like the one observed by Hinde (2006). Interestingly, synchrony of the nest visits is one form of pair coordination that is likely to produce correlated temporal heterogeneity in provisioning, as synchrony implies that parents tend to return at the nest together (Mariette and Griffith, 2015; Mariette and Griffith, 2012; Raihani et al., 2010; van Rooij and Griffith, 2013). Moreover, synchrony of the nest visits was also found to correlate with alternation in another species with biparental care (Bebbington and Hatchwell, 2016) and with coordination during the foraging activity (Mariette and Griffith, 2015), supporting the idea that other form of pair coordination than turn-taking can create significant patterns of alternation.

In addition to within nest behavioural processes, we also found indication that alternation is, to a smaller extent, created by general environmental factors driving the provisioning behaviour of multiple pairs within a breeding area. These encompass rainfall, predator presence and circadian rhythms in provisioning rates [although in great tits there is no evidence of diurnal changes in parental provisioning rates (Barba et al., 2009; Pagani-Nunez and Senar, 2013)] and these factors do not require any form of interaction between the parents, as the latter can potentially respond directly, and independently from each other, to the these external stimuli. Lastly, we observed a minor effect of the refractory period on alternation even though visual exploration of the distribution of the inter-visit intervals reveals its presence (Figure 2.S1).

In conclusion, our study indicates that biological processes occurring at the nest level are major contributors to alternation of parental nest visits. However, some level of alternation is also accounted for by correlated temporal heterogeneity due to the wider environment and the presence of a refractory period. Although parents alternate more than expected by chance, we cannot discriminate the potential presence of turn-taking negotiation strategy between the parents. We therefore strongly promote further investigations on the behavioural mechanisms underlying negotiation over offspring provisioning.

Acknowledgements

We thank the board of the National Park ‘De Hoge Veluwe’ for the permission to conduct our research in their site. We are grateful to Henri Bouwmeester for his help in the field work, Jorina Boer for her help collecting data, Mathias Cox for scoring the video recordings, and James Savage for providing the script for the randomization test. This research was supported by the Netherlands Organization for Scientific Research (NWO).

Supplementary material

2.S1 - Calculation of alternation score

We expected the observed amount of alternation in a sequence of provisioning visits to be function of the proportion of visits by either sex. We therefore calculated an alternation score that represents the deviation of the observed amount of alternation from the expected amount generated by the difference in parental provisioning rates. Within the four-hour recorded period, we scored the status (alternated (av) or non-alternated (nav)) of all the visits (focal visit) except the first one, depending of the sex of the preceding visit. It is important to notice that in a sequence on *n* visits, the first visit can only be used as preceding visit in the scoring of the status, whereas the last visit can only be used as focal visit. From the status of the visits we constructed a two-by-two contingency table with the sex (male or female) of the focal visits in relation to the sex of the preceding visits (Table 2.S1).

Table 2.S1. Two by two contingency table containing the number of visits given the sex of the focal and preceding individual visiting the nest in an observed sequence.

		Preceding visit		
		Male	Female	Row total
Focal visit	Male	nav_m	av_m	$nav_m + av_m$
	Female	av_f	nav_f	$av_f + nav_f$
	Column Total	$nav_m + av_f$	$av_m + nav_f$	n-1

The cells of the tables contains the occasions in which the four types of visits (abbreviated to av_m: alternated visit by the male; nav_m: non-alternated visits by the male; av_f: alternated visit by the female; nav_f : non-alternated visits by the female) occurred in the sequence. Note that the first visit of the sequence has no status, therefore in a sequence of *n* visits the grand total of the contingency table is *n*-1.

From the contingency table we calculated the observed number of alternated and non-alternated visits as:

$$Observed\ number\ of\ alternated\ visits = \text{av}_m + \text{av}_f \ ;$$

$$Observed\ number\ of\ non - alternated\ visits = \text{nav}_m + \text{nav}_f \ \ ;$$

and the expected number of alternated and non-alternated visits as:

$$Expected\ number\ of\ alternated\ visits$$
$$= \frac{(\text{nav}_m + \text{av}_m)(\text{av}_m + \text{nav}_f) + (\text{av}_f + \text{nav}_f)(\text{nav}_m + \text{av}_f)}{n-1} \ ;$$

$$Expected\ number\ of\ non -\ alternated\ visits$$
$$= \frac{(\text{nav}_m + \text{av}_m)(\text{av}_m + \text{av}_f) + (\text{av}_f + \text{nav}_f)(\text{av}_m + \text{nav}_f\ )}{n-1} .$$

For example, the number of alternated visits expected by chance is given by the proportion of focal visits made by the male multiplied by the proportion of preceding visits made by the female plus the proportion of focal visits made by the female multiplied by the proportion of preceding visits made by the male, all divided by the observed number of visits minus one.

The two types of expected number of visits therefore depends on the observed proportion of visits by the two sexes, and therefore account for the observed differences in provisioning rates. These expected values assume that visits by each sex occur at a constant rate over time (so that inter-visit intervals follow a negative exponential distribution).

From these values derived from the contingency table, we calculated the alternation score which expresses the deviation of the observed amount of alternation from the expected. This deviation is calculated as the difference in log odds between the observed and expected odds of alternated visits, as log odds are additive, as follows:

$$\begin{aligned} & Alternation\ score \\ & \quad = \log\left(\frac{Observed\ number\ of\ alternated\ visits}{Observed\ number\ of\ non-alternated\ visits}\right) \\ & \quad - log\left(\frac{Expected\ number\ of\ alternated\ visits}{Expected\ number\ of\ non-alternated\ visits}\right). \end{aligned}$$

In this way, alternation score assumes a value of zero if there is no difference between the observed and expected amount of alternation, positive values when the observed amount is greater than the expected, and negative values when the observed amount of alternation is smaller than expected.

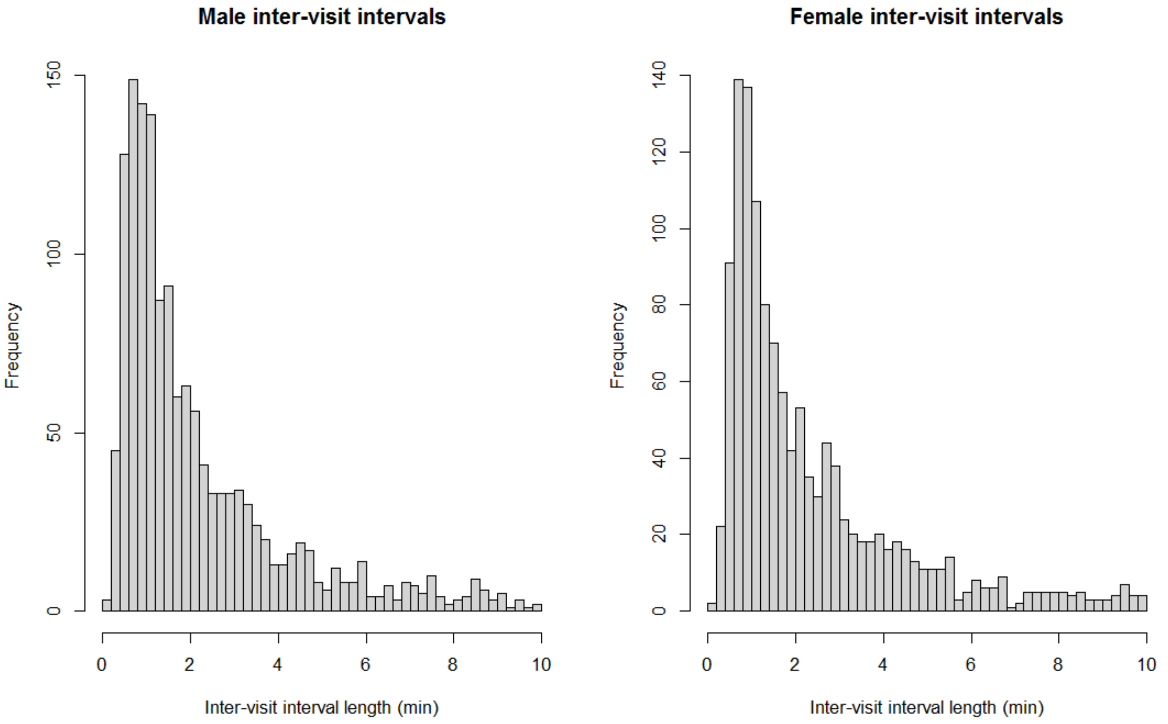

Figure 2.S1. Distribution of the inter-visit intervals for male and female parents.

Chapter 3

Alternation of nest visits varies with experimentally manipulated workload in brood-provisioning great tits

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Teja Curk

Camilla A. Hinde

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Submitted

Abstract

In species with biparental care, the amount of care devoted to offspring is affected by the negotiation rules that the parents adopt. Recently, turn-taking in provisioning visits has been proposed as a negotiation rule by which parents respond to their partner's behaviour, and empirical evidence suggests that in some species, parents alternate their visits more than expected by chance. However, we currently do not know whether alternation of the nest visits varies with environmental or social factors, such as workload. To test this, we manipulated the workload in 15 great tit (*Parus major*) pairs using a short-term brood size manipulation. When workload was experimentally reduced, parental nest visits alternated more than in control and enlarged broods. To understand this variability in alternation, we explored the rate and regularity of the parents' intervisit intervals. Treatment differences in alternation were still present when controlling for the rate and regularity of the visits by each of the two parents, suggesting that workload also affected alternation via the temporal sequence of the intervals. Our results show that alternation of nest visits varies in response to workload and is not merely a by-product of variation in visit rate.

3.1 Introduction

Parents often invest heavily in their dependent offspring (Royle et al., 2012). While this provides direct fitness benefits via the current offspring, parental care is costly for parents and often comes at the detriment of reduced survival and future reproduction (Williams, 1966), so that there is a trade-off between the production of current and future offspring (Roff, 2002; Stearns, 1992). In species with biparental care, however, parents share the benefit of their joint investments but only pay the cost of their own care (Lessells, 2006; Wedell et al., 2006). This asymmetry in the benefits and costs between the parents produces sexual conflict, a conflict of interest over how much each parent should invest in the joint offspring (Houston et al., 2005; Trivers, 1972), because each parent would have higher fitness when its partner did a larger share of the care.

How this sexual conflict is resolved evolutionarily has been the topic of extensive theoretical work. The first model, proposed by Houston and Davies (1985), assumed that each parent makes an independent single decision about its parental investment, and that this decision can change only over an evolutionary time scale. The model predicts lower overall parental investment and parental fitness than in a purely cooperative situation in which each parent maximizes the sum of its own and its partner's fitness (McNamara et al., 2003). Later models have considered situations in which there are multiple bouts of investment in a given set of offspring, allowing the parents to 'negotiate' their level of investment in relation to that of their mate over a behavioural timescale (Lessells and McNamara, 2012; McNamara et al., 1999; McNamara et al., 2003). Initially, McNamara et al. (1999) modelled the negotiation between the parents when each of the two parents can only change their investment in relation to their mate's investment in the previous bout. Subsequently, Lessells and McNamara (2012) developed a negotiation model where each parent modifies its effort according to the current state of the offspring, which reflects the joint amount of investment by both parents until that moment. Although these models differ in the negotiation rules, they support the original prediction from the Houston and Davies' model that sexual conflict leads to a lower parental care than a cooperative situation (McNamara et al., 2003). These theoretical predictions that sexual conflict can be costly to offspring seem to have empirical support, because offspring are sometimes better off raised by one parent than by two (Royle et al., 2002). However, Johnstone et al. (2014) proposed that sexual conflict may be reduced by a form of conditional cooperation between the parents while provisioning offspring. They modelled a negotiation strategy in which the rates at which each parent visited the brood to provide care when they were, or were not, the last parent to make a visit could evolve. The evolutionarily stable rate at which they provision when they were the last to feed is zero, so that there is strict turn-taking by the parents resulting in the most efficient overall level of care (Johnstone et al., 2014).

The idea that some level of coordination between the pair might reduce the cost of sexual conflict and increase parental fitness is also supported by studies of synchrony of provisioning visits to the nest (Bebbington and Hatchwell, 2016; Mariette and Griffith, 2015; Mariette and Griffith, 2012; Raihani et al., 2010; Shen et al., 2010; van Rooij and Griffith, 2013). In several avian species, nest visit synchrony has been related to increased reproductive success through increased brood survival (Raihani et al., 2010), more equal allocation of resources to the offspring (Shen et al., 2010) and greater offspring mass (Mariette and Griffith, 2015). Moreover, Mariette and Griffith 2015 showed in wild zebra finches *Taeniopygia guttata* that nest synchrony may be a flexible behaviour that pairs adjust to increase parental effort when workload was experimentally increased.

Compared to nest visit synchrony, alternation has recently received more empirical attention (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Johnstone et al., 2014; Khwaja et al., 2017; Koenig and Walters, 2016; Savage et al., 2017). In particular, Johnstone et al. (2014, 2016a) investigated whether great tit *Parus major* parents tend to alternate their nest visits, and whether a turn-taking negotiation rule might be responsible for this effect. They found that alternation was greater than expected by chance, but also realised that 'alternation' could be due to other biological mechanisms instead of, or in addition to, 'turn-taking.' In the current paper, we use the term 'alternation' to refer to the observed pattern in sequences of visits by male and female parents, and 'turn-taking' to refer to a specific type of parental negotiation strategy. Specifically, Johnstone et al. (2014, 2016a) realised that the presence of a refractory period, the lack of short intervisit intervals (at least partially caused by the impossibility for parents of returning immediately to the nest with a new food item) would create some degree of alternation without needing any form of behavioural interaction between the parents. However, Johnstone et al. (2014) accounted for the effect of the refractory period by carrying out randomization tests, in which the sequences of the intervisit intervals by the male and the female were randomly rearranged and showed that alternation in the real sequences was higher than in the randomized ones. This procedure maintains the same frequency distribution of the length of the intervals, and therefore the presence of the refractory period, in both types of sequences but the randomization of the intervals excludes the effect of any form of coordination of visits by the parents. The difference in alternation between the real and randomized sequences therefore indicates that alternation is dependent on the temporal order of the intervals and was interpreted by Johnstone et al. (2014) as evidence of turn-taking. However, as Schlicht et al. (2016) pointed out, other biological factors than turn-taking (e.g. brood size, begging intensity, foraging strategy, weather conditions) can affect the temporal order of the intervals in real provisioning sequences. These factors can in turn generate alternation higher than the values observed in Johnstone et al (Schlicht et al., 2016) with the consequence that there is not yet firm empirical evidence of negotiation via turn-taking (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Khwaja et al., 2017; Koenig and Walters, 2016; Savage et al., 2017).

Another aspect emerging from observational studies is that alternation is not strict and varies among breeding pairs within the same population (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Johnstone et al., 2014; Khwaja et al., 2017; Koenig and Walters, 2016; Savage et al., 2017) and within pairs over the chick rearing period (Iserbyt et al., 2017). This contrasts with the theoretical prediction of Johnstone et al. (2014), but these authors explained this discrepancy as a product of the impossibility for parents of continuously monitoring each other's provisioning behaviour, combined with the risk of not noticing a visit by a mate, which would result in a complete suspension of feeds by both individuals. An alternative explanation for why there is no strict alternation could result from the potential time costs associated with maintaining this negotiation rule. For instance, maintaining a turn-taking provisioning strategy by ceasing or reducing visit rates might be non-beneficial if parents face time constraints because of environmental (e.g. rainfall) or social factors (e.g. workload) or have to devote time to other activities (e.g. predator protection, self-maintenance). In this regard, Iserbyt et al. (2017) showed that alternation of the nest visits was lower in Fife Fancy canaries (*Serinus canaria*) at early stages of the chick rearing period when parents devoted time to other parental activities. This suggests that turn-taking might be a flexible behaviour and responsive to different environmental or social factors (Royle et al., 2014), rather than being consistent within the breeding pair.

In this study, we tested whether alternation of nest visits varies in relation to workload. Furthermore, we explored the rate and regularity of the parent's intervisit intervals to investigate whether the temporal order of the visits caused the observed changes in alternation.

3.2 Methods

Data were collected in 2015 from a great tit, *Parus major*, breeding population in Roekel, a mixed woodland area in Ede, the Netherlands (52°07'N, 5°72'E). This area contains around 250 nest-boxes that were checked weekly from the beginning of April to determine the onset of egg laying and incubation. Active nests were then checked daily from the day before predicted hatching to determine the exact hatch date (day 0).

Experimental set-up

A brood size manipulation experiment was carried out to determine the effect of workload on alternation in provisioning visits to the nest by parents. Parental nest visits were recorded at each of 15 nests for three consecutive days on days 9, 10 and 11 after hatching. For each of the experimental days a brood size manipulation was carried out after 1700h on the previous day to produce brood sizes that were the original size, or reduced or enlarged by about 50% (brood size (mean size ± SE): reduced: 3.3 ± 0.17; original size: 6.5 ± 0.39; enlarged: 9.6 ± 0.59), with manipulation treatments at each nest being in a random sequence. Nestlings swapped into an experimental brood came from broods with the same hatching date. Nestlings were swapped among experimental broods of approximately the same size (the experimental groups were organized in four triplets and one duo), and in the remaining case chicks came from a brood not included in the experiment. Four of the 15 experimental nests were subsequently excluded from the analysis because data were incomplete as a result of nest desertion (n = 3) or failure to record parental provisioning behaviour on one day (n = 1). All broods included in the analysis were composed of an approximately equal number of original and foster chicks and the proportion of foster chicks did not differ among the treatment groups (GLMM on proportion of foster chicks, $\chi^2 = 0.04$, d.f. = 2, P = 0.981, N = 30; data are not available for 3 broods). This experimental design allowed a complete within-nest comparison and eliminated the effect of potential confounding factors (e.g. treatment order and the presence of foster chicks).

Parental nest visit data were obtained using a small video camera (Birdhouse Spy Cam BCAMHEW) mounted in the roof of the nest-box and connected to a portable video recorder (Lawmate PV-500L2) at the foot of the tree. The camera was placed on or before day 8, and recordings (720 x 576 pixels of resolution) started before 0730h on the recording days. Two hours of video (0800h – 1000h) were scored for each day by TC, and parental sex (determined from the blackness of the crown feathers) and time that the bird entered the nest-box (to the nearest s) recorded for each parental visit. Parents brought food for the chicks at 100% of the scored nest visits (n = 2118).

Ethical statement

Permission for this study was granted by the Dutch legal entity: KNAW Dier Experimenten Commissie (DEC) no. NIOO-14.17 to C.M.L.

Calculation of alternation of the nest visits

We defined alternated nest visits as visits of one individual that followed a visit of its mate. For each sequence of nest visits, we scored the status (alternated or non-alternated) of all the visits within the two-hour sampling period. Different amounts of alternation are expected to arise by chance, i.e., assuming constant instantaneous rates in each of the parents, in a sequence of visits as a function of the proportion of visits by the two parents. If one parent makes all the visits in a sequence, no alternated visits can occur and as the proportions of visits by the parents become more equal, the proportion of alternated visits expected by chance increases to 50% when the two parents make the same number of visits. To account for this effect, we used an alternation score (see supplementary material 3.S1 for calculation of alternation score) that measures the deviation of the observed amount of alternation from that expected given the proportion of visits by the two parents. An alternation score of zero represents the amount of alternation expected by chance, a value of less than one means that the observed alternation of the visits is lower than expected by chance, and a value greater than one means that the observed alternation of the visits is greater than expected by chance.

Statistical analysis

All linear mixed models (LMMs) were performed with the *lmer* function in the *lme4* package (Bates et al., 2015) in R environment (version 3.2.3). Unless otherwise stated, we used a backward removal procedure, starting with the full models containing all the main effects and the interaction terms, then dropped the predictor (the interaction terms were tested first) with the highest *P* value in each step until only significant effects remained in the final models. To test significance of the main effects and interaction terms in the linear mixed models, degrees of freedom and *P* values of the F-tests were estimated with the Kenward-Roger approximation implemented in the *pbkrtest* package (Halekoh and Hojsgaard, 2014). Significance was taken at $\alpha = 0.05$ and all models assumptions were met.

We investigated the effect of the brood size manipulation on provisioning rate. We fitted a LMM with individual provisioning rate as the response variable, ‘individual ID’ nested in ‘nest ID’ as random effects, and ‘treatment’, ‘sex’, and their interaction as fixed effects. Provisioning rate was log transformed to improve the normality of model residuals. Furthermore, we explored whether alternation of nest visits changed in response to workload. Here, we first fitted a linear mixed model with alternation score as response variable, ‘treatment’ as fixed effect and ‘nest ID’ as random effects. Next, we carried out Tukey post-hoc tests to investigate differences in alternation among the treatment groups.

To understand how any treatment effect on alternation arose, we investigated the effect of treatment on parameters of the inter-visit interval distributions (IVIs) of each parental sex (mean and standard deviation for males and females), and on the relationship between the standard deviation and mean of the IVIs for each sex (see supplementary material 3.S2 for calculation of standard deviation and mean of the IVIs). IVIs are the intervals between arrivals of two consecutive visits made by the same parent. For the individual parameters, we used LMMs similar to that for alternation score. For example, we tested whether the mean and standard deviation of the IVIs are affected by treatment by fitting separate LMMs with ‘mean’ and ‘standard deviation’ of the IVIs for males and females as the response variable, ‘treatment’ as a fixed effect and ‘individual ID’ nested in ‘nest ID’ as random effects. For the relationship between the standard deviations and means, we first tested whether this relationship differed between the sexes. In the following analyses, we log transformed mean and standard deviation of the IVIs to meet model assumptions. We fitted a model with ‘standard deviation’ as the response variable, ‘individual ID’ nested in ‘nest ID’ as random effects, ‘mean’ of the IVIs, and ‘sex’ as fixed effects.

Next, we investigated whether the relationship between standard deviation and mean of the IVIs is affected by treatment for each sex. Here we fitted models with ‘standard deviation’ as response variable, ‘individual ID’ nested in ‘nest ID’ as random effects, ‘mean’ of the IVIs, and the ‘treatment’ as fixed effects.

Lastly, we explored whether the treatment effect on alternation score can be entirely accounted for by its effect on the IVI distribution. Because the standard deviation of the IVIs is strongly correlated with the mean of the IVIs in both sexes, we used the coefficient of variation (CV) of the IVIs (i.e. standard deviation/mean) to express regularity. As a measure of male and female visit rate, we used the reciprocal of the mean IVIs. This variable has a much less skewed distribution than that of the mean IVIs. We fitted a LMM with alternation score as the response variable and ‘male CV’, ‘reciprocal of male mean IVIs’, ‘female CV’, ‘reciprocal of female mean IVIs’ and ‘treatment’ as fixed effects. ‘Nest ID’ was included as random effect. The presence of a significant ‘treatment’ over and above the effect of the other variables was tested by comparing the full model with a model without ‘treatment’ that retains all the other variables. Significance of the other fixed effects were also tested by removing the single term of interest from the full model.

3.3 Results

Provisioning rate varied in response to manipulated brood size, with male and female parents increasing their provisioning rates as brood size increased (Figure 3.1A, Table 3.1). There was no significant interaction between sex and treatment, suggesting the two parents responded similarly to the change in workload. Nor was there a significant sex effect, showing that the two sexes had similar provisioning rates. In addition, alternation score varied significantly across the treatments (LMM: $F_{2,20} = 6.39$, $P = 0.007$; Figure 3.1B). Tukey post-hoc tests showed that alternation score was higher in the reduced broods than original size ($P = 0.014$) and enlarged broods ($P = 0.002$) with no difference between original size and enlarged broods ($P = 0.86$).

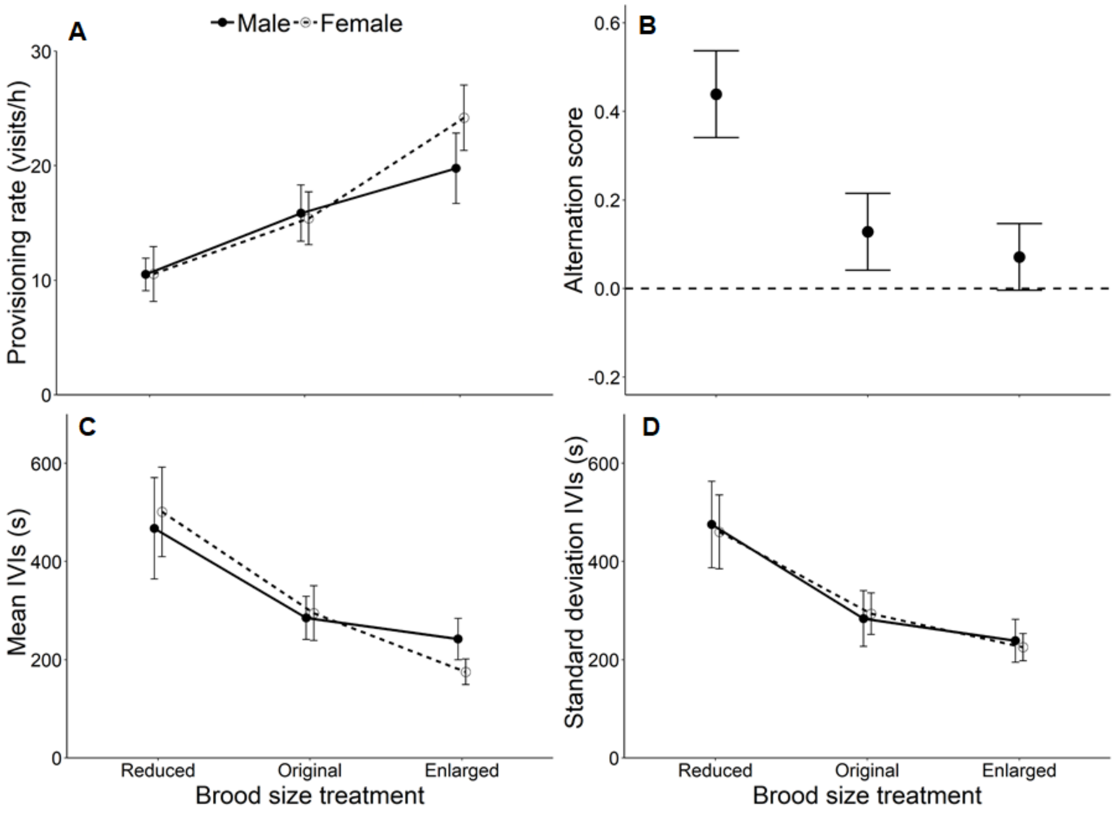

Figure 3.1. Effect of brood size manipulation on provisioning rate, alternation score, mean and standard deviation of the intervisit intervals (IVIs). **A** - Provisioning rate (nest visits/h) for males and females are shown separately. **B** - Alternation score in reduced, original and enlarged broods. An alternation score of zero (dashed line) represents the amount of alternation expected by chance, assuming that the probability of a nest visit by a parent is constant with respect to time. **C** - Mean of the IVIs for males and females. **D** - Standard deviation of the IVIs for males and females. Bars show means and SE of the mean.

To explore why treatment affected alternation score, we first analysed the effect of treatment on characteristics of the distributions of the parents' intervisit intervals that are expected to affect alternation: the rate (represented by the mean of the IVIs) and regularity (represented by the standard deviation of the IVIs). Male and female mean and standard deviation of the IVIs increased at reduced brood size (LMM for male mean: $F_{2,20} = 5.10$, $P =$

0.016; LMM for female mean: $F_{2,20} = 8.63$, $P = 0.002$; Figure 3.1C; LMM for male standard deviation: $F_{2,20} = 6.16$, $P = 0.008$; LMM for female standard deviation: $F_{2,20} = 6.99$, $P = 0.005$, Figure 3.1D), indicating that workload changed the distribution of the intervisit intervals.

Table 3.1. Effects of brood size manipulation (treatment) and sex on log transformed provisioning rate. F, df, and P values are given for the point of exclusion from the model. Estimates and standard errors are given for a model only containing significant terms (shown in bold).

Variable		**Estimate**	**s.e.**	**F**	**df**	**P**
Treatment (brood size)				19.55	2,42	**< 0.001**
	Reduced	0.95	0.06			
	Original	1.14	0.06			
	Enlarged	1.29	0.06			
Sex				0.16	1,10	0.70
Treatment x Sex				1.07	2,40	0.35

Table 3.2. Linear mixed model investigating the presence of a treatment effect on alternation score while controlling for male and female rate and regularity [expressed as the coefficient of variation (CVs)] of the intervisit intervals (IVIs). For each variable, statistics are given by removing the variable of interest from the full model containing all the fixed effects. Significant terms are shown in bold. Estimates and standard errors are given only for significant terms.

Variable		**Estimate**	**s.e.**	**F**	**df**	**P**
Treatment (brood size)	Reduced	0.85	0.17	4.08	2,19.83	**0.033**
	Original	0.55	0.10			
	Enlarged	0.62	0.11			
Female CV of the IVIs		-0.41	0.14	4.94	1,24.69	**0.036**
Reciprocal of female mean IVIs (s^{-1})				0.15	1,23.64	0.70
Male CV of the IVIs				0.28	1,23.31	0.60
Reciprocal of male mean IVIs (s^{-1})				0.07	1,17.77	0.80

We then investigated the relationship between the standard deviation and the mean IVIs for each sex and whether there is a treatment effect. We found that mean and standard deviation of the IVIs were highly positively correlated for males ($F_{1,23.3} = 257.8$, $P < 0.001$) and females ($F_{1,30.6} = 102.7$, $P < 0.001$). There was no treatment effect on this relationship for males ($F_{2,22} = 1.12$, $P = 0.343$) and for females ($F_{2,23} = 0.65$, $P = 0.532$) and the relationship did not differ between the sexes ($F_{1,9.76} = 1.89$, $P = 0.200$).

Lastly, in the final model exploring the effect of treatment while controlling for rate and distribution of the IVIs, treatment remained significant when controlling for the other variables (Table 3.2).

3.4 Discussion

In this study, we experimentally investigated how workload affected alternation of the nest visits in provisioning great tit pairs. Our study indicates that alternation is variable and increases at lightened workloads. More importantly, treatment differences in alternation could not be explained solely by a change in the rate and regularity of the visits.

Our experiment of changing parental workload through a manipulation of brood size was effective. Both males and females similarly increased feeding rates when brood size was increased and decreased feeding rates when brood size was decreased, which is widely shown in previous studies (see table 1 in Gow and Wiebe, 2014). In addition to changing provisioning rate, parents also change the size and type of the prey delivered at the nest (Garcia-Navas and Sanz, 2010; Lifjeld, 1988) and foraging patterns (Gow and Wiebe, 2014; Mariette and Griffith, 2015). Only recently, however, researchers have started to investigate how variation in parental provisioning involves changes in the behavioural interactions between the parents (Mariette and Griffith, 2015).

For example, zebra finch pairs increase synchrony of the nest visits and presence at the foraging patch at higher brood demand (Mariette and Griffith, 2015). To investigate another form of parental coordination, we looked at whether alternation of the nest visits changed as a result of changing parental workload. We found a significant difference in alternation score in relation to workload; alternation increased at reduced workloads. The question is whether this variation in alternation can be attributed to a change in the behavioural interactions between the parents, e.g., turn-taking, and what the functional significance of increased turn-taking at reduced workloads might be.

Johnstone et al. (2014) showed that, if a turn-taking negotiation rule is occurring, it acts at the level of sequence of the intervals and it created a significant amount of alternation in the pattern of the nest visits. In this study, we found that the difference in alternation between the treatment groups is not accounted for solely by a change in the distribution (rate and regularity) of the provisioning intervals of the two parents. Provided that the rate and regularity (means and CVs) adequately represent the frequency distributions, a significant treatment effect after controlling for these variables implies that there must also be an effect of treatment on sequencing. Therefore, the observed change in alternation at reduced brood sizes could be a result of a change in turn-taking.

Why might parents increase turn-taking at reduced workloads? Johnstone et al.'s (2014) turn-taking strategy of food provisioning implies that parents should refrain from further feedings as long as they are the last to feed and provision only when their partner was the last to feed. This implicitly can only occur when there is continuous monitoring of the partner's activity to prevent visits by the partner being overlooked, which would otherwise lead to the complete cessation of feeding. In Johnstone et al. (2014) model, turn-taking and monitoring bear no costs. In practice, however, parental provisioning is likely to be subject to time constraints (Grieco, 2002a, b; Lucas, 1987; Ydenberg et al., 1994) so that parents must allocate their time between different competing activities (Hurtrez et al., 2000; Tóth et al., 1998). In our experiment, parents may thus face a trade-off between maintaining strict turn-taking and provisioning rate, which might explain why we observed more alternation at reduced workload. At higher workloads, parents might discontinuously monitor and respond to the partner, resulting in a lower alternation rate. The original turn-taking model with no cost of negotiation does not allow for flexibility in parental interactions. It would interesting to include potential time costs of turn-taking to allow predictions of how pair coordination might vary in relation to changes in the social and non-social environment (Royle et al., 2014).

An alternative and non-mutual explanation for the observed increase in alternation in reduced broods can involve the offspring's behaviour. Johnstone et al.'s (2014) negotiation model assumes that parents negotiate directly in response to their partner's behaviour. Empirical studies, however, show that negotiated decisions about how much to care involve complex interactions between all family members. Parental provisioning rate indeed has been shown to be affected by factors other than their mate's provisioning rate (Hinde, 2006; Meade et al., 2011; Paredes et al., 2005; Wright and Cuthill, 1989), including brood size (Neuenschwander et al., 2003; Sanz and Tinbergen, 1999; Verhulst and Tinbergen, 1997) and offspring begging (Kilner, 1995; Kilner et al., 1999; Kölliker et al., 2000; Ottosson et al., 1997). In great tits, in particular, parents have been shown to simultaneously integrate all these different cues, with brood size being the main determinant of provisioning behaviour (Hinde and Kilner, 2007). Therefore, variation in alternation in relation to workload could be explained by parents varying the extent to which they respond to the different family members, responding more to each other when the workload is reduced. However, our experiment does not enable us to independently assess the effects of brood size *per se* and partner activity in parental provisioning rules. Experiments with simultaneous manipulation of brood size and provisioning rate of one parent, e.g., by handicapping or playback, might be a reasonable approach to shed light on the behavioural mechanisms underlying provisioning rules.

Despite the fact that turn-taking has been suggested as a mechanism generating alternation in nest visits (Johnstone et al., 2014), other biological factors might be responsible for significant alternation of the visits and for our changes in alternation at different workloads. In particular, Schlicht et al. (2016) argued that biological factors can determine significant levels of alternation. For instance, differences in alternation between treatment groups could be explained by a change in foraging behaviour, which might also contribute to the rate or regularity of the nest visits. In central-place foraging species like the great tit, intervisit intervals consist of travel time (both ways) and residence time at the food patch (Charnov, 1976; Lessells and Stephens, 1983; McNair, 1982; Orians and Pearson, 1979). This time increases with distance of the foraging patch from the nest site and might affect the variation in IVIs and the refractory period. Another aspect of foraging behaviour is whether parents are spatially coordinated in foraging movements. If parents are foraging independently, their visit patterns to the nest box are likely to be independent as well. These patterns will reduce alternation. If they forage together, there is likely correlated temporal heterogeneity in their visit patterns, which would increase alternation.

In conclusion, our study demonstrates that alternation of nest visits is variable and affected by workload in great tit parents. In particular, processes that affect visit sequencing, e.g., turn-taking, are responsible for variation in alternation. However, further studies are necessary to investigate which biological mechanisms generate the observed differences in alternation. We strongly encourage the study of the behavioural mechanisms underlying negotiation rules.

Acknowledgements

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Supplementary material

3.S1 - Calculation of alternation score

We used an alternation score which represents the deviation of the observed amount of alternation from that expected assuming that the parents feed at a constant instantaneous rate equal to their observed provisioning rate. The alternation score therefore corrects for the expected variation in alternation with the proportion of feeds by each of the two parents. Because the score is based on log odds, the scores are expected to be normally distributed on an additive scale.

We calculated the alternation score by classifying each visit (focal visit) during the two-hour observation periods as alternated or non-alternated depending on the sex of the parent(s) making the focal and preceding visits. (To assign the status of the first visit within each two-hour observation period, we used the last visit before the sampling period started as the preceding visit.) From the status of the visits we constructed a two-by-two contingency table classified by the sex of the parent(s) making the focal visit and the preceding visit (Table 3.S1). The cells of the tables contains the number of occasions on which each of the four types of visits were made ($v_{a,m}$: no. of alternated visits by the male; $v_{na,m}$: no. of non-alternated visits by the male; $v_{a,f}$: no. of alternated visits by the female; $v_{a,f}$: no. of non-alternated visits by the female).

Table 3.S1. Two-by-two contingency table containing visits classified by the sex of the parent(s) at the focal and preceding visits.

		Preceding visit:		
		Male	Female	Row total
Focal visit:	Male	$v_{na,m}$	$v_{a,m}$	$v_{na,m} + v_{a,m}$
	Female	$v_{a,f}$	$v_{na,f}$	$v_{a,f} + v_{na,f}$
	Column total	$v_{na,m} + v_{a,f}$	$v_{a,m} + v_{na,f}$	V

From these values, the expected number of alternated visits is

$$\frac{\left(v_{na,m} + v_{a,m}\right)\left(v_{a,m} + v_{na,f}\right) + \left(v_{a,f} + v_{na,f}\right)\left(v_{na,m} + v_{a,f}\right)}{V},$$

and the expected number of non-alternated visits

$$\frac{\left(v_{na,m} + v_{a,m}\right)\left(v_{na,m} + v_{a,f}\right) + \left(v_{a,f} + v_{na,f}\right)\left(v_{a,m} + v_{na,f}\right)}{V}.$$

We then calculated the alternation score as the following difference in log odds:

$$\begin{aligned} Alternation\ score\ = \\ \log\left(\frac{Observed\ no.\ alternated\ visits}{Observed\ no.\ non\text{-}alternated\ visits}\right) \\ -\ \log\left(\frac{Expected\ no.\ alternated\ visits}{Expected\ no.\ non\text{-}alternated\ visits}\right)\ . \end{aligned}$$

Alternation score has a value of zero if there is no difference between the observed and expected amount of alternation, a positive value when the there is more alternation than expected, and a negative value when there is less alternation than expected.

Based on calculated values using the supplementary data in Johnstone et al. 2014, the alternation score is directly proportional to the Pearson contingency coeffcient (see Figure 3.S1), with a proportionality constant of about -1.77.

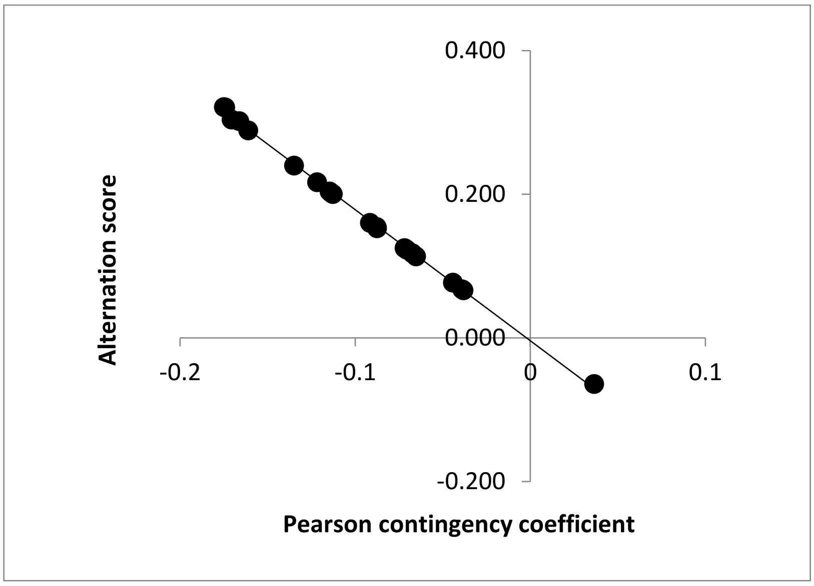

Figure 3.S1. The relationship between the alternation score and the Pearson contingency coefficient. Points are calculated values using the supplementary data in Johnstone et al. 2014 for 21 nests (data for chick ages 9 and 10 days combined when both were available).

3. S2 - Calculation of the mean and standard deviation of the length inter-visit intervals (IVIs)

For each sex separately, we calculated the mean and standard deviation of the length of inter-visit intervals (IVIs) as a measure of the rate and regularity of the provisioning visits. We used all the intervals occurring within the two-hour sampling period plus, for each sex, the IVIs at the ends of the two hour sampling period that were cut by the start and end of the sampling period (0800h and 1000h). We expected these ‘cut IVIs’ to be longer on average than the uncut IVIs within the sequence because they present larger targets for arbitrarily chosen points in time (in our case, 0800h and 1000h). For example, an individual 500s IVI should be five times as likely to be cut as an individual 100s IVI. We confirmed that in both sexes cut IVIs (first and last cut IVIs separately) were significantly longer than the median length of the uncut IVIs (Table 3.S2). We also confirmed that the lengths of the first and last cut IVIs did not differ significantly, nor did the lengths of the initial and final ‘bookends’ (the parts of the cut IVIs within the two hour sampling period), suggesting that the lengths of the cut IVIs and bookends at the ends of the sampling period were not affected by disturbance at the nest when the video recording was started, or by some diurnal trend (Table 3.S2).

Because cut IVIs are longer on average than uncut IVIs, their exclusion would bias the means and standard deviations of the length of the IVIs. This would be especially a problem in our experiment because provisioning rates (and hence the proportion of IVIs that are cut) varied between brood size treatments. However, although the uncut IVIs within a sampling period are a *biased* sample of IVIs, the visits within a sampling period are an *unbiased* sample of visits. Hence they are also an unbiased sample of the start, or of the end, of IVIs. This means that unbiased estimates of the mean and standard deviation of the length of IVIs can be calculated based on the uncut IVIs in a sampling period plus either the first, or last, cut IVI. Because there was no evidence for a difference in the length of the first and last cut IVIs in our data (see above), we calculated the mean and standard deviation of the lengths of the IVIs twice for each two-hour observation – including either the first, or last, cut IVI – and then took the average of the two estimates. By doing this, we increased the precision of the estimates above that gained by using either only the first, or last, cut IVI.

We measured IVIs as the intervals between arrivals at the nest on successive visits by a parent, so that visit duration is included as part of the round trip time. If visit duration is short relative to the total round trip time and/or nest departure is independent of visits by the other member of the pair, visit duration should not greatly affect alternation. However, if neither of these conditions hold, visit duration, and the timing of nest departure, might affect the alternation score. In great tits, females with 10 day old chicks sometimes spend several minutes in the nest brooding or carrying out ‘nest sanitation’ behaviour, and the arrival of the male often precipitates the female’s departure (C.M.L. personal observation of videos). If the amount of brooding or nest sanitation varies with brood size, this might have contributed to the treatment effect that we observed. It is not clear how this could be addressed in the analyses presented here, or in analyses such as the randomisation tests carried out by Johnstone et al (2014).

Table 3.S2. Pairwise comparisons for males and females between the lengths of the first or last cut IVIs and the median of the uncut IVIs, first and last cut IVIs, and initial and final bookends.

	Paired t-test, t(*P*)[a]		Wilcoxon rank test, V(*P*)[a]	
	males	females	males	females
first cut IVI[b] *vs* median of the uncut IVIs	3.45 (**<0.001**)	4.25 (**<0.001**)	99.0 (<**0.001**)	47.0 (**<0.001**)
last cut IVI *vs* median of the uncut IVIs	3.22 (**0.003**)	4.08 (<**0.001**)	42.0 (<**0.001**)	40.5 (<**0.001**)
first *vs* last cut IVI	1.13 (0.265)	0.81 (0.423)	224.5 (0.321)	250.5 (0.598)
initial *vs* final bookend[c]	0.27 (0.790)	0.27 (0.785)	294.5 (0.809)	228.5 (0.357)

[a] N = 33 comparisons (3 observation periods at each of 11 nests) for all tests.

[b] a ‘cut IVI’ is an IVI that straddles the start or end of the observation period.

[c] a ‘bookend’ is the part of a cut IVI within the observation period.

Chapter 4

Spatial coordination of foraging is related to alternation of the nest visits in a biparental songbird

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In preparation

Abstract

Parents caring for offspring face an evolutionary conflict of interest over how much to invest, leading to a sexual conflict. Parents are therefore expected to negotiate their efforts. Recently, 'turn-taking' in provisioning visits, by which parents respond to their partner's behaviour, has been proposed as a negotiation rule. Several empirical studies in birds indicate that parents do indeed alternate their nest-visits more than expected by chance, with evidence that within nest factors (e.g. turn-taking, foraging coordination between parents, offspring begging) are the main processes generating the observed pattern of visits. However it is still not known whether parents coordinate their foraging activity while provisioning the offspring and how this coordination is related to the pattern of visits at the nest. We combined video recordings at the nests with an automated radio-tracking technology, to remotely monitor provisioning activity of eight wild great tit (*Parus major*) pairs during chick rearing. We explored i) whether parents coordinate their foraging movement in space and time, and ii) whether alternation of the nest visits relates to spatial coordination of the parents. We found that during nest provisioning, foraging behaviour of the parents was highly coordinated in space and time and that the degree of parental coordination was positively associated with nest visit alternation. Despite the fact that we cannot discriminate between two explanations (alternation is produced by active turn-taking between the parents or results from foraging coordination alone), this study underlines the importance of foraging coordination for studying the behavioural mechanisms underlying negotiation over offspring care.

4.1 Introduction

Raising a family is not free of conflict for parents when they have to decide how much to invest in the current offspring (Parker, 1979; Royle et al., 2012; Trivers, 1972). In species with biparental care, this decision does not only depend on a trade-off between the production of current and future offspring (Roff, 2002; Stearns, 1992) but also on sexual conflict, an evolutionary conflict of interest between the two sexes (Trivers, 1972). The consequence of this conflict is that each parent is selected to exploit its mate, leaving a larger share of the care, as parents caring for common offspring share the benefit of their joint investment but only pay the cost of their own care (Lessells, 2006; Wedell et al., 2006).

How this sexual conflict is resolved evolutionarily and how it affects the final amount of care that parents devote to offspring has been the topic of extensive theoretical work. While the first theoretical model assumed that each parent makes an independent single decision about its parental investment and that this decision can change only over an evolutionary time scale (Houston and Davies, 1985), later models allowed parents to behaviourally respond to each other, so to negotiate their investment in response to that of their mate over multiple investment bouts (Johnstone and Hinde, 2006; Lessells and McNamara, 2012; McNamara et al., 1999; McNamara et al., 2003). Both types of models, however, predict that sexual conflict and negotiation lead to a suboptimal level of care, which seems to find empirical support in a zebra finch *Taeniopygia guttata* study, where offspring received greater *per capita* parental investment when raised by single females than when feed by both parents (Royle et al., 2002).

In addition, the other main prediction of all the above mentioned negotiation models is that, to avoid exploitation by the partner, a reduction in parental care by one parent should be only partially compensated by the other. This theoretical prediction has been intensively studied by experimental manipulations of the level of investment of one parent and the overall results of these studies are in line with the prediction of partial compensation, as suggested by a meta-analysis in birds species (Harrison et al., 2009). However there was substantial variation across studies and species in the direction and magnitude of the response: in some studies parents were observed to match the response of the partner (Hinde, 2006; Hinde and Kilner, 2007), in others was observed no response (Rauter and

Moore, 2004; Smiseth et al., 2005; Suzuki and Nagano, 2009), or complete (Paredes et al., 2005; Tomotani et al., 2018), or even over-compensation (Griggio and Pilastro, 2007). This variation in the behavioural responses was thought to occur when parents use the negotiation process to gain information about the current need of their offspring (Johnstone and Hinde, 2006). This implies that parents may negotiate their investment by gathering information from multiple family members. Parents may indirectly asses partner’s contributions via offspring condition (Lessells and McNamara, 2012) or may directly respond to each other’s behaviour (Hinde, 2006; Johnstone and Hinde, 2006; Johnstone et al., 2014). In birds for instance, parents are seen to negotiate via the offspring, by positively responding to an increase of begging calls by the young (Hinde and Kilner, 2007; Ottosson et al., 1997), and via the observation of their partner’s behaviour (Hinde, 2006). There is also evidence that parental investment is simultaneously affected by both of these stimuli (Hinde and Kilner, 2007).

More importantly, the adopted negotiation mechanism critically affects the exact evolutionary outcome of sexual conflict (Johnstone et al., 2014; Lessells and McNamara, 2012). The prediction of an overall decrease in parental care of the early theoretical models (Houston and Davies, 1985; Lessells and McNamara, 2012; McNamara et al., 1999; McNamara et al., 2003) in fact, implicitly assumes that parents cannot make a ‘binding agreement’ about how much each parent should invest in the offspring so that any form of investment made by one individual is open to exploitation by the other. Intriguingly, Johnstone et al. (2014) proposed that simple forms of conditional cooperation may be able to create this ‘binding agreement’. They proposed a new negotiation mechanism where parents take turns of investment (e.g. provisioning the offspring) over time (Johnstone et al., 2014). Specifically, this negotiation rule was modelled with parents visiting the brood to provide care at two different rates, depending on whether they were, or were not, the last to visit the nest. The evolutionary stable outcome of this strategy is a turn-taking rule where each parent does not provision when it was the last to feed, but only after a visit by the mate, leading to a strict alternation of the nest visits. Implicitly this turn-taking rule creates this ‘binding agreement’ between the parents as it guarantees that each investment bout by one parent will be later reciprocated by the other.

The hypothesis that parents may adopt this form of conditional cooperation have triggered new studies on parental behaviour in avian species (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Johnstone et al., 2014; Khwaja et al., 2017; Koenig and Walters, 2016; Savage et al., 2017), showing that in different species, parents tend to alternate the visits at the nest more than expected by chance (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Johnstone et al., 2014; Koenig and Walters, 2016; Savage et al., 2017). However, only few of these studies tried to investigate the biological processes generating the higher-than-random alternation of the nest visits (Bebbington and Hatchwell, 2016; Savage et al., 2017) and in particular whether parents actively take turns of the visits. In this regard, Schlicht et al. (2016) argued that significant amount of alternation can be produced by processes other than turn-taking (e.g. weather conditions, offspring begging) affecting the sequence of visits.

In chapter two of this thesis we used provisioning sequences of great tit parents and explored the contribution of these different processes on alternation. We showed that nest-specific factors (perhaps due to e.g. turn-taking, foraging coordination, offspring begging) were the main contributors to alternation of the nest visits explaining up to 80% of the observed pattern of visits. However in that study we could not explore the effect of these factors individually as we only had data of provisioning at the nest available. Recent advancements in technology, however, allow monitoring spatial movements and social interactions between individuals (Krause et al., 2011; Mennill et al., 2012) and can be applied to investigate the relationship between alternation of the nest visits and spatial movements of the provisioning parents. Evidence that pattern of nest visits can result from foraging coordination between the parents come from wild zebra finches. For this species Mariette and Griffith (2015) showed a positive association between synchrony of the parental visits at the nest and synchrony of the visits to artificial feeders scattered inside the foraging territory (Mariette and Griffith, 2015).

In this study, we tested the hypothesis that alternation of the nest visits is related to spatial coordination between the parents in their provisioning. For this, we used the automated Encounternet tracking technology (Maynard et al., 2015; Mennill et al., 2012; Rutz et al., 2012; Snijders et al., 2017a; Snijders et al., 2014) to remotely monitor spatial movements of wild great tit (*Parus major*) pairs during chick rearing. Firstly, we explored whether great tit parents coordinate their foraging movements in space and time. Secondly, we investigated the relationship between alternation of the nest visits and spatial coordination of the parents.

4.2 Methods

Study population and data collection

This study was conducted in 2016 in a great tit population in Roekel, a mixed woodland area in Ede, the Netherlands (52°07'N, 5°72'E). This area contains around 250 nest-boxes that were checked weekly from the beginning of April to determine the onset of egg laying and incubation. During early egg laying, active great tit nests were pre-selected to be radio-tracked based on their position in the field site. Specifically, we selected nests in which the array would contain relatively homogenous tree coverage, i.e., avoiding nests close to human paths and open fields. 16 birds (8 pairs) were caught and fitted with radio tags during incubation or the chick rearing period. Five males were caught during the incubation period using a mist net nearby the nest site. During incubation, three females were also caught when leaving the nest-box early in the morning with 'box nets' (te Marvelde et al., 2011) placed around the nest box. All the remaining individuals were caught and tagged at the nest during chick provisioning. There was no effect of how adults were trapped or stage of trapping on nest characteristics (Mann-Whitney tests, all $P > 0.3$).

We collected radio tracking data with the automatic tracking system Encounternet (Encounternet LLC, Portland, OR, U.S.A.). Encounternet consists of small radio transmitters of approximately 1 g, fitted to the bird with a leg-looped backpack harness (Rappole and Tipton, 1991). These tags broadcast a radio signal every 5s, which is recorded by small wireless receivers logging the ID number, time and received signal strength indication (RSSI) of every tag pulse they receive (Mennill et al., 2012). To track spatial movements of the eight tagged pairs during chick provisioning, for each pair we deployed 38 receivers around the nest site in a 25-m array. These receivers were placed in a triangular array consisting of three 'rings' at 25, 50 and 75 meters from the nest containing six, twelve and 18 receivers, respectively (Figure 4.1). The receivers were positioned in trees or plastic poles at *ca.* 3.5m height at a regular distance of approximately 25m distance one another. On average, parents spent 63% of their time within the array (mean ± SE: 0.63 ± 0.02) and the proportion of time inside of the array negatively correlated with the number of chicks (GLMM, estimate ± SE: -0.60 ± 0.26, $\chi^2 = 4.21$, d.f. = 1, $P = 0.04$, N = 168). The coordinates of the receivers were located and surveyed in the field with a survey-grade GPS (Ashtech ProMark 800, Santa Clara, CA, U.S.A.). At the nest site, two more receivers were placed, one on the front side of the tree *ca.* 50 cm above the nest-box, and the other on the back side of tree trunk at the same height off the ground. In addition to the Encounternet array, a small video camera was mounted in the roof of the nest-box and connected to an external video recorder at the foot of the tree. Video recordings (720 x 576 pixels of resolution) started before 0730h and ended on the recording days and were synchronized (to the nearest second) to the Encounternet array to simultaneously monitor spatial movements and visits at the nest of the provisioning parents. Although Snijders et al. (2017b) found no effect of Encounternet tags on provisioning rate and visit duration at the nest in great tit parents, all parents were tagged at least two days before data collection to reduce possible effects of tagging on provisioning activity of the parents. The array of receivers and the video set-up were also placed on the day prior to data collection to habituate parents to their presence.

For each Encounternet nest, we collected video recordings and radio tracking data of both parents for five consecutive days. In this study, we used 84 hours of data collected on the first (eight hours of data from 0800h to 1600h for all nests) and last (four hours of data from 0800h to 1200h for five nests) day of the five day period. The nests differed in the brood size (mean ± SD: 7.5 ± 0.93) and age of the chicks (mean ± SD: 11 ± 1.77). Brood size, chick age and April day did not differ between the nest recorded only on one day or on two days (Mann-Whitney tests, all $P > 0.3$). A total of 2661 nest visits were detected from the video recordings, for which we scored the parental sex (determined from the blackness of the crown feathers) and the times that the bird entered and left the nest-box (to the nearest second). We used a triangulation algorithm implemented in MATLAB (The MathWorks, Natick, MA, U.S.A.) to locate the position inside the array of each tagged parents every five seconds from the radio signals logged by the receivers (see supplementary material 4.S1 for validation of triangulation algorithm). A total of 76067 locations were estimated within the 84 hours used in this study.

Calculation of similarity in spatial utilization distribution between parents

To investigate spatial coordination between provisioning parents, for each hour we used dynamic Brownian bridge movement models (Kranstauber et al., 2012) to estimate the utilization distributions (UDs) of each parent (Figure 4.1). This method uses the time series of sequential locations for each individual and summarizes its movement into a 2-dimensional spatial representation referred to as utilization distribution (Worton, 1989). A comparison between UDs of different individuals, via indices of spatio-temporal overlap (Fieberg and Kochanny, 2005), have been used to explicitly quantify interactions between individuals (Lewis et al., 2017; Robert et al., 2012; Schauber et al., 2015). In this study, we compared male and female UDs by using the earth mover’s distance (EMD), a measure that quantifies the similarity between two UDs (Kranstauber et al., 2017a). EMD can be interpreted as the amount of work necessary to shape one UD into the other, thus the EMD increases with increasing dissimilarity between two UDs whereas it assumes a value of 0 when two identical UDs are compared. We use EMD as measure of spatial coordination (similarity) of male and female parents in their provisioning. Calculations of the parental UDs via dynamic Brownian bridge movement models (Kranstauber et al., 2012) and EMD (Kranstauber et al., 2017a) were performed in the R package ‘*move*’ (Kranstauber et al., 2017b).

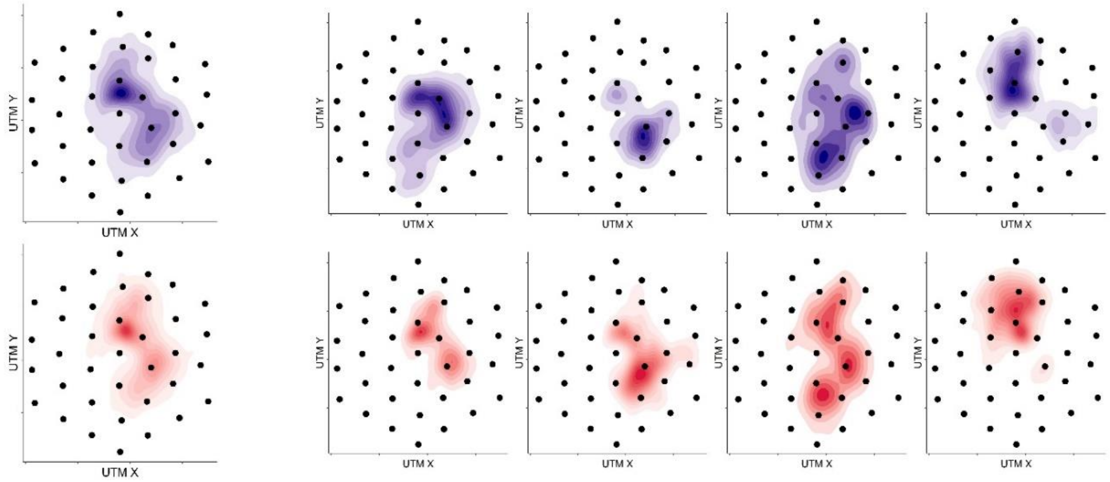

Figure 4.1. Example of utilization distributions (UDs) of a single pair (male and female respectively in blue and red). The two leftmost UDs represent male and female daily space use over the full period of eight hours. The other four UDs, from left to right, represent the hourly UDs of the first four hours (from 0800h to 1200h) for each sex. The black dots are the Encounternet receivers.

Calculation of alternation of the nest visits

The nest visits were scored from the video recordings and used to calculate alternation of the nest visits for each one-hour period. We defined alternated nest visits as visits of one individual that followed a visit of its mate. For each one-hour sequence of nest visits, we scored the status (alternated or non-alternated) of all the visits except for the first visit. Different amounts of alternation can arise by chance in a sequence of visits as a function of the proportion of visits by the two parents. If one parent, for instance, makes either all or none of the visits in a sequence, no alternated visits can occur. Conversely, as the proportions of visits by both parents become equal, the proportion of alternated visits expected by chance increases. To account for this effect, we calculated and used in the statistical analysis an alternation score (see supplementary material 2.S1 for calculation of alternation score) that measures the deviation of the observed amount of alternation from that expected given the proportion of visits by the two parents.

Statistical analysis

To investigate whether parents coordinate foraging in space and time we carried out analyses at different temporal scales: a broad scale (among hours) analysis and a fine scale (within hour) analysis.

In the broad scale analysis, we used male and female hourly utilization distributions (UDs). For each nest and each day, we calculated the EMD between the male and female UDs of the same one-hour period (e.g. male UD from 0800h to 0900h with female UD from 0800h to 0900h). These EMD values are defined as “lag 0” EMD as they represent the observed similarity in the parental space use. To explore whether parents are coordinated in space and time, within each nest and day, we calculated the EMD between all combinations of male and female hourly UDs, by progressively lagging the UDs of one parent relative to the other. For instance, by lagging the female UDs by one hour, we compared the EMD between male UDs from 0900h to 1000h with female UD from 0800h to 0900h and so on. These EMD values are defined as “lagged” EMD, as they represent the similarity in the parental space use when one individual provisioning activity is lagged relative to the other. In this way, we expected that if parents are coordinated in space but also in time, the EMDs at lag 0 would be smaller (higher similarity in the UDs) compared to the lagged values in absence of any periodicity in foraging territory use. To test for spatio-temporal coordination between parents, we investigated whether the EMD value of a parental UD (focal UD) was smaller when matched with its own partner's UD at the same time (lag 0) or with its own partner's UD at different time (lagged EMD). This approach created a pseudoreplication issue since the same UD was present multiple times in the dataset. To resolve this problem, this analysis was performed using linear mixed models with EMD as response variables, "lag" as fixed effect and "parental UD" nested in "Nest ID" as random factor. The variable "lag" was used as factor in the analysis. We ran this comparison twice, one using "male UD ID" and one using "female UD ID" as random factor to test whether the difference between EMD types depended on the sex of the focal UD. We also run post-hoc tests to investigate the differences in EMD between the different lag classes.

In the fine scale analysis, we further investigated spatio-temporal coordination between parents by decomposing parental hourly UDs into six UDs, each consisting of a ten-minute period. In this fine scale analysis, we applied the same methodology and statistical models of the broad scale analysis (in the fine scale analysis the lag between UDs occurred in steps of 10 min periods instead of one-hour periods) to explore whether pair coordination in space also occurred at a smaller temporal scale.

In addition to the previous analysis, we explored whether pair coordination varied over time (time of day, period during breeding season) or was related to brood characteristics, such as number and age of the chicks. We used linear mixed models with EMD as response variables, 'hour of the day', 'April day', 'number of chicks' and 'chick age' as fixed effects. 'Nest ID' was included as random effect to account for repeated measures. All the fixed effects were included as covariates in the models. The correlation coefficients among these variables were small, so we included the independent variables in the same model ($R^2 < 0.3$).

Lastly, we investigated whether alternation of the nest visits is related to parental spatial coordination. We fitted linear mixed models with alternation score as response variable, 'EMD, 'hour of the day', 'April day', 'number of chicks' and 'chick age' as fixed effects. 'Nest ID' was included as random effect. All the fixed effects were included as covariates in the models. The amount of variance explained by 'EMD' was estimated by calculating the marginal R^2GLMM(m) of the reduced model, using the methods described by Nakagawa and Schielzeth (2013).

All the statistical analyses were performed in R environment (version 3.2.3). All mixed models were performed with the *lme4* package (Bates et al., 2015) and the significance of the main effects was calculated with the Kenward-Roger approximation implemented in the *pbkrtest* package (Halekoh and Hojsgaard, 2014). To define the minimal adequate models, we used backwards model selection, dropping non-significant terms in each step. In all models, the proportion of available locations for each period used to generate the UDs were included to weight the cases (Bates et al., 2015; Wood and Scheipl, 2017).

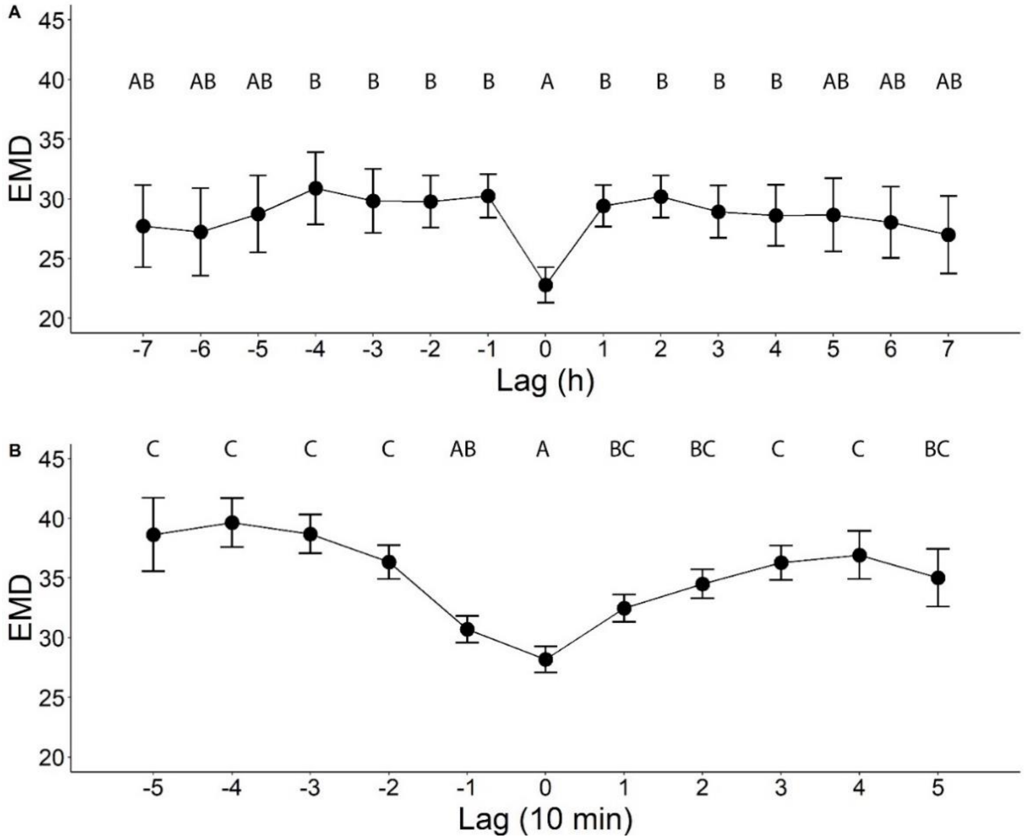

Figure 4.2. Broad (**A**) and fine (**B**) scale analysis of similarity in parental spatial utilization distribution (EMD) in relation to lag. **A –** Effect of lag on EMD in the broad scale analysis. Positive lag values refer to a situation when female UDs are compared with male UDs at an earlier point in time (e.g. female UD at 1000h with male UD at 0800 has a +2 lag). Negative values of lag refer to the opposite situation when male UDs are compared with female UDs at an earlier point in time (e.g. male UD at 1000h with female UD at 0800 has a lag value of -2). **B –** Effect of lag on EMD in the fine scale analysis. Also in here, positive values of lag occur when female UDs are compared with male UDs occurred earlier in time, whereas negative values of lag occur when male UDs are compared with female UDs occurred earlier in time. Mean ± SE are given. Different letters indicate significant differences among lag classes in the post-hoc tests.

4.3 Results

In the broad scale analysis, earth mover's distance (EMD) between male and female utilization distributions (UDs) differed between lags ($F_{14,83}$ = 2.66, *P* = 0.001 when "male UD ID" was used as random factor; $F_{1,83}$ = 2.94, *P* < 0.001 when "female UD ID" was used as random factor, Figure 4.2A). EMD was significantly smaller at lag 0 indicating that parental provisioning activity was coordinated in space and time. Note that the EMD values at lags farther away from 0 show a declining trend and might be due to lower sample sizes at longer lag comparison that create larger variances. The same pattern was found in the fine scale analysis ($F_{10,379}$ = 11.46, *P* < 0.001 when "male UD ID" was used as random factor; $F_{10,424}$ = 13.31, *P* < 0.001 when "female UD ID" was used as random factor, Figure 4.2B) showing that parental coordination occurred at short temporal scale (minutes). In the post-hoc tests, EMD did not differ between lag -1 and lag 0, suggesting that female might act as leader in foraging movement.

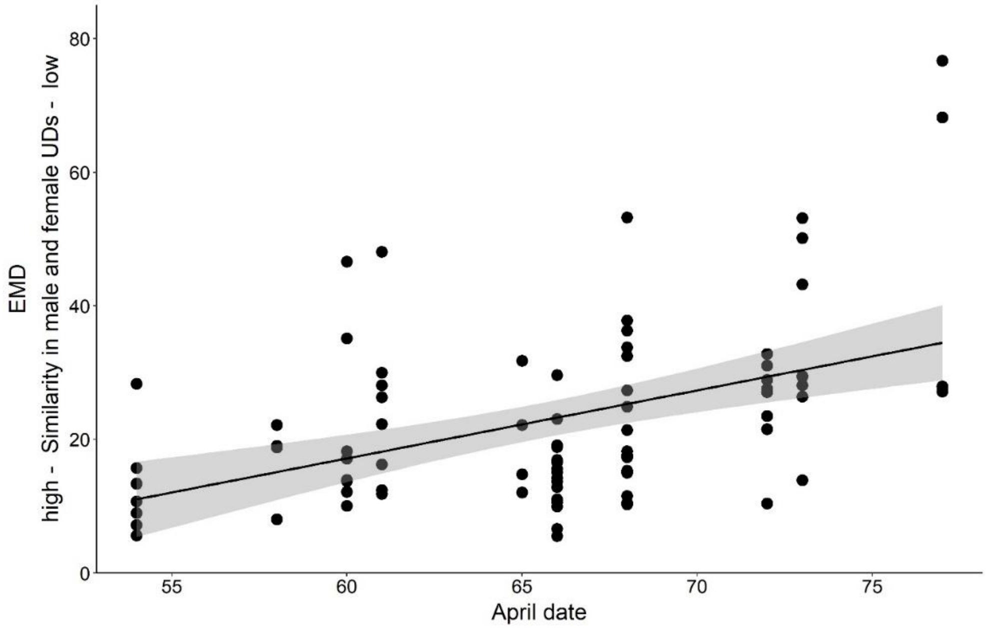

Figure 4.3. Relation between similarity in male and female spatial utilization distributions (EMD) and April date. The line represents fitted values with its SE represented by shaded areas.

Table 4.1. Estimated parameters of the linear mixed models investigating the effect of nest variables on EMD. 'Nest ID' was included as random effect in the models. For each variable, statistics are given for the point of exclusion from the model. Significant terms are shown in bold. Estimates and standard errors are given only for significant terms.

Variables	Estimate	s.e.	F-test	ndf	ddf	P-value
our			0.87	1	34.80	0.358
April day	0.15	0.02	6.97	1	7.30	**0.032**
number of chicks			0.01	1	3.27	0.914
chick age			0.81	1	14.23	0.383

In addition, EMD significantly increased with April date (Figure 4.3; Table 4.1) suggesting that pair coordination decreased (higher EMD) at the end of the breeding season. Furthermore, alternation score was related to EMD (Figure 4.4; Table 4.2), suggesting that higher alternation of the nest visits was associated to spatial coordination of the provisioning activity. EMD explained 12% of the variance of alternation score (R^2GLMM(m) of the reduced model: 0.12).

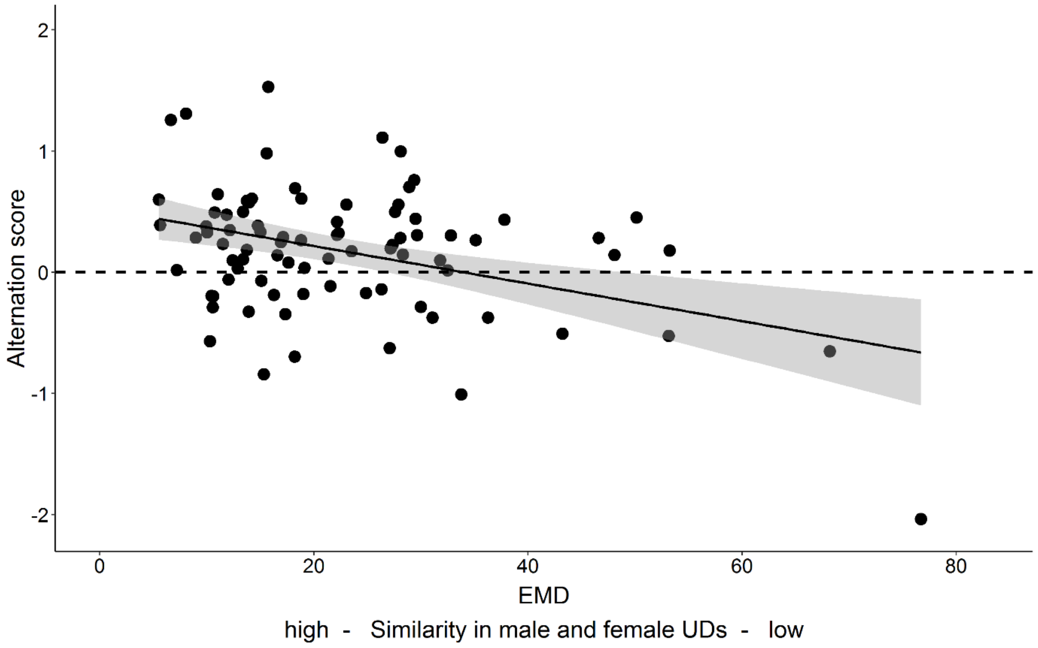

Figure 4.4. Alternation score in relation to similarity in male and female spatial utilization distribution (EMD). An alternation score of zero (dashed line) represents the amount of alternation expected by chance, assuming that the probability of a nest visit by a parent is constant with respect to time. The line represents fitted values with its SE represented by shaded areas.

Table 4.2. Estimated parameters of the linear mixed models investigating the effect of EMD on alternation score. 'Nest ID' was included as random effect in the models. For each variable, statistics are given for the point of exclusion from the model. Significant terms are shown in bold. Estimates and standard errors are given only for significant terms.

Variables	Estimate	s.e.	F-test	ndf	ddf	P-value
hour			1.18	1	34.60	0.285
April day			0.03	1	3.01	0.872
number of chicks			0.75	1	1.85	0.485
chick age			5.35	1	6.53	0.057
EMD	-0.011	0.005	5.33	1	26.11	**0.029**

4.4 Discussion

We used two days of continuous Encounternet and video recordings to investigate whether parents were coordinated in space and time, as measured by similarity in parental space-use. We found that during nest provisioning, parents were indeed coordinated both spatially and temporally more than expected by chance. Additionally, high parental coordination correlated with more alternated nest visits.

To the best of our knowledge, this is the first time we have concrete spatial data on pair coordination of songbirds in their natural setting. In zebra finches, Mariette and Griffith (2015) found that pairs coordinated their foraging by synchronizing their visits to feeders deployed in their territory. Our study reinforces their findings showing that pair coordination also occurs in completely natural situation where individuals were free to search for food in their environment. Furthermore, by lagging the provisioning activity of one parent over the other by blocks of ten minutes, we also showed that this spatio temporal coordination takes place on the order of few minutes. Because foraging trips also occur at a similar time scale (in this dataset parents visits on average every 2 minutes), these findings suggest that parents might be able to continuously monitor and promptly respond to the visits and spatial movements of the partner. This fine scale coordination could therefore represent the behavioural mechanism underlying the negotiated responses of parents observed when the provisioning rates of their partners were experimentally manipulated (Griggio and Pilastro, 2007; Hinde, 2006; Hinde and Kilner, 2007; Paredes et al., 2005; Tomotani et al., 2018). In addition to spatio temporal coordination, we found indication that females might be leading the foraging movements compared to males, as coordination at lag -1 did not differ from lag 0 in the fine scale analysis. This finding also suggests that males might be responsible for maintaining coordination and respond to the movements of the females. Previous studies on pair coordination did not directly investigate, within breeding pairs or groups, which individual leads the provisioning and who maintains the pair/group coordination (Koenig and Walters, 2016; Mariette and Griffith, 2015).

Why females might act as leaders in the provisioning movement? One possible explanation is that females are more responsive to or better informed about offspring condition and needs (Kölliker et al., 2000) and then foraging in different locations, depending on the density/quality of the prey to deliver at the nest (Kacelnik, 1984). Alternatively, different sexes might simply use different foraging strategies and female switch foraging patches more often than males (Naef-Daenzer and Keller, 1999). We note, however, that our method of pair coordination does not directly measure the exact distance between the two parents over time. We showed that pairs are using the same space at the same time, but in this study we did not measure directly how often parents occurred in close proximity to each other. However, Robert et al. (2012) showed that indices of spatio-temporal overlap correlated with contact rate between individuals. The main reason why our approach is more valid for the current dataset than calculating the actual distances between the parents over time is because our birds can occur (individually or both at the same time) outside of the array, resulting in missing locations. Using only periods where both parents were inside the array to estimate an averaged measure of distance between the individuals, would have created a bias for pairs that had most of their foraging locations outside the array because their actual mean proximity would have been underestimated. If for instance parents were foraging in two different directions outside the array (and therefore non-coordinating their movement) but only occur inside the array when returning at the nest to feed the offspring we would have substantially underestimate their average proximity and overestimate their pair coordination. Instead our UDs take into account the direction of the foraging trips and in the above circumstance the EMD would correctly estimate lower coordination between the parents. Further analysis on the directions of the foraging trips on this dataset would thus be useful to support that similarity in UDs correctly estimates coordination in the foraging movement.

We found that parents were less coordinated towards the end of the breeding season. This result corroborates with studies that have shown that caterpillar abundance decreases towards the end of the great tit breeding season (Naef-Daenzer and Keller, 1999; Naef-Daenzer et al., 2000; Visser et al., 2006). For the parents that bred later in the season in poorer environments, they may need to sample different areas to find food sources, which may result in parents breaking the spatial and temporal coordination of their movements. Alternatively but not mutually exclusive, less pair coordination at the end of the breeding season might be related to less quality individuals (Verhulst et al., 1995). This opens up the intriguing hypothesis that pair coordination might be an intrinsic property of the pair and be less dependent on the environmental components. However in this study we have data from a limited number of pairs to draw firm conclusions on the effect of life history traits (e.g. clutch size, time of reproduction), pair composition (age of the parents, pair bond duration) and environmental factors (prey abundance and patchiness) on pair coordination.

More importantly, we found that periods with more spatial coordination between the parents were also characterized by more alternated visits at the nest. We expected that one way to produce a higher-than-random amount of alternation of the visits would be by parents coordinating their activity. The spatial analysis presented in this study however cannot discriminate whether parents actively respond to each other’s visits by taking turns or if instead the observed pattern of visits at the nest is the product of foraging coordination alone. We found indication that alternation at the nest correlates with more similar space use by parents but this only explained 12% of the variation in alternation. Why did we find only a reduced effect of similarity in space use on alternation? One explanation could be that parents actively take turns visiting by mainly monitoring the visits by the other individual at the nest (e.g. by waiting at the nest before or after its own visit). In this scenario parents do not need to monitor and coordinate the foraging movements with those of the partner, and thus alternation would not strongly correlate with our measure of similarity in space use. Alternatively, parents may mainly respond to each other by coordinating foraging and not by taking strict turns of visits. For instance parents may forage together in the same area but returning at the nest independently from the partner. In this case, parents would have similar space use but this coordination would not predict alternation at the nest. To have better insight on the behavioural mechanisms generating alternation of the nest visits we need to examine the spatial movements of the parents at the level of the single foraging trips and investigate whether parents occur in spatial proximity to each other at the foraging site and/or the nest and how these forms of coordination relate to the pattern of the nest visits.

In conclusion, our study indicates that parents are highly coordinated in space and time and that this coordination relates to alternation of the nest visits, suggesting that parents are able to monitor and respond to partner’s activity. However further investigations of the spatial movements of the parents are necessary to discriminate the effect of a potential turn-taking of the provisioning. We therefore strongly promote further studies on spatial movements of provisioning parents to better understand the behavioural mechanisms underlying negotiation over offspring provisioning.

Acknowledgements

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Supplementary material

4.S1 - Validation of triangulation algorithm

The algorithm we used to estimate the initial locations (before correction) of tags consists of two steps:

i) The RSSI (received signal strength indication) values from a single pulse recorded by the base stations (receivers) are used to estimate the distance between each base station and the given tag using an RSSI-to-distance conversion function. The function we used was:
distance (in m) = 4 – 0.2 RSSI.

ii) The distances to each base station estimated in step 1 are used in a Muti-Dimensional Scaling (MDS) analysis (Matlab function “mdscale”) to generate the tag location coordinates that best match the distances to each base station.

To validate the Encounternet triangulation algorithm, in autumn 2014 we carried out a field test with the Encounternet tracking system in a forest of mixed wood located at Westerheide, The Netherlands (52°01’N, 5°85’E). In two different areas within the field site, we built an array of 38 receivers in a 25-m array. The receivers were placed on plastic poles at *ca.* 3.5m off the ground in a triangular array with the same configuration of the arrays deployed during the field season in 2016 (see Methods section). Within each array, we randomly selected 40 tag sites distributed at different distances (ranging from to 4.9 to 72.8m) from the centre of the array. The coordinates of the receivers as well as those of the tag sites were located and surveyed in the field with a survey-grade GPS (Ashtech ProMark 800, Santa Clara, CA, U.S.A.). Each tag site was visited for two minutes with two active radio-tags mounted on a plastic support at the top of a 4m glass fiber pole. The tags could be oriented in six different orientations: the antenna could point upwards, downwards, towards the centre of the array, towards the border of the array, or positioned horizontally with a 90 or 270 degree angle from the centre of the array). The orientation of the two tags differed from one another in each tag site and was randomized across tag sites.

For each tag in each tag site, we estimated the locations of the tag from one pulse randomly selected within the two-minute intervals with the algorithm described above. We found that, with the original version of the algorithm, the inaccuracy in locating the tag increases as the distance between tag location and centre of the array increases ($F_{1,157.01}$ = 322.71, *P* < 0.001). This effect was created by the algorithm which biases the estimated locations towards the centre of the array ($F_{1,157.91}$ = 16.30, *P* < 0.001) so that locations at the edge of the array were estimated more inside the array (Figure 4.S1A).

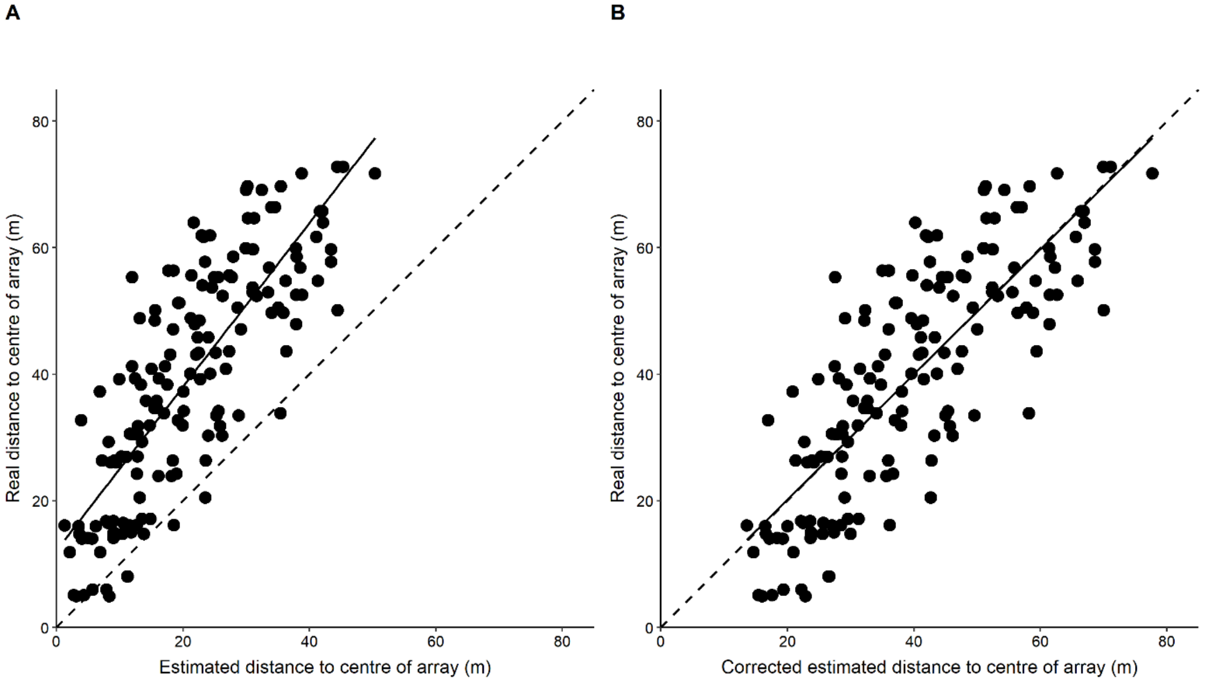

Figure 4.S1. Relationship between real and estimated distance to the centre of the array for 160 tag locations before (**A**) and after (**B**) the correction of the triangulation algorithm.

To correct for this systematic error, we corrected the estimated distance of the tag from the centre of the array by adding the bias, estimated from the regression line of bias and estimated distance to the centre. With this correction, we improved the accuracy of the triangulation algorithm (inaccuracy of the algorithm before correction (mean ± SE): 20.69 ± 0.80; inaccuracy of the algorithm after correction (mean ± SE): 14.13 ± 0.55), and we eliminated the effect of the original bias (the accuracy does no longer depend on the distance from the centre of the array: $F_{1,157.06} = 1.59$, $P = 0.209$; no bias toward the centre: $F_{1,157.91} = 0$, $P = 1$, Figure 4.S1B). The accuracy of the estimated locations did not differ between the tags ($F_{1,152} = 0.19$, $P = 0.66$) and did not depend on their orientation ($F_{5,149.30} = 1.88$, $P = 0.10$).

Lastly we found that the corrected version of the triangulation algorithm correctly estimates the angle of the tag locations relative to the centre of the array as the estimated angle of the tag sites strongly correlated with the actual angle [$r = 0.91$, $P < 0.001$; calculated with the function cor.circular in the R package ‘circular’ (Agostinelli and Lund, 2017)].

Chapter 5

Is alternation of nest visits due to a response to the partner's provisioning behaviour in a biparental care songbird?

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Camilla A. Hinde
C.M. Lessells

In preparation

Abstract

In species with biparental care, raising a family leads to a conflict of interest between the parents as both parents benefit when the other parent cares more for the shared offspring. Solving this conflict requires cooperation between the parents. One way to achieve such cooperation is for parents to adopt a ’turn-taking’ strategy where they respond to each other’s offspring provisioning visits. Empirical studies in birds indicate that alternation of nest visits is common and there is evidence that this pattern is related to parental coordination of their foraging activity away from the nest. However, it is not clear whether alternation of visits is the result of foraging coordination alone or whether parents directly respond to the nest visits of the partner. We study two mechanisms that could be used by parents to monitor and respond to each other visits: they may synchronise their nest visits or they may wait at the nest for the partner when returning from a foraging trip. We combined video recordings at the nests with Encounternet, an automated radio-tracking technology, to remotely monitor provisioning trips of wild great tit (*Parus major*) pairs during chick rearing. Firstly, we explored whether alternation of nest visits is related to more synchronous visits and/or to more frequent waiting at the nest. Next, we explored for the cases of synchronized visits whether the order of entrance at the nest depends on whether the last visit was made by the same or the other parents. Lastly, we investigated whether waiting at the nest could be attributed to parents taking turns. We found that higher nest visit alternation was associated with parents synchronizing their nest visits. During synchronized visits, the order of entrance at the nest was not random: the parent which arrived first at the nest site was more likely to enter first and males were more often the first parent to arrive. This order of entrance, however, did not depend on the identity of the last visiting parent. Higher alternation scores were also associated with more frequent waiting at the nest. Waiting occurred more frequently when a parent came back to the nest from a longer trip and its partner had not visited in the meantime. In addition, waiting at the nest occurred more often when parents foraged further away from each other. These results indicate that the visit pattern at the nest is associated with parental coordination of the visits and suggest that parents are responsive to the provisioning behaviour of the partner. This study demonstrates the importance of including parental coordination when studying the behavioural mechanisms underlying parental care.

5.1 Introduction

Raising a family is not free of conflicts for parents (Royle et al., 2012; Trivers, 1972). While both parents benefit from the joint investment to the offspring, providing care is costly in terms of reduced survival and future reproduction (Williams, 1966) and these costs are paid by the individual parents. In species with biparental care, each parent would therefore benefit from doing a smaller share of the care if the partner works harder to compensate (Lessells, 2006). This generates a conflict of interest between the parents over how much each individual should invest in the current offspring (Trivers, 1972), and theoretical studies have explored how this conflict ultimately affects offspring care.

Parental investment has been typically modelled using either “sealed bid” (Houston and Davies, 1985) or “negotiation” (Lessells and McNamara, 2012; McNamara et al., 1999) approaches to determine the optimum level of care and the response rule that parents should adopt to changes of investment levels of the partner. Both models predict that sexual conflict and negotiation lead to lower levels parental care than in a cooperative situation (McNamara et al., 2003) because when parents work together, each individual is expected to withhold part of their potential investment to avoid being exploited by their mate (Lessells and McNamara, 2012). This prediction is also supported by a zebra finch *Taeniopygia guttata* study, in which offspring experimentally reared by single females received greater *per capita* parental investment than offspring raised by both parents (Royle et al., 2002).

The prediction of an overall decrease in parental care of the early theoretical models (Houston and Davies, 1985; Lessells and McNamara, 2012; McNamara et al., 1999; McNamara et al., 2003) implicitly assumes that parents cannot make a ‘binding agreement’ about how much each parent should invest in the offspring so that any form of investment made by one individual is open to exploitation by the other. Recently, Johnstone et al. (2014) proposed that simple forms of conditional cooperation may be able to create this ‘binding agreement’. They modelled a new negotiation mechanism where parents take turns to invest (e.g. provisioning the offspring) over time providing care to the offspring only when the other parent was the last to do so. The evolutionary stable strategy of this response rule supports an efficient level of investment in which parents work at their optimal rates as investments can no longer be exploited by the partner. In a situation of offspring provisioning, this turn-taking rule predicts that each parent does not provision when it was the last to feed, but only after a visit by the mate, leading to strict alternation of the nest visits.

This turn-taking negotiation rule has triggered several empirical studies in addition to the one included in the original paper, showing that in different avian species parents alternate their visits at the nest more than expected by chance (Bebbington and Hatchwell, 2016; Iserbyt et al., 2017; Savage et al., 2017). Although the higher-than-random alternation of the nest visits was originally considered as evidence of turn-taking (Johnstone et al., 2014), only few of the successive studies tried to investigate the biological processes generating the observed pattern of nest visits (Schlicht et al., 2016). In particular, Schlicht et al. (2016) argued that the observed amount of alternation could result from mechanisms other than turn-taking (e.g., weather conditions, offspring begging) affecting the sequence of visits. In chapter two of this thesis, we explored the contribution of these different processes on alternation. We showed that both nest-specific (perhaps due to turn-taking, foraging coordination, offspring begging) and general environmental (perhaps due to weather conditions) factors contribute to the observed pattern of visits with the first group accounting for up to 80% of nest visit alternation. In chapter three and four of this thesis, we further explored the nest-specific factors responsible for alternation of the nest visits and we found that the pattern of nest visits varies in relation to manipulated workload and that is related to pair coordination in the foraging movements. In particular, more alternation of the nest visits was associated with parents foraging together in the same area.

It is not clear whether alternation of the visits is the result of foraging coordination alone or whether parents directly respond to the nest visits of the partner by taking turns. On the one hand, parents may forage together in the same area but return at the nest independently from the partner. On the other hand, parents may also monitor the foraging trips of the partner and respond to their partner's nest visits. How can parents monitor each other's visits and possibly maintain turn-taking of provisioning? We propose two mechanisms by which parents may be able to monitor and respond to the visits of the partner: synchrony of the nest visits and waiting at the nest at the end of the foraging trip.

Synchrony of the nest visits is a form of pair coordination where parents arrive together at the nest to feed the offspring and this pattern of visits has been found in different bird species (Bebbington and Hatchwell, 2016; Mariette and Griffith, 2012; Raihani et al., 2010; Shen et al., 2010; van Rooij and Griffith, 2013). By synchronizing the visits at the nest with the partner, parents may be able to continuously monitor the provisioning activity of the partner and maintain high levels of alternation of the nest visits simply because when parents visit together, the second individual to visit will always make an alternated visit. Not surprisingly, in the long tailed tit *Aegithalos caudatus* synchrony of nest visits positively correlates with alternation of the nest visits (Bebbington and Hatchwell, 2016). Furthermore, when parents do not synchronize their visits but arrive alone at the nest site at the end of a foraging trip, sometimes they briefly wait outside the nest before entering, often producing contact calls (personal observations). In a turn-taking framework, one possible explanation for this behaviour is that parents, when they are the last one to feed, wait at the nest for the partner to feed the offspring before entering the nest itself. In this study, we investigated whether alternation of nest visits is related to parents synchronizing their visits or waiting at the nest, and whether these two behaviours suggest that parents actively take turns to feed. In this paper, we use the term 'alternation' to refer to the observed pattern in sequences of visits by male and female parents, and 'turn-taking' to refer to a specific type of parental negotiation strategy (Johnstone et al., 2014). To address our research questions, we conducted a follow-up study to chapter 4 where we used the automated Encounternet tracking technology (Maynard et al., 2015; Mennill et al., 2012; Rutz et al., 2012; Snijders et al., 2017a; Snijders et al., 2014) to remotely monitor provisioning trips of wild great tit (*Parus major*) pairs during chick rearing. In particular, we addressed the following questions:

i) Does alternation of the nest visits correlate to more synchronous visits and/or to more frequent waiting at the nest?

ii) When parents synchronize their visits, is there an order of entrance? If so, does it depend on the identity of the parent visiting in the preceding visit?

iii) Is waiting at the nest related to the provisioning behaviour of the partner?

5.2 Methods

Study population and data collection

This study was conducted in 2016 in a great tit population in Roekel, a mixed woodland area in Ede, the Netherlands (52°07’N, 5°72’E). This area contains around 250 nest-boxes that were checked weekly from the beginning of April to determine the onset of egg laying and incubation. During early egg laying, active great tit nests were pre-selected to be radio-tracked based on their position in the field site. Specifically, we selected nests in which the array would contain relatively homogenous tree coverage, i.e., avoiding nests close to human paths and open fields. 16 birds (8 pairs) were caught and fitted with radio tags during incubation or the chick rearing period. Five males were caught during the incubation period using a mist net nearby the nest site. During incubation, three females were also caught when leaving the nest-box early in the morning with ‘box nets’ (te Marvelde et al., 2011) placed around the nest box. All the remaining individuals were caught and tagged at the nest during chick provisioning. There was no effect of how adults were trapped or stage of trapping on nest characteristics (Mann-Whitney tests, all $P > 0.3$).

We collected radio tracking data with the automatic tracking system Encounternet (Encounternet LLC, Portland, OR, U.S.A.). Encounternet consists of small radio transmitters of approximately 1 g, fitted to the bird with a leg-looped backpack harness (Rappole and Tipton, 1991). These tags broadcast a radio signal every 5s, which is recorded by small wireless receivers logging the ID number, time and received signal strength indication (RSSI) of every tag pulse they receive (Mennill et al., 2012). To track spatial movements of the eight tagged pairs during chick provisioning, for each pair we deployed 38 receivers around the nest site in a 25-m array. These receivers were placed in a triangular array consisting of three ‘rings’ at 25, 50 and 75 meters from the nest containing six, twelve and 18 receivers, respectively. The receivers were positioned in trees or plastic poles at *ca.* 3.5m height at a regular distance of approximately 25m distance one another. On average, parents spent 63% of their time within the array (mean ± SE: 0.63 ± 0.02) and the proportion of time inside of the array negatively correlated with the number of chicks (GLMM, estimate ± SE: -0.60 ± 0.26, $\chi^2 = 4.21$, d.f. = 1, $P = 0.04$, N = 168). The coordinates of the receivers were located and surveyed in the field with a survey-grade GPS (Ashtech ProMark 800, Santa Clara, CA, U.S.A.). At the nest site, two more receivers were placed, one on the front side of the tree *ca.* 50 cm above the nest-box, and the other on the back side of tree trunk at the same height off the ground. In addition to the Encounternet array, a small video camera was mounted in the roof of the nest-box and connected to an external video recorder at the foot of the tree. Video recordings (720 x 576 pixels of resolution) started before 0730h and ended on the recording days and were synchronized (to the nearest second) to the Encounternet array to simultaneously monitor spatial movements and visits at the nest of the provisioning parents. Although Snijders et al. (2017b) found no effect of Encounternet tags on provisioning rate and visit duration at the nest in great tit parents, all parents were tagged at least two days before data collection to reduce possible effects of tagging on provisioning activity of the parents. The array of receivers and the video set-up were also placed on the day prior to data collection to habituate parents to their presence.

For each Encounternet nest, we collected video recordings and radio tracking data of both parents for five consecutive days. In this study, we used 84 hours of data collected on the first (eight hours of data from 0800h to 1600h for all nests) and last (four hours of data from 0800h to 1200h for five nests) day of the five day period. The nests differed in the brood size (mean ± SD: 7.5 ± 0.93) and age of the chicks (mean ± SD: 11 ± 1.77). Brood size, chick age and April day did not differ between the nest recorded only on one day or on two days (Mann-Whitney tests, all $P > 0.3$). A total of 2661 nest visits were detected from the video recordings, for which we scored the parental sex (determined from the blackness of the crown feathers) and the times that the bird entered and left the nest-box (to the nearest second). We used a triangulation algorithm implemented in MATLAB (The MathWorks, Natick, MA, U.S.A.) to locate the position inside the array of each tagged parents every five seconds from the radio signals logged by the receivers (see supplementary material 4.S1 of chapter three for validation of triangulation algorithm).

Calculation of alternation of the nest visits

The nest visits were scored from the video recordings and used to calculate alternation of the nest visits for each one-hour period. We defined alternated nest visits as visits of one individual that followed a visit of its mate. For each one-hour sequence of nest visits, we scored the status (alternated or non-alternated) of all the visits except for the first visit. Different amounts of alternation can arise by chance in a sequence of visits as a function of the proportion of visits by the two parents. If one parent, for instance, makes either all or none of the visits in a sequence, no alternated visits can occur. Conversely, as the proportions of visits by both parents become equal, the proportion of alternated visits expected by chance increases. To account for this effect, we calculated and used in the statistical analysis an alternation score (see supplementary material 2.S1 for calculation of alternation score) that measures the deviation of the observed amount of alternation from that expected given the proportion of visits by the two parents.

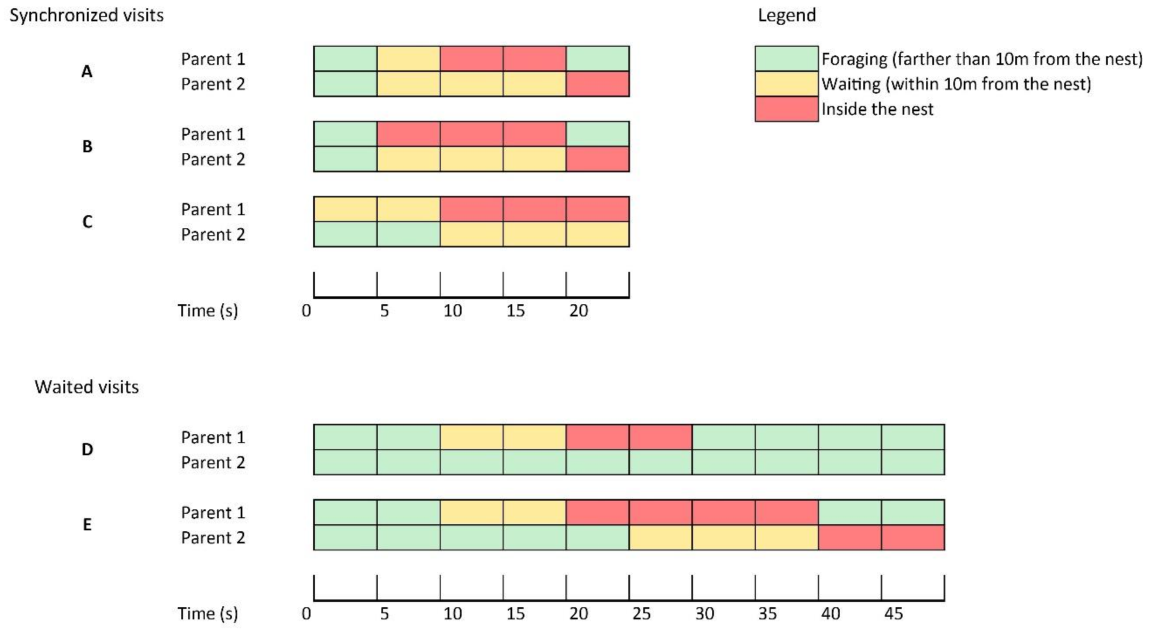

Figure 5.1. Examples of synchronized (**A**-**C**) and waited (**D**-**E**) visits. Synchronized visits were defined as visits where both individuals arrived at the nest site (within 10m from the nest location) within three tag pulses of one another. During synchronized visits, parents may arrive during the same tag pulse (actual arrivals of the parents occur within four seconds) within the nest site (**A** and **B**), or the second parent may arrive within three pulses (in this case the second parents' actual arrival occurs within 14 seconds; **C**). Waited visits were defined as all the non-synchronized visits, which, at the end of the foraging trip, the parent was within the nest site for more than two tag pulses (shortest time of waiting is 6 seconds) before entering the nest (Parent 1 in **D** - **E**). During a waited visit by parent 1, the partner may (**E**) or may not (**D**) arrive at the nest site within three tag pulses (maximum 14 seconds). In the case that the second parent arrived after three tag pulses and waited at the nest for the partner to exit before entering, the visit was not considered as a waited visit (Parent 2 in **E**).

Characterization of synchronized and waited nest visits

We defined synchronized visits as a duet of visits (one by the male and one by the female) in which the parents arrived at the nest site (within 10m from the nest) within three tag pulses (within 14 seconds) of one another (Figure 5.1A-C). We chose our measure of synchrony based on the time of arrival at the nest site instead of using an arbitrary time window between two visits inside the nest (Bebbington and Hatchwell, 2016; Mariette and Griffith, 2015; Mariette and Griffith, 2012; van Rooij and Griffith, 2013), as parents during synchronized visits rarely enter the nest at the same time but the second parent almost always enters once the first individual leaves the nest. We defined "waited visits" as all the non-synchronized visits, which, at the end of the foraging trip, the parent was within the nest site for more than two tag pulses (more than six seconds) before entering the nest (Figure 5.1D-E). During a waited visit, the partner may or may not arrive at the nest site. In the case that the second parent arrived after three tag pulses and waited at the nest for the partner to exit before entering (ca 3% of the visits), the visit was not considered as a waited visit. This visit was excluded from the analysis because the partner was inside the nest.

Statistical analysis

To investigate whether alternation of the nest visits was related to more synchronous visits and/or to more frequent waiting at the nest, for each one-hour period, we calculated the proportion of synchronized and waited visits. We fitted linear mixed models (LMMs) with alternation score as the response variable, 'proportion of synchronized visits, and 'proportion of waited visits' as fixed effects. Despite our restricted time window used to determine synchrony of the nest visits (ten seconds), we expected that the proportion of synchronized visits would increase when the provisioning rate increases. Therefore, we also included 'parental provisioning rate' as a fixed effect as a control. 'Nest ID' was included as a random effect.

We expected that higher synchrony between parental visits would lead to higher alternation simply because for each duet of synchronized visits the second visit is always alternated. If parents always synchronize their visits, we would expect at least half of the visits to be alternated because of this effect. Moreover, even if there is no strict order about which individual enters first in the duets of synchronized visits but the identity of the first entering bird is completely random, we would expect up to 75% of the visits to be alternated. For this reason, for each duet of synchronized visits, we investigated whether there is an order of entrance and whether it depends on the identity of the parent visiting in the preceding visit. First, we tested whether, both within each nest and across nests, a specific sex is more likely to enter first. Separately for each pair, we carried out binomial tests on the first sex to enter. We then used Fisher's method (Rosenthal, 1978) to combine the p-values from each binomial test using the sumlog function implemented in the R package *metap* (Dewey, 2017). Second, we investigated whether the order of entrance at the nest depended on the order of arrival at the nest site. For each pair separately, we used Fisher's exact test for 2x2 contingency tables on the first individual to enter in relation to the first individual to arrive. P-values of each test were then combined using Fisher's method. Third, we investigated whether a specific sex is more likely to enter the nest first when both parents arrived at the same time (i.e. within 4s) within the nest site. Also in this case, for each nest we carried out independent binomial tests and combined the p-values with Fisher's method. Last, we explored whether the first individual to enter depended on the identity of the parent visiting in the preceding visit. Also here, we applied the same methodology described for the previous analysis.

Furthermore, we investigated whether waiting at the nest could be related to the provisioning behaviour of the partner. We modelled the probability of waiting at the nest site when returning from a foraging trip as a binomial response (logit link) by using generalized linear mixed models (GLMMs). We hypothesized that waiting at the nest could be a mechanism by which parents may monitor their partner and maintain alternation. For this reason, we used "Identity of the last visiting parent" as the response variable. We also included "Time since partner's last visit" and "Average distance to the partner" in the foraging trip because parents might not be able to continuously monitor the visits at the nest but respond to the provisioning behaviour of the partner. "Time since its last visit" was also included as a response variable, as the individual might not be responsive to its partner's behaviour but only wait around the nest when coming back from a long foraging trip. This last variable was also tested in an interaction with "Identity of the last visiting parent", whereas "Time since its last visit" and "Distance to the partner" were also tested in interaction with "Sex". 'Individual ID' nested in 'Nest ID' was included as the random effect.

All the statistical analyses were performed in R environment (version 3.2.3). All mixed models were performed with the *lme4* package (Bates et al., 2015). The significance of the main effects in the LMMs was calculated with the Kenward-Roger approximation implemented in the *pbkrtest* package (Halekoh and Hojsgaard, 2014). For the GLMMs, the significance of the main effects was instead calculated using the *car* package (Fox and Weisberg, 2011). To define the minimal adequate models, we used backwards model selection, dropping non-significant terms in each step starting from the interaction terms.

5.3 Results

Alternation score was positively related to both synchrony of the nest visits ($F_{1,79.58}$ = 7.79, *P* = 0.007, Figure 5.2A) and to more frequent waited visits at the nest ($F_{1,75.31}$ = 4.51, *P* = 0.037, Figure 5.2B), while controlling for the effect of parental provisioning rates ($F_{1,56.94}$ = 0.51, *P* = 0.479).

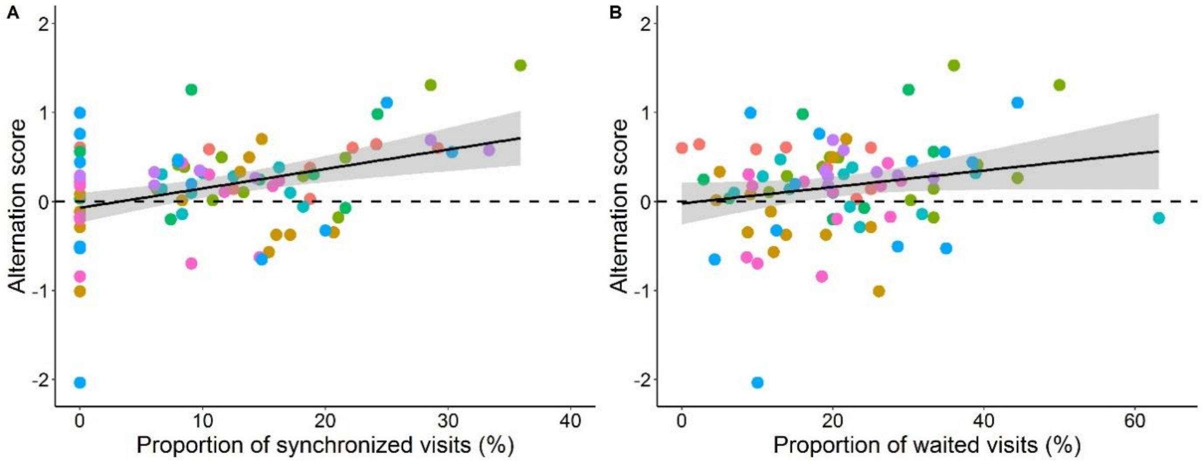

Figure 5.2. Alternation score in relation to proportion of synchronized (**A**) and waited (**B**) visits at the nest. An alternation score of zero (dashed line) represents the amount of alternation expected by chance, assuming that the probability of a nest visit by a parent is constant with respect to time. The line represents fitted values with its SE represented by shaded areas. Data points collected from the same nest in different periods of one hour are colour coded.

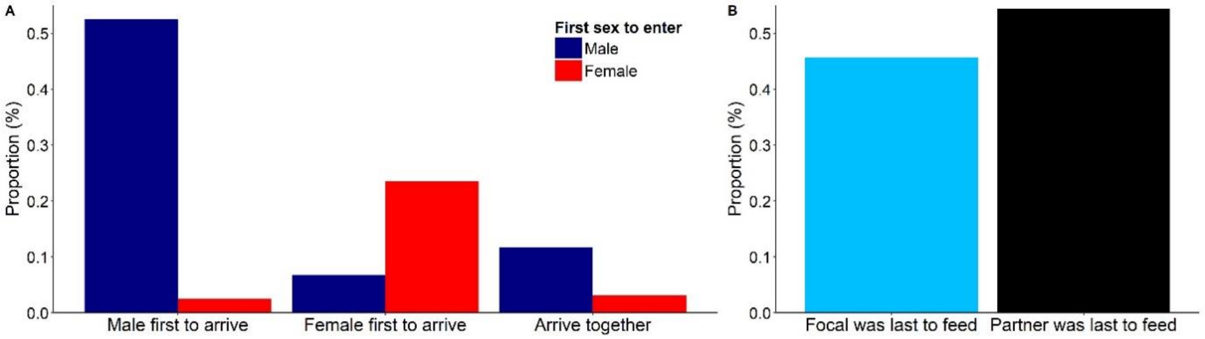

Figure 5.3. A - Proportion of the synchronized visits divided according to the identity of the first individual arriving at the nest site and identity of the first individual entering the nest. Synchronized visits (n= 324) were defined as visits where both individuals arrived at the nest site (within 10m from the nest location) within 14 seconds one another. **B** - Proportion of the synchronized visits where the first individual to enter the nest was or was not also the last individual to feed in the preceding visit.

When parents synchronized their visits at the nest (arrived at the nest site within 14 secs of each other), males were most often the first parent to enter (Fisher's combined probability test: χ^2 = 38.44, df = 16, P = 0.001). This is because males were more often the first individuals to arrive at the nest site, and the first individual to arrive was also more often the first individual to enter (Fisher's combined probability test: χ^2 = 92.23, df = 16, P < 0.001, Figure 5.3A). When both parents arrived together at the nest site (arrived within four second one another), there was no difference in the sex to enter first (Fisher's combined probability test: χ^2 = 12.70, df = 16, P < 0.695, Figure 5.3A). However, we may lack power to detect differences because at three of the nests, the occurrence of both parents arriving at the exact same time was very low (n ≤ 3). Across nests, the order of entrance, however, did not depend on the identity of the last visiting parent (Fisher's combined probability test: χ^2 = 6.54, df = 16, P < 0.98, Figure 5.3B).

Lastly, we found that the probability of waiting at the nest for a focal parent increased with an interaction between the time since the focal individual's last visit and the identity of the last visiting parent (Figure 5.4A; Table 5.1). This means that parents were more likely to wait at the nest when returning from a longer foraging trip and the partner did not visit in the meantime. Furthermore, the probability that the focal parent waited at the nest site increased with the average distance to the partner during the foraging trip interacting with sex: males were more likely to wait than females when foraging further apart (Figure 5.4A; Table 5.1).

Table 5.1. Estimated parameters of the generalized linear mixed models investigating the waiting behavior at the nest for the dataset where distance to the partner was available (n = 688 visits). For each variable, statistics are given for the point of exclusion from the model. Significance levels of the variables did not vary from the full dataset (n = 2299 visits). Significant terms are shown in bold. Estimates and standard errors are given only for significant terms.

Variables	**Estimate**	**s.e.**	**χ^2**	**df**	**P-value**
Time since its last visit			25.63	1.00	**< 0.001**
Time since partner's last visit			0.01	1.00	0.905
Identity of the last visiting partner			0.08	1.00	0.780
Sex (male)	-4.974	0.643	0.17	1.00	0.674
Sex (female)	-3.650	0.595			
Distance to the partner			13.30	1.00	**< 0.001**
Time since its last visit x Identity of the last visiting parent (Focal was last to feed)	0.015	0.003	12.45	1.00	**< 0.001**
Time since its last visit x Identity of the last visiting parent (Partner was last to feed)	-0.011	0.003			
Identity of the last visiting parent x Sex			0.42	1.00	0.517
Distance to the partner x Sex (female)	0.013	0.010	4.21	1.00	**0.040**
Distance to the partner x Sex (male)	0.030	0.015			
Time since its last visit x Sex			0.20	1.00	0.654
Time since partner's last visit x Sex			0.33	1.00	0.568

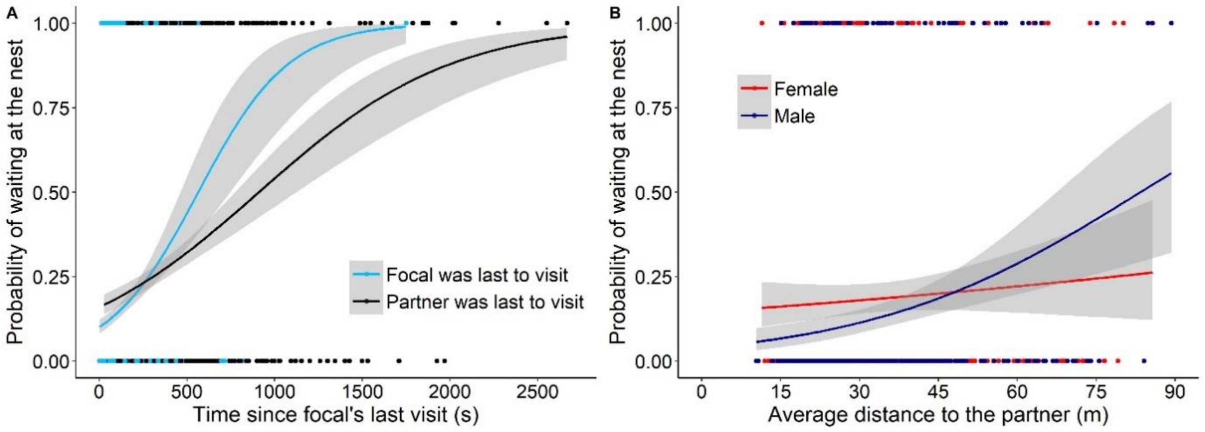

Figure 5.4. Probability of waiting at the nest at the end of a foraging trip for a focal individual as function of time since its own last visit (**A**) and average distance to the partner in that foraging trip (**B**). The lines represent fitted values with its SE represented by shaded areas.

5.4 Discussion

We explored whether alternation of nest visits could be attributed to parents responding to each other's visits. Specifically, we focused on two behavioural mechanisms: synchrony of the visits and waiting at the nest site. We found that more alternated visits were related to more synchrony and more waited visits. When parents synchronized their arrival at the nest site, males were more often the first sex to enter the nest but the entrance order did not depend of the identify of last visiting partner. Waiting at the nest occurred more frequently at the end of long foraging trips and the partner did not visit in the meantime or when the parents foraged further away from each other. Therefore, alternation may be the result of parents responding to the visits of their partner.

In biparental great tits, synchrony of the nest visits is highly correlated with alternation. Periods in which parents were synchronizing their nest visits were also periods of high levels of alternation. Since synchrony is a measure of coordination, our results indicate that alternation may be the result of a form of temporal coordination of the nest visits. Other studies have observed an association of alternation and synchrony proposing that synchrony might be the behavioural mechanism by which parents can take turns (Bebbington and Hatchwell, 2016). However, parents can synchronize their nest visits without using a turn-taking negotiation rule, and still generate a higher than expected alternation of the nest visits. The relationship between alternation (the observed pattern of visits), synchrony and turn-taking can have two pathways. In one pathway, parents adopt a turn-taking negotiation rule, and achieve this is by synchronizing their visits, resulting in alternation of the nest visits higher than expected by chance. The alternative pathway is that parents do not follow a turn-taking rule but instead synchrony as a behavioural mechanism may be another responsive rule, and the observed amount of alternation can simply be the by-product of this rule. Even though it is difficult to disentangle the causal pathway between turn-taking, synchrony and alternation without experimental manipulation, what do our data suggest about the two possible pathways? We found indication that during synchronized visits, the first individual that enters the nest is most often the first individual to arrive at the nest site and does not depend on whether it was the last individual to feed. This pattern suggests that the parents do not visit through a strict turn-taking mechanism.

Why might parents synchronize their nest visits? There are two explanations for this behaviour. The first is that synchrony improves offspring fitness (Mariette and Griffith, 2015; Raihani et al., 2010), possibly by decreasing predation risk at the nest (Bebbington and Hatchwell, 2016; Leniowski and Węgrzyn, 2018; Raihani et al., 2010), and by allowing more equal allocation of resources to the offspring (Shen et al., 2010). The other reason for why parents may be synchronous during nest visits is to monitor each other's investments. Therefore, synchrony as a behavioural mechanism may be another responsive rule, either with or without a turn-taking rule, which reduces exploitation by the mate and guarantees an equal level of investment between the parents. Synchrony of the visits therefore could also be the mechanism by which, in some negotiation studies where the provisioning rate of one parent was experimentally manipulated, the partner matched the change in provisioning rate observed in the manipulated parent (Hinde, 2006; Hinde and Kilner, 2007).

Another possible mechanism by which parents may monitor each other is waiting at the nest for the other parent to arrive. We found that alternation of the nest visits is related to more waiting at the nest site. However, this relationship can arise not because parents are actively waiting for the partner but indirectly because by waiting, the individual increases the chance that the partner would arrive and visit. Why would a bird wait at the nest? We modelled the probability of waiting at the end of a foraging trip based on characteristics of the focal and its partner’s provisioning behaviour. We found that the focal individual was more likely to wait at the nest site at the end of longer provisioning trips and when it was the last the visit the nest. This means that its partner did not visit in the meantime. The focal individual may wait at the nest to check for predators, especially if they have been away for a long time (Fontaine and Martin, 2006; Yasukawa et al., 1992). In addition, waiting at the nest can perform the dual function of checking also for the partner. Therefore, finding an interaction between the time since the last visit and the identity of the last visitor suggests that waiting might be related to monitoring for not only predators but also the partner. For instance, we also found that waiting increases as foraging distance between the pair increased. This pattern may result in the focal individual having no information on where its partner is and on its provisioning behaviour. Moreover, there is a sex difference in this behaviour. Although both males and females increase waiting behaviour when foraging farther from their partners, males are more responsive to the difference in distance. It could be that females are more responsive to offspring condition and begging behaviour (Kölliker et al., 2000) rather than the partner’s behaviour, whereas males are more responsive to the partner’s behaviour.

If waiting occurs to monitor the partner, we may expect that this waiting behaviour ends when the partner arrives. However, only in few occasions (4%, Figure 5.S1), this waiting behaviour stopped due to the arrival of the partner. Most of the time, the focal individual entered alone without the arrival of the partner. One possible explanation is that parents also monitor each other via vocal communication (Elie et al., 2010; Logue, 2007). For example, the waiting individual can make a long-distance contact call and the partner responds, triggering the individual to enter the nest (Halkin, 1997). The other reason that the waiting may be related to the partner’s behaviour is because in the few occasions in which the partner did arrive, the focal individual always stopped waiting and visited the nest. This indicates that the cessation of waiting is triggered by the arrival of the partner.

In conclusion, we combined spatial and temporal data on provisioning behaviour to investigate how provisioning parents respond to their partner. Experimental manipulations on parental behaviour are now needed to understand how parents negotiate their efforts.

However, at this stage we propose that alternation of the nest visits can arise due to parents responding to each other via synchrony and waiting behaviour. We strongly promote further investigations on the behavioural mechanisms underlying negotiation over offspring provisioning.

Acknowledgements

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Supplementary material

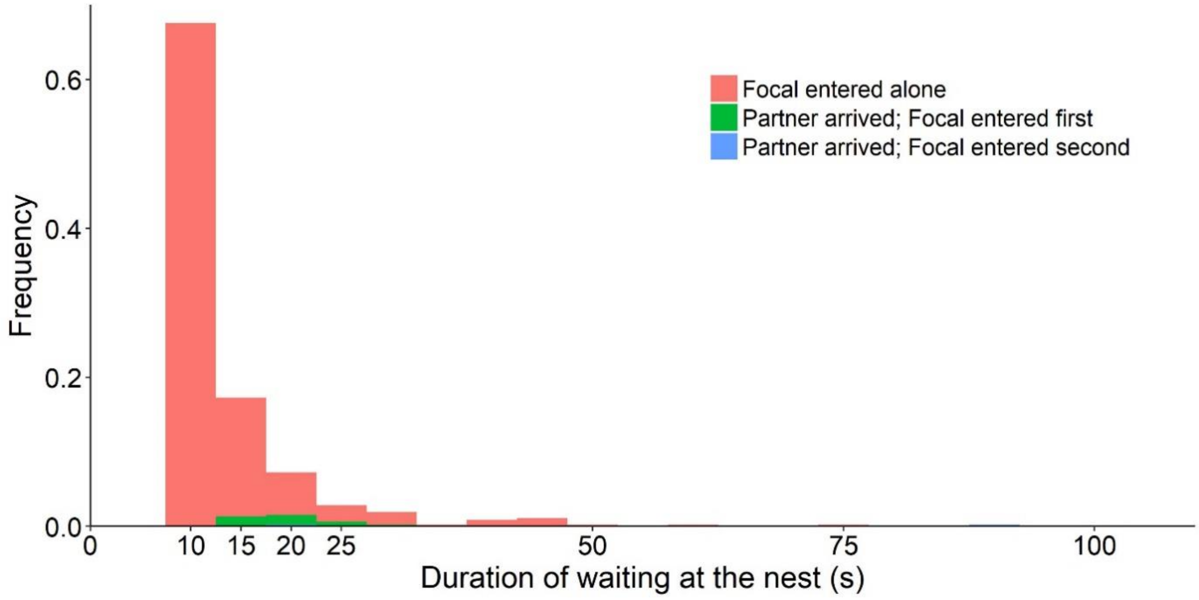

Figure 5.S1. Frequency distribution of the duration of waiting at the nest for the focal parent and whether the waiting was interrupted by the arrival of the partner at the nest site. On no occasion did the focal parent persist waiting after the partner arrived. Cases in which the focal individual entered second (blue) are extremely rare and only occurred twice.

Chapter 6

General discussion

6.1 Determinants of alternation of the nest visits

For three decades, it was commonly accepted that parental care, as well as parent and offspring fitness, inevitably suffer as result of sexual conflict over parental investment (Houston and Davies, 1985). Johnstone et al. (2014) completely changed our view of parental investment by suggesting that parents may overcome sexual conflict by adopting forms of conditional cooperation, which promote efficient levels of care. Although Johnstone's study prompted new research in this field, specifically on alternation of the nest visits, it is still debated whether the observed pattern of care is the result of a turn-taking strategy, as proposed in Johnstone et al. (2014) or whether other mechanisms are responsible.

In this thesis, I focused on the ecological and behavioural mechanisms generating the pattern of provisioning in great tit parents. In **chapter two**, I partitioned the observed amount of alternation into different processes expected to generate alternation. I found that biological processes occurring at the nest level (perhaps due to pair coordination or offspring begging) were the main contributors of the alternating pattern of visits. In addition, global environmental factors (perhaps due to predation risk, weather conditions) were responsible for a significant portion of the observed alternation (about 20%). In **chapter three**, I investigated whether alternation of the nest visits was affected by a nest-specific factor, specifically parental workload. Using a short-term brood size manipulation**,** I found that alternation increased at reduced workloads. I proposed that this effect could be attributed to changes in negotiation rules.

The finding in **chapters two** and **three** that nest-specific factors are the main causes of the observed pattern of visits suggested that pair coordination might have a pivotal role in determining alternation of the nest visits. For this reason, I used Encounternet, an automated radio-tracking system, to monitor spatial movements of the parents during chick provisioning (Figure 1 in **chapter four**). In **chapter four**, I tested whether parents spatially coordinate their movements more than expected by chance and whether this coordination was related to the pattern of nest visits. I found that parents were highly coordinated in space and time and that the degree of parental coordination positively correlated with nest visit alternation. Furthermore, in **chapter five** I investigated whether parents directly respond to the nest visits of the partner and whether this response occurred in relation to the identity of the last visiting parent. I focused on two mechanisms: nest visit synchrony and waiting at the nest for the partner when returning from a foraging trip. I looked at parental movements at the level of single foraging trips and found that alternation of nest visits was associated with both higher synchrony of visits and more frequent waiting at the nest. In the synchronized visits, however, the order of entrance did not depend on the identity of the last visiting parent. On the other hand, waiting occurred more frequently when the parents returned at the nest from longer trips and the partner did not visit in the meantime. In addition, waiting at the nest was also more frequent when parents foraged farther away from each other.

The main finding of my thesis is that the alternation of nest visits is the result of multiple processes governing the provisioning behaviour of the parents. Some of these processes do not relate to any form of interaction between the parents, e.g., weather or predation risk. However, the largest portion of alternation appears to be created by interaction between the parents, e.g., coordination. I discuss how my findings relate to different forms of coordination in the next two sections.

6.2 Turn-taking as a negotiation rule

The turn-taking negotiation rule proposed by Johnstone et al. (2014) differs from previous negotiation rules (Lessells and McNamara, 2012) and assumes that parents respond to each other only via the provisioning behaviour of the partner by only feeding when the other individual was the last to do so. Therefore, to find evidence that provisioning parents adopt a turn-taking rule, we need to find indications that: i) parents respond to partner’s provisioning behaviour and ii) this response depends on the identity of the last visiting parent, i.e., whether the individual itself was the last to feed or the partner.

In **chapter four,** we found evidence that parental movements are highly coordinated in time and take place on the order of a few minutes. For example, even experimentally lagging one parent’s movements by ten minutes resulted in less coordination than observed. This suggests that parents might be able to continuously monitor the activity of the partner and possibly respond to it. The results of **chapter five** also suggest that parents indeed respond to each other's visits by synchronizing their arrival at the nest or by waiting at the nest before entering. In particular, waiting at the nest occurred more frequently when parents were foraging farther apart but also at the end of long trips in which the partner did not visit in the meantime. While this latter finding does not provide direct evidence of turn-taking, it suggests that some part of the provisioning activity (in this case whether to wait at the nest site or not) may depend on whether the partner had visited or not. However, when we looked into another aspect of the provisioning, the identity of the first individual entering the nest during synchronized visits, we did not find support that parents controlled the order of visits.

In my thesis, I collected spatial data on parental provisioning behaviour only in non-experimental settings, where one observed behaviour might be linked to other covariates. To test the causal relationship between parental behaviour and partner's responses, experimental approaches are essential to test whether parents actively control the order of visits via a turn-taking mechanism. Such experiments should focus on decreasing the provisioning of one parent, e.g. by handicapping, and to the negotiated responses of the partner. In these circumstances, a turn-taking mechanism would predict the unmanipulated individual to control the order of the visits, i.e., waiting or synchronizing its own visits more often with those of the manipulated individual.

Even with experimental studies on negotiation rules, providing evidence of turn-taking might still not be easy because parents are known to respond to multiple stimuli when negotiating the amount of care (Hinde and Kilner, 2007). In **chapter three** I found that lower workloads were associated with more alternation of the nest visits. I argued that one potential explanation of the observed increase in alternation could be the fact that parents might change their responsiveness to different family members at different workloads. At normal or increased workloads, parents might be more responsive to offspring begging calls than to the partner and vice versa. Alternatively, I proposed that the change in alternation could be caused by costs of the turn-taking rule. The current theoretical framework of Johnstone et al. (2014) does not consider costs associated to the suspension or reduction of provisioning visits when one individual was the last to feed. Nor does take into account that parental activity might be subject to time constrains (Grieco, 2002a) or composed of different tasks that might affect the potential for parents to respond to each other (Iserbyt et al., 2017). Therefore, more theoretical work that encompasses the costs of negotiation is needed.

6.3 Alternative negotiation rules

In addition to or instead of turn-taking strategies, parents may employ other negotiation rules. In **chapter four,** I found that great tit parents coordinate their movements and forage in the same locations more than expected by chance. In addition to foraging coordination alone, in **chapter five** I also provide evidence that the pattern of visits at the nest is also related to synchronized visits by the parents. Although foraging coordination and synchrony of the visits might be the behavioural mechanisms by which parents take turns, these two forms of coordination might also be negotiation rules, independently from turn-taking, by which parents respond to each other.

There are currently a few studies that have investigated coordination and synchrony as mechanisms that increase parental effort and therefore reproductive success. Why parents coordinate is an important question to answer to understand the evolution of parental care. A study in zebra finches found that, after an experimental increase in parental workload, parents raised their level of foraging and nest visit coordination (Mariette and Griffith, 2015). This suggest that pair coordination might increase foraging efficiency. In addition, synchrony of the nest visits also correlates with offspring fitness (Mariette and Griffith, 2015; Raihani et al., 2010; Shen et al., 2010) and has been proposed to decrease predation risk at the nest (Bebbington and Hatchwell, 2016; Leniowski and Węgrzyn, 2018; Raihani et al., 2010). My findings in **chapters four** and **five** add to these prior studies by showing that parents are not only temporally but also spatially coordinated. Therefore, parental coordination and synchrony might have multiple functions, i.e., they are mechanisms that increase offspring fitness through increased foraging efficiency and that parents use to negotiate and monitor each other.

If parental coordination achieves multiple functions, we will need a comprehensive framework that investigates parental provisioning from both sexual conflict and foraging efficiency perspectives. In models of sexual conflict, theoretical work has focused on the best response rule given the effort of the partner. In **chapter four,** I found that parents change their foraging locations together over time, but my focus was from a sexual conflict perspective on how parents might monitor each other. However, foraging movements of the partner are likely to follow foraging rules, such as those posited by central place foraging theory (Charnov, 1976; Orians and Pearson, 1979). Classical models of central place foraging are aimed at quantifying the best strategy to maximize energy delivered to the offspring over energy consumed by the parents (Charnov, 1976; Lessells and Stephens, 1983). For instance, Kacelnik (1984) modelled provisioning activity in several decisional steps that maximized energy available to the offspring. We should combine central place foraging and sexual conflict frameworks to develop a comprehensive model that explains food provisioning by parents, in which parental decisions result from a combination of foraging efficiency and response to partners (Figure 6.1).

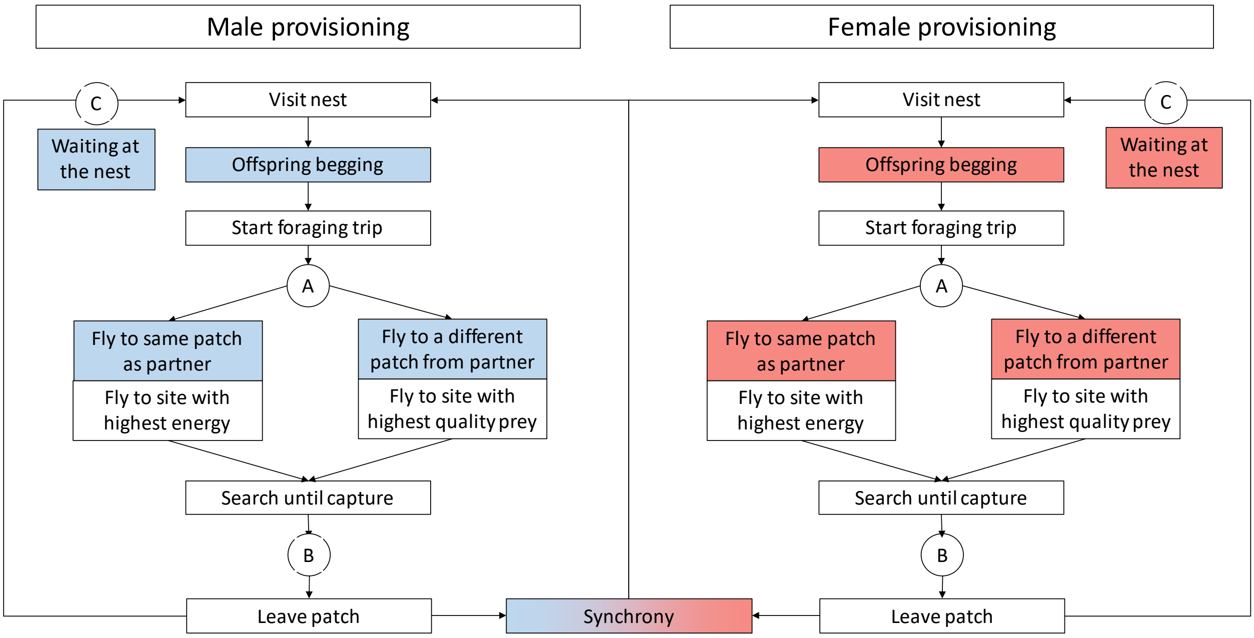

Figure 6.1. Proposed scheme of the activity cycle of great tits feeding young. A, B, and C refer to foraging and coordination decisions by the two parents feeding with single prey loads. Coloured boxes (male in blue and female in red) represent new modelling steps incorporating negotiation rules in classical models of foraging theory. Modified from Kacelnik 1984.

If parental coordination during foraging and synchrony are alternative negotiation rules, would they support the same level of care as the turn-taking model does? Johnstone et al.'s (2014) model works because by taking turns, parents have an equal level of investment as well as working at their optimal rate. This optimal level of investment is ensured because an increase of feeding rates by one parent produces a similar increase in its partner, which results in an overall incentive to work harder. Synchrony guarantees equal provisioning rates without a need of ordered visits. For instance, in **chapter five,** I found that the first individual that enters in a synchronized visit does not follow a complete alternated pattern. However, we do not know whether parents will work at their maximal rate with a synchrony rule. Therefore, having a model that encompasses these other forms of parental coordination rules would shed light on: i) whether these strategies would be evolutionarily stable and ii) provide efficient levels of care.

6.4 Thesis conclusions and future directions

The possibility that coordination between the parents may reduce sexual conflict has important evolutionary implications. Johnstone et al.'s model in 2014 proposed a novel perspective in which sexual conflict can be resolved through cooperation rather than conflict. This perspective propelled a new research frontier looking into different mechanisms of parental care. My thesis aimed to investigate the mechanisms underlying negotiation over parental care. I found that the pattern of alternated nest visits is mainly a result of nest-specific factors and some contribution of environmental factors. Parental coordination and parental workload both contribute to nest-specific factors. In particular, foraging coordination and nest visit synchrony contribute to the level of alternated visits. Therefore, the observed pattern of alternation is the result of a complex series of processes. We should be cautious of equating alternation to “turn-taking” until we have a better understanding of these processes.

To have a better understanding of the evolutionary causes and outcomes of parental care, there are three areas for future work. First, experimental approaches are necessary to test for evidence of turn-taking and in general of how parents negotiate their investment. For example, manipulation of a focal individual and observing the reaction of its partner in terms of foraging movements will give insight into the mechanisms underlying negotiation rules. Second, we need theoretical work on alternative negotiation strategies, i.e., implementing a turn-taking strategy that includes costs of the negotiation phase and investigating synchrony as a novel rule. Third, we need both theoretical and empirical work that studies provisioning behaviour from both a negotiation and foraging point of view. Only by studying the mechanisms that underlie the interactions of parents, offspring, and environmental conditions can we have a more complete understanding on the evolution and persistence of biparental care systems.

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English and Dutch summaries

The evolution of parental care is one of the central topics in behavioural ecology and evolutionary biology. For almost three decades, it was commonly accepted that parental care, as well as parent and offspring fitness, inevitably suffer as result of a conflict of interest between parents over their level of investment. However, a recent theory proposed that a form of parental coordination, turn-taking during offspring provisioning, could lead to conflict resolution between parents and increase the level of parental care. Although this new theory prompted new research in the field of parental care, it is still debated whether the observed pattern of care is the result of a turn-taking strategy or whether parents respond to each other via other negotiation rules.

In this thesis, I investigated in detail the ecological and behavioural mechanisms underlying parental provisioning patterns in great tits, a songbird well-known for its bi-parental care. In the first chapters of my thesis, I explored provisioning sequences of nest visits and focused on the mechanisms generating alternation of the nest visits. In chapter two, I partitioned the amount of alternation of nest visits into different processes and found that biological factors occurring at the nest level (perhaps due to pair coordination or offspring begging) were the main contributors of the alternating pattern of visits. In addition, global environmental factors (perhaps due to predation risk or weather conditions) were responsible for a smaller but significant portion of the observed alternation. The results of chapter two led to a field experiment, in which I explored the effect of a nest specific factor, parental workload, on provisioning behaviour at the nest. In chapter three, using a short-term brood size manipulation**,** I found that alternation increased at reduced workloads and proposed that this effect could be attributed to changes in negotiation rules between the parents.

The finding in chapters two and three that nest-specific factors are the main causes of the observed pattern of visits suggested that pair coordination might have a pivotal role in determining alternation of the nest visits. For this reason, I used Encounternet, an automated radio-tracking system, to simultaneously monitor spatial movements of the parents during chick provisioning. In chapter four, I found that parents highly coordinated their foraging movements in space and time and that the degree of parental coordination correlated with nest visit alternation. In chapter five, I examined in more detail single foraging trips of the parents to test whether the pattern of nest visits could be attributed to parents responding to each other. I found evidence that parents often synchronized their visits at the nest with those of the partner and also waited more often at the nest site when their activity was less coordinated, indicating that parents monitor and respond to the provisioning behaviour of the partner.

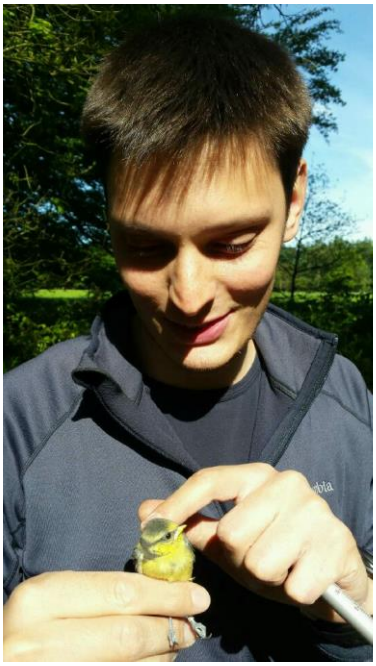
The main finding of my thesis is that the alternation of nest visits is a flexible behaviour and results from multiple processes. Some of these processes do not relate to any form of interaction between the parents, e.g., weather or predation risk. However, the largest portion of alternation is created by coordination between the parents during both foraging movements and nest visits. Parental coordination is an often-overlooked behaviour in studies of parental care. My thesis provides support for a novel perspective in which sexual conflict can be resolved through cooperation rather than conflict. Coordinated behaviour can be adaptive by reducing sexual conflict and should be investigated both empirically and theoretically to fully understand the persistence of bi-parental systems.

De evolutie van ouderlijke zorg is een centraal thema in gedragsecologie en evolutionaire biologie. Gedurende bijna drie decennia werd aangenomen dat zowel individuele ouderlijke zorg, als de vitaliteit van het nageslacht, te lijden hadden onder het onderlinge conflict tussen de ouders over hun respectievelijke bijdrage aan de zorg. Een recente theorie propageert echter dat een vorm van ouderlijke samenwerking, het wisselen van beurt tijdens de opvoeding, dit conflict teniet kan doen en kan leiden tot een verhoogde zorg door beide ouders. Hoewel, deze theorie heeft geleid tot nieuwe onderzoeken in het onderzoeksveld van ouderlijke zorg, wordt nog steeds betwist of het patroon van ouderlijke zorg dat we waarnemen daadwerkelijk een 'beurtenwisselstrategie' betreft of dat ouders op elkaar reageren door middel van andere onderhandelingsstrategieën.

In dit proefschrift onderzoek ik in detail welke ecologische en gedragsmatige mechanismen ten grondslag liggen aan patronen van ouderlijke zorg bij de koolmees, een zangvogel waarvan beide ouders zorg voor hun jongen dragen. In het eerste hoofdstuk van mijn proefschrift verken ik de volgordes van nestbezoeken tijdens de voerfase en spits ik toe op het mechanisme dat de afwisseling van bezoeken genereert. In hoofdstuk 2 verdeel ik de hoeveelheid afwisseling van nestbezoeken onder verschillende processen en laat ik zien dat biologische factoren in relatie tot het nest (wellicht door coördinatie tussen de ouders of door bedelende jongen) de belangrijkste bijdrage leveren aan de afwisseling van nestbezoeken. Daarnaast leveren andere, globale omgevingsfactoren (wellicht gerelateerd aan predatierisico of weersomstandigheden) een kleinere maar significante bijdrage aan de waargenomen afwisseling. Het resultaat van hoofdstuk 2 heeft geleid tot een veldexperiment, waarin ik het effect van een nest-specifieke factor, ouderlijke werkdruk, heb bestudeerd op het voergedrag bij het nest. In hoofdstuk 3 vond ik door middel van een kortdurend broedselmanipulatie-experiment dat afwisseling in nestbezoeken toeneemt bij aflopende werkdruk en bepleit ik dat dit effect kan worden toegeschreven aan veranderingen in de onderhandelingsregels tussen de ouders.

De bevindingen in hoofdstuk 2 en 3, dat nest-specifieke factoren de hoofdoorzaak vormen van het waargenomen nestbezoekpatroon, suggereren dat paarcoördinatie een belangrijke rol kan spelen bij het bepalen van afwisseling. Om deze reden heb ik gebruik gemaakt van Encounternet, een geautomatiseerd *radio-tracking*-systeem, om ruimtelijke bewegingen van de ouders tijdens het voeren simultaan te monitoren. In hoofdstuk 4 ontdekte ik dat ouders hun foerageerbewegingen sterk coördineren in ruimte en tijd en dat de mate van coördinatie correleert met de mate van afwisseling in nestbezoeken. In hoofdstuk 5 onderzoek ik in meer detail afzonderlijke foerageertochten van de ouders om te testen of het nestbezoekpatroon verklaard kan worden doordat ouders op elkaar reageren. Ik vond bewijs dat ouders hun bezoeken vaak bij het nest synchroniseren met die van de partner en dat ze vaker wachten bij het nest wanneer hun activiteiten minder gecoördineerd zijn, hetgeen aangeeft dat ouders het voergedrag van hun partner in de gaten houden en erop reageren.

De belangrijkste bevinding van mijn proefschrift is dat de afwisseling van nestbezoeken een flexibele gedragsvorm is en het resultaat van meerdere processen. Sommige van deze processen zijn op geen enkele manier gerelateerd aan de interactie tussen de ouders, zoals weersomstandigheden en predatierisico. Het grootste aandeel van afwisseling kan echter worden toegeschreven aan coördinatie tussen de ouders tijdens zowel foerageerbewegingen als nestbezoeken. Ouderlijke coördinatie is een gedragsvorm die vaak over het hoofd wordt gezien bij studies naar ouderlijke zorg. Mijn proefschrift levert bewijs voor een nieuw perspectief waarbij het conflict tussen de seksen kan worden opgelost door samenwerking tussen de ouders. Gecoördineerd gedrag kan adaptief zijn doordat het conflicten tussen de seksen kan verminderen, en zou daarom zorgvuldig bestudeerd moeten worden—zowel empirisch als theoretisch—om te begrijpen hoe een systeem waarbij beide ouders de zorg dragen kan voortbestaan.



Davide Baldan was born on the 12th of July 1987 in Venice, Italy. He grew up together with his identical twin brother with a strong passion for animals, especially birds. He spent hours observing birds feeding in the garden from his home window. In 2006, Davide started his bachelor's in biology at the University of Padua, Italy.

Following his BSc degree in 2008, he continued his study in Padua with his MSc in Evolutionary Biology. To complete his master thesis, Davide obtained an Erasmus scholarship to visit the Konrad Lorenz Institute of Ethology and carried out three projects on social network and foraging behavior in house sparrows and bearded reedlings in collaboration with Dr. Herbert Hoi and Prof. Matteo Griggio. In that period, fascinated by the study on animal social interactions, Davide decided to continue his academic career with a PhD, after obtaining his MSc degree in 2013 with the distinction *cum laude*.

In 2014, Davide started his PhD in the Department of Animal Ecology at the Netherlands Institute of Ecology (NIOO) studying sexual conflict and negotiation over parental care. In the same institute, Davide also met Jenny Ouyang, his wife.

Davide now has moved to Reno, Nevada, US with his wife and in 2019 he is excited to pursuing the next great adventure of becoming a father.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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Behavioural mechanisms underlying negotiation over parental care (2014-2015)

Post-graduate courses (3.7 ECTS)

- Introduction to R for Statistical Analysis; PE&RC (2015)
- Generalized linear models; PE&RC (2015)
- Life History theory; Groningen University (2015)

Invited review of (unpublished) journal manuscript (1 ECTS)

- Oecologia: predation rate and parental care (2017)

Deficiency, Refresh, Brush-up courses (3.4 ECTS)

- Laboratory animal science; KNAW (2014)

Competence strengthening / skills courses (1.6 ECTS)

- Reviewing a scientific paper; PE&RC
- The essentials of scientific writing and presenting; PE&RC
- Career assessment; PE&RC

PE&RC Annual meetings, seminars and the PE&RC weekend (1.9 ECTS)

- PE&RC Day (2014)
- NAEM Meeting (2018)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- NIOO AnE Journal Club and Science Club and NIOO seminars (2014-2017)
- R Users meetings; PE&RC (2015-2016)

International symposia, workshops and conferences (11.3 ECTS)

- Behaviour conference; Cairns (2015)
- ISBE International society for behavioural ecology; Exeter (2016)
- Behaviour conference; Estoril (2017)
- SICB Society for Integrative and comparative biology; San Francisco (2017)

Supervision of MSc students (6 ECTS)

- Food provisioning and parental coordination
- Daily variation in parental care pattern

Colophon

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