Evolution of brood parasitism in passerine birds: Constraints related to prey type

Master of Science Thesis 2007

Tea Turtumøygard

Photo: Rim Tusvik

Department of Biology, P.O. Box 1066 Blindern

NO-0316 Oslo, Norway

Forord

Jeg vil først få takke min veileder, Tore Slagsvold, for kyndig veiledning, råd, vink og forslag til forbedringer, og ikke minst for uvurderlig hjelp i forbindelse med feltarbeid og filming! Ditt engasjement og din kunnskap har virkelig inspirert meg!

Jeg vil også takke Lise Heier for hjelp med statistikk; da jeg ble kastet ut på dypt vann viste du meg veien til grunna! Uten deg ville jeg aldri ha blitt fortrolig med R.

Vil også rette en takk til de små fjærkledde i Sørkedalen, fluesnapperne og kjøttmeisene, for at jeg fikk låne kasser og unger til filming, håper at mitt lille eksperiment ikke voldte for stor skade!

Takk også til mamma og pappa, for støtte og hjelp gjennom hele studietiden! Jeg er så glad jeg har dere! Vil også takke vennene mine, særlig Marijana, Svetlana og Ingvild, for all moro vi har hatt gjennom studietiden; selv om det ikke har blitt så mye av det i det siste! Kristin Vesterkjær og Synne Folsland Olsen, "my partners in crime", det hadde ikke blitt det samme uten dere! Masse lykke til videre, begge to!

Takker også Lena Kristiansen og Lars Erik Johannesen for hjelp under feltarbeid!

Tea Turtumøygard

16 april 1007

Table of contents

Abstract

Great tit nestlings won't thrive in nests of the pied flycatcher, even though the opposite has been proven successful. In order to explore some of the reasons why, we performed cross fostering experiments where great tit nestlings were temporarily placed in nests of the pied flycatcher. The experiments were conducted in two sessions, at three and ten days of age. To be able to compare nestlings of the two species directly, we created mixed groups consisting of nestlings of both species, in addition to groups consisting of great tit nestlings only. This ensured grounds for comparison with regards to prey types and feeding rate featured when parents encounters great tit nestlings only.

Filming inside the nest boxes enabled us to assess properties of food loads brought to the nest by the parents, such as type and size of prey, and comparing the nestlings of the two species with regards to swallowing time.

Due to the fact that great tits are considered to be caterpillar specialists, we expected great tit nestlings to measure up to pied flycatcher nestlings in their handling time of caterpillars. We also expected the great tit nestlings to experience difficulties when handling other prey items such as flies and wasps.

This held true, the species differed markedly in their handling time of prey; both when considering all prey types as a whole and when segregated into groups. Great tit nestlings were slower than their nest mates in both age groups, and this difference was correlated to prey type. While the pied flycatcher nestlings improved their ability to swallow flies with age, this did not apply to great tit nestlings. Although the species differed in their swallowing time of larvae at three days of age, this difference was non-existent at ten days of age, which was as predicted from the assumption that great tits are adapted to swallowing larvae. In addition, testing, cases where the parent lowered its bill into that of the nestling and then raised it again without delivering the food, was by far more extensive during feeding attempts to great tit nestlings than to pied flycatcher nestlings. This applied to both age groups, indicating that the parents assessed the swallowing ability of the former nestlings to be poorer.

Problems related to prey type and swallowing constraints may help to explain why great tits do not parasitize pied flycatcher broods.

Introduction

Interspecific brood parasitism, the reproductive strategy where birds lay eggs in the nests of another species, can be either facultative or obligate. In the case of facultative brood parasitism, the female lays some of her eggs in the nests of others, but also have a nest of her own. Obligate brood parasitism, on the other hand, means that members of the species do not build their own nests and always rely on other species to raise their young. The best known examples of the latter is the European cuckoo (*Cuculus canorus*), and the brown-headed cowbird (*Molothrus ater*), which parasitize a wide variety of hosts (Davies 2000).

Obligate interspecific brood parasitism occurs in about 100 species of birds, and only in four orders: Cuculiformes, Piciformes, Passeriformes and Anseriformes (Yom-Tov and Geffen 2006). It is not difficult to imagine the advantages of this reproductive strategy. By laying eggs in the nest of another bird, the parasite will save all the effort usually invested in the nesting process; nest building, incubation and parental care. More importantly, it allows females to increase their fitness by increasing the number of young that are raised.

Despite these obvious advantages, interspecific brood parasitism in birds is surprisingly rare (Slagsvold 1998; Davies 2000). There are probably a number of constraints that prevent this reproductive strategy from evolving, such as (1) problems with species recognition of juveniles reared as parasites when subsequently trying to mate and breed (Slagsvold *et al.* 2002), (2) discrepancy between timing and duration of egg laying and incubation periods (Slagsvold 1998), (3) low access to host nests, and (4) inadequate parental care and food quality provided by hosts (Hamilton and Orians 1965; Payne 1977).

(1) Learning from parents during early development may crucially influence future mate choice decisions of young birds, because in birds, sexual imprinting seems to be the rule rather than the exception (ten Cate and Vos 1999). There is substantial evidence that the rearing environment may be important for the development of mating preferences, indicating that they may be learnt, or modified by experience. The relative importance of such sexual imprinting across species remains largely unexplored, and little is known about the relative importance of learning and genes in shaping the sexual preferences and how this may vary among species (Slagsvold *et al*. 2002). It is plausible that sexual imprinting, and its impact on subsequent mate choice decisions in young birds, can act as a restraint on the evolution of interspecific brood parasitism. A recent, large-scale study conducted by Slagsvold *et al.* (2002) in the wild showed that sexual imprinting may in fact have a negative effect on pairing success in two species of tits, but not in the pied flycatcher.

(2) For parasitism to be able to arise in a natural population, the parasite needs to have somewhat overlapping egg laying periods, if not fully then at least partly, with their host. Many brood parasites tend to lay their eggs early in the host's egg laying period, preferably after the host has laid its first egg (Davies 2000). This is to ensure that the nestling will receive the greatest possible amount of parental care, and to minimize the risk of rejection. It is crucial that the host nest is found at precisely the right time since an egg deposited during incubation would hatch after those of the host species, if at all, and the young would be at a strong competitive disadvantage (Hamilton and Orians 1965).

Interspecific nest parasites are in general larger than their hosts (Hamilton and Orians 1965; Rothstein 1990), and have shorter incubation periods (Hamilton and Orians 1965; Payne 1977). Hence, parasitic nestlings tend to be larger than the host nestlings already at hatching, thus taking advantage of the fact that larger and older nestlings receive priority (Hamilton and Orians 1965; Slagsvold 1997). In altricial birds however, length of the incubation period tends to increase with body size across species (Clutton-Brock 1991). Hence, larger species may not always succeed parasitizing smaller species, due to their demand for a longer incubation period. This was demonstrated by Slagsvold (1998), who simulated nest parasitism within three species of titmice, great tit, blue tit and coal tit. Great tit nestlings parasitizing blue tit nestlings suffered if hatching a few days after the host nestlings. Blue tit nestlings in great tit nests survived well if hatching a few days before the host. The latter also held true after host nestlings had become much larger than the parasite. It was suggested that relative hatching time may be of equal or even greater importance than size of species for origin parasitism (Slagsvold 1998). One would, considering the evidence, expect it to be a trade-off between the size of the parasitic nestling and the duration of the incubation period.

(3) Access to host nests may be a serious constraint. Many bird species are territorial, making it difficult for parasitic species to obtain access to their nests. In addition, there could arise problems with locating host nests, obtaining information about stage of breeding, and of gaining access to nests defended by hosts (Hamilton and Orians 1965).

(4) Yet another obstacle standing in the path of successful parasitism is the food quality provided by the host. The food delivered to the young by the host species must be suitable for normal growth and development; eggs of insectivorous species deposited in nests of species with markedly different diets would probably have little chance of success (Hamilton and Orians 1965).

A recent cross-fostering experiment has shown that pied flycatcher nestlings can be fostered with tits as parents, displaying very similar growth curves and fledgling body mass when compared to data from unmanipulated pied flycatcher nests in the same area (Slagsvold 2004). However, it has been shown that the opposite fails, tit nestlings will not thrive in nests of the pied flycatcher (Slagsvold, unpublished results). The reason for this discrepancy is not known, but possible candidates could be prey types and degree of prey preparation. The present study explores the feeding pattern of pied flycatcher parents, with hopes of revealing some of the causes affecting the failure in the previously mentioned pilot trials. This is done through heterospecific cross-fostering experiments, with nestlings of both the pied flycatcher (*Ficedula hypoleuca*) and great tit (*Parus major*) being fostered by pied flycatcher parents.

Filming inside the nest boxes enables us to analyse the prey items brought to nestlings by pied flycatcher parents, assessing prey type and make assumptions about their size. In addition, it makes it possible to compare the two species in a feeding situation, to see whether or not there is a deviation in their manner of coping with different prey items.

A number of factors related to prey type and size could influence or limit great tit nestling survival in nests of the pied flycatcher, by exceeding their confines of tolerance. Do the parents provide enough food, and in the right proportions, to meet their calorific requirements?
Do they share the prey load equally between nestlings, or simply neglect the great tit nestlings? Is the quality of the prey items sufficient for the great tit nestlings, conceivably requiring nutrients in a different ratio than pied flycatcher nestlings?
Is the size of prey within the limits of what great tit nestlings can swallow?

Considering the failure of pilot trials, some critical limits are exceeded. Knowing that great tit nestlings are fed mostly small spiders and larvae, we expect the great tit nestlings to experience some difficulties swallowing prey items such as flies and wasps (i.e. winged insects with a tougher exterior), but being more equal to pied flycatchers in their ability to swallow larvae. We further expect three day old nestlings to be slower than ten day old nestlings, and parents to adjust prey type and size to nestling age to meet their physiological restraints, such as gape size and swallowing ability.

The study species

The pied flycatcher and the great tit both belong to the order Passeriformes, and exert several similarities when it comes to breeding ecology and feeding (Lack 1966). They are both non-parasitic and altricial species, meaning that they are in need of some parental care after fledging. The pied flycatcher will nest in holes and cavities, but strongly prefer nest boxes (Lundberg and Alatalo 1992); this also applies to the great tit (Perrins 1979).

The pied flycatcher, belonging to the family Muscicapidae, weighs about 12-13 g. The pied flycatcher is a migratory bird, and spends the winter in western and central Africa, and arrives at Scandinavian breeding grounds late April/beginning of May. They lay only one clutch with 5-7 eggs each summer (latitude and density dependent), and incubation time is 13-16 days depending on time of season, clutch size and weather conditions. Fledging time can be variable, but is often 14-16 days (Lundberg and Alatalo 1992).

The great tit is, with a mean body mass of about 17-19 g, the largest member of the tit family Paridae. The great tits are mostly resident, with a few exceptions, and commonly join mixed flocks of other species in the late summer and through to spring. Their mean clutch size is about 9 eggs, incubation time is about 13-14 days, while fledging time is often 21 days.

Most species within the passerine order lay their eggs shortly after sunrise, with some exceptions (Schifferli 1979). In the matter of the pied flycatcher, it has been shown that most eggs are being laid between 05.30 and 07.00 hours (Creutz 1955). After laying, the female stays in the nest for up to half an hour, but then very rarely visits the nest-hole during the rest of the day (Lundberg and Alatalo 1992). With a nest that is unattended for most of the day, it is not unlikely that the pied flycatcher could be a potential host for brood parasites, who easily could seize this possibility to drop their eggs.

Even though both species are mainly insectivorous during the nestling period, they differ slightly in the composition of prey types fed to the nestlings. Studies on the pied flycatcher uniformly show that flies, spiders, butterflies, moths, wasps and beetles are the most common prey categories fed to nestlings (Slagsvold 1975; Dornbusch 1981; Alatalo *et al*. 1988; Lifjeld and Slagsvold 1988; Moreno et al. 1995; Siikamaki *et al*. 1998). The proportions of different prey species, and of imagines and larvae, differ between habitats (Lundberg and Alatalo 1992). According to the studies mentioned above, and some other studies mentioned in Lundberg (1992), the proportion of Lepidoptera in the nestling diet (mostly larvae) ranges from 9.4 % in a pine forest (Dornbusch 1981) to 50.2 % in a deciduous forest (Lifjeld and Slagsvold 1988). In comparison, Siikamaki *et al.* (1998) found the

proportion of flies (Diptera), lepidopteran larvae (i.e. larvae of butterflies and moths) and spiders (Araneae) to constitute 37 %, 18 % and 16 % of all prey items, respectively.

Several studies show that lepidopteran larvae are the preferred food of nestling tits (Betts 1955; van Balen 1973; Perrins 1991; Gosler and Riddington 1995; Nour *et al*. 1998), and they exhibit a preference for caterpillars whenever possible ((Perrins 1965; Perrins and McCleery 1989). As this caterpillar diet is especially characteristic during the nestling period of tits, it could be assumed that caterpillars are both nutritionally optimal and easy to handle, swallow and digest by the nestling tits (Gosler 1993). However, caterpillars are likely to differ in the content of various nutrients essential for the nestlings (Tinbergen 1981; Graveland and Drent 1997; Zandt 1997), and this could possibly explain why tits, even in the richest of habitats, never restrict themselves to an exclusive diet of caterpillars (Royama 1966; Royama 1970; Cowie and Hinsley 1988; Gosler and Riddington 1995). Naef-Daenzer (2000) investigated nestling diet and the foraging performance of great tits in relation to prey abundance in the field, and found that winged insects were caught in remarkably lower proportions than expected from their relative density on trees, which was comparable to the density of caterpillars. The reason for this was thought to be that this group (i.e. winged insects) comprises different species; their highly variable proportion in the diet makes it probable that they were used as a supplement in the diet (Naef-Daenzer *et al*. 2000).

A problem with insect diets is the presence of large amounts of chitin, a polysaccharide that forms fibrous molecules, which is generally indigestible (Bell 1990). The chitin, forming the exoskeleton, can be hard and tough, as in beetles, or soft and flexible, as in caterpillars and other insect larvae. Chitin is also the major component of the wings of winged insects such as flies, wasps and moths. It has been shown that great tits prepare the prey items before feeding them to their nestlings (Dahlsten and Grundel 1991; Kaspari 1991; Ponz *et al.* 1999). This is probably done to make it easier for the nestlings to swallow prey, as cylindrical prey items are less difficult to swallow than prey items with protruding appendages (Kaspari 1990). Other factors, such as nutrient concentration (through removal of low-quality or deleterious parts) or palatability (considering scaly moth wings unpalatable), is also thought to determine the degree of preparation. In great tits, prey preparation increased with prey size and decreased as the nestlings grew older, as brood size increased and as the season progressed (Barba *et al*. 1996). This suggests that preparation was a way of facilitating ingestion and digestion of the prey items. This notion was sustained by the observation that the tits removed at least half of the legs and wings of 71 % of Lepidoptera imagines fed to nestlings (Barba *et al*. 1996). Another passerine, the magpie (*Pica pica*) has been shown to do

the same, readily removing legs and other appendages from beetles (57 % of cases) and grasshoppers (71 % of cases) before feeding them to their young (Ponz *et al.* 1999). Pied flycatchers do not seem to prepare prey items before feeding them to their young.

Materials and methods

The study site

The study was conducted in June 2005 at a study site in Sørkedalen (10° 48' N, 59° 58' E), near Oslo, Norway. The site consists of about 300 nest boxes spread out over an area of approximately 75 ha. The nest boxes are attached to tree trunks about 1.5 m above ground, which makes them easy to inspect. The field site consists of a mixture of deciduous forest, mainly comprised of birch (*Betula sp.*), maple (*Acer sp.*), ash (*Fraximus excelsior*), elm (*Ulmus sp.*) and hazel (*Corylus avellana*). In addition, one will also find areas with coniferous forest, both pine (*Pinus sp.*) and Norway spruce (*Picea albies*).

The experiment

The experiment was performed under license from the Directorate for Nature Management, and the National Animal Research Authority in Norway.

Ethical note: Cross fostering and filming was only conducted in short intervals of time to minimize stress and to reduce potentially harmful effects on the nestlings. In addition, nest boxes used in the experiment were in close proximity to each other (i.e. within walking distance), to decrease stress associated with the relocation of the nestlings between nests.

The ten nest boxes included in the experiment were occupied by pied flycatcher adults and nestlings, and were all replaced with specially designed nest boxes. These boxes have an opening on the right hand side to allow filming inside. The camera (Sony Hi-8) was mounted on a tripod and adjusted to fit in the opening and the small gap between the camera and the nest box was covered with plastic.

The filming was conducted in two sessions for each nest, at both three days and at ten days of age. First, half of the pied flycatcher nestlings were removed and replaced with great tit nestlings collected from nearby great tit nests, in which the pied flycatcher nestlings were temporarily placed. This was done to create a mixed group comprising of nestlings of both species, and of the same age, termed mixed broods below. Mixed broods consisted of equal numbers of nestlings of each species, constituting groups of either four or six nestlings. This was done in order to be able to compare the nestlings of the two species directly in the same nests and with the same parents; both with regards to swallowing behaviour and to assess

10

whether or not parents discriminated between the species. For example, one could imagine that the parents were more easily triggered by the gapes of the pied flycatcher species than that of the great tit nestlings, thus feeding the former more frequently. The nest box was then left with the camera running for approximately 1.5 hour before switching it off upon arrival. The remaining pied flycatcher nestlings were then removed as well, and replaced with great tit nestlings, to form a uniform brood (only great tit nestlings). This was done to observe whether or not the feeding rate and prey composition differed from that of the mixed broods, and to be able to compare data from the two sessions. Say, parents did not feed the great tit nestlings at all in the mixed broods, how would they react to a brood consisting of great tit nestlings only? The camera was then switched on, running for another 1.5 hour, before the nestlings were returned to their nests of origin. Of the ten nest boxes, only five were used in both sessions. Of a total of 15 video sessions, eight were within the three day old group and seven within the ten day old group.

Nestlings included in the experiments were measured with regards to bill length and width and tarsus length, and weighed to assess body mass. Length of the bill was the distance between the corners of the mouth to the tip of the bill. Bill width was the distance between the corners of the mouth. Tarsus length was the length from the lower notch at the back of the leg to the distal edge of the last tarsal scale. Body mass was measured in grams.

Video analysis

A number of variables were measured, although not all were employed in the following data analysis. The tapes were analysed in random order to avoid bias in prey measurements and group design.

Several variables were measured: (1) Number of great tit and (2) pied flycatcher nestlings fed in a single feeding visit. The nestlings of the different species were identified by comparing the shape of their beaks. The great tit nestling has a thin fleshy brim in the corners of its beak, whereas the pied flycatcher beak has a more clean-cut, pointy shape. (3) Occurrence of attempted feedings (testing) of the pied flycatcher nestlings and (4) great tit nestlings. Testing was defined as cases where the parent lowered its bill into that of the nestling and then raised it again without delivering the food (Slagsvold and Wiebe 2007). (5) Type of prey, denoted by specific initial letters for each prey type. The different prey items were identified by appearance, and then categorized. Prey items ranged from spiders (Araneae), flies (Diptera), wasps and ants (Hymenoptera), beetles (Coleoptera), butterflies and moths (Lepidoptera). Prey items were not categorized strictly by order; due to the

similarities between winged insects such as flies, hoverflies and wasps, these were grouped together. Lepidopteran larvae and other caterpillars were categorized by colour (brown, green or white). Crane flies (Tipulidae) and harvestmen (Opiliones) were grouped together because of their physical similarities (long legs, small body). Finally, a group dubbed "others" was used for all prey items that were not identified, and items that did not fit in under the previous categories (e.g. spider eggs, grasshoppers, unknown prey items). (6) Time taken to swallow a prey item. The latter was measured in seconds, and defined as the time passed from the beak holding the prey were in between the tips of the nestlings' beak, until the beak was closed for at least two seconds, or until the nestlings' beak was reopened for a new feeding. (7) Length and (8) width of prey. Prey length and width were assessed by comparing them to the length of the adult bill, and the values were then used to estimate prey volumes by using the formula $V = \pi (0.5\ w)^2 l$, where l is the length and w is the width (Slagsvold and Wiebe 2007). This estimation assumes an equal length of the male and female bill. Legs and other appendages of the prey were not taken into account. (9) Number of prey in a load and (10) if prey was alive or not.

Statistical procedure

The data extracted from the video analysis were processed further to facilitate the following statistical analysis. Totals, means and percentages were calculated, as well as standard deviations. Swallowing times and prey volumes of prey items fed to nestlings of both species were log transformed. Due to the small sample size and data which rarely followed the normal distribution, non-parametrical tests were used. Sample sizes did occasionally differ from the source data (three days $n = 8$, ten days $n = 7$) due to missing values, hence sample size is specified in each case. Computer programs R, Minitab and Excel were used in the statistical analysis. Tests used include Mann-Whitney U-test, Wilcoxon signed-rank test and Spearman's rank correlation coefficient.

Results

Size of nestlings

When comparing the bill lengths of the nestlings of the two species, no significance was found for three day old nestlings (Wilcoxon signed-rank test $V = 30$, $n = 8$, $P = 0.10$). At ten days of age however, pied flycatcher nestlings had significantly longer bills ($V = 28$, $n = 7$, $P = 0.022$).

Great tit nestlings had significantly wider bills at both tree days of age ($V = 0$, $n = 8$, $P = 0.007$), and ten days of age ($V = 0$, $n = 7$, $P = 0.022$).

The body mass of nestlings did not differ significantly between the species at three days of age ($V = 15$, $n = 8$, $P = 0.93$), but at ten days of age great tit nestlings were significantly heavier ($V = 2$, $n = 7$, $P = 0.046$). Tarsus length did not differ between nestlings in either age group ($P > 0.05$, data not shown).

Prey type

Prey items fed to nestlings mainly comprised of spiders, flies and larvae, but a substantial amount of prey items did not fall in any of these categories. These were compiled in a group dubbed "others", containing prey items such as spider eggs, butterflies, moths, ants, beetles, harvestmen, crane flies, wasps, grasshoppers and hover flies, together with unidentified items.
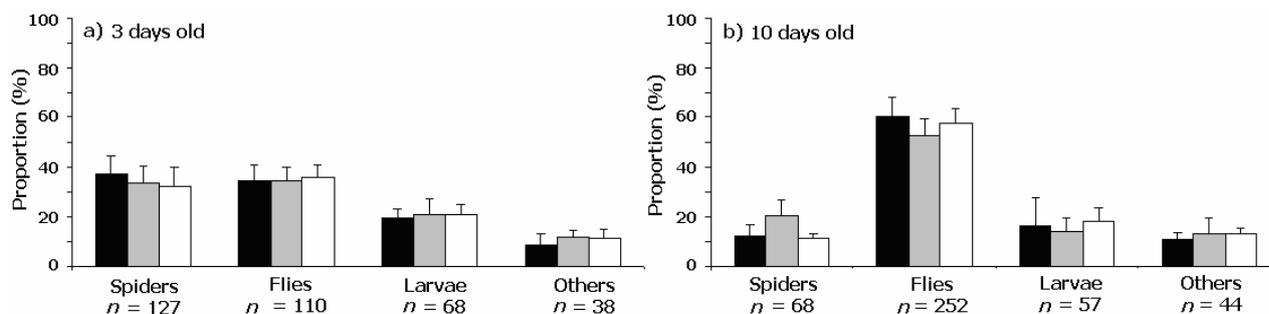

Figure 1 Relative proportions of prey items fed to pied flycatcher nestlings (black columns), great tit nestlings (grey columns) in the same mixed broods, and great tit nestlings only (white columns) at a) three days of age and b) ten days of age. Sample size is shown below bars.

The relative proportions of each prey category did not differ within age groups (**fig. 1**), and pied flycatcher nestlings and great tit nestlings were fed approximately equal proportions of spiders, flies, larvae and other prey items ($P > 0.05$, data not shown).

When comparing proportions between age groups however, differences were found. With regards to spiders, proportions did not differ in great tit nestlings (U-test $W = 15.5$, $n_1 = 8$, $n_2 = 7$, $P = 0.16$), but there was a difference in the pied flycatcher nestlings

13

($W = 7$, $n_1 = 8$, $n_2 = 7$, $P = 0.017$), receiving more spiders at three days of age. In flies however, differences were found in both species (great tit nestlings $W = 46$, $n_1 = 8$, $n_2 = 7$, $P = 0.040$, pied flycatcher nestlings $W = 50$, $n_1 = 8$, $n_2 = 7$, $P = 0.012$), with a substantially higher proportion of flies at ten days of age.

In larvae, there were no discrepancies between species (great tit nestlings $W = 19$, $n_1 = 8$, $n_2 = 7$, $P = 0.32$, pied flycatcher nestlings $W = 12$, $n_1 = 8$, $n_2 = 7$, $P = 0.072$). This also held true for other prey items (great tit nestlings $W = 26$, $n_1 = 8$, $n_2 = 7$, $P = 0.86$, pied flycatcher nestlings $W = 38$, $n_1 = 8$, $n_2 = 7$, $P = 0.26$).

Prey size

Prey loads fed to nestlings mainly consisted of one prey item, with only about 8 % and 5 % of the total number of prey loads containing two or more prey items in the three day old and ten day old group, respectively.
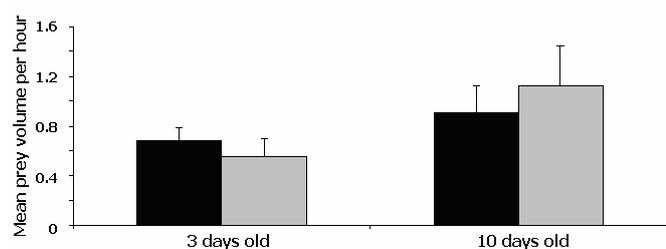

Figure 2 Mean prey volume (+ SE) given to each pied flycatcher nestling (black columns) and great tit nestling (grey columns) per hour in the same mixed broods, at both three days ($n = 8$) and ten days ($n = 7$) of age. Numbers have been log reversed.
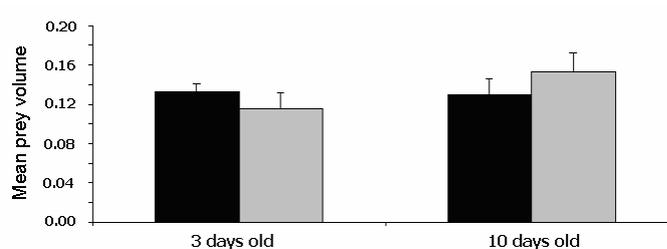
Figure 3 Mean prey volume per visit in pied flycatchers (+SE) feeding pied flycatcher nestlings (black columns) and great tit nestlings (grey columns) in the same mixed broods, at both three days ($n = 8$) and ten days ($n = 7$) of age. Numbers have been log reversed.

When comparing sizes of prey items fed to the different age groups, no differences were evident for spiders (U-test pied flycatcher $W = 14.5$, $n_1 = 8$, $n_2 = 6$, $P = 0.22$, great tit $W = 14$, $n_1 = 7$, $n_2 = 5$, $P = 0.61$), flies (pied flycatcher t = 0.2542, df = 12.772, $n_1 = 8$, $n_2 = 6$, $P = 0.80$, great tit $W = 20.5$, $n_1 = 8$, $n_2 = 5$, $P = 0.41$), or larvae (pied flycatcher $W = 30$, $n_1 = 8$, $n_2 = 7$, $P = 0.4767$, great tit $W = 20$, $n_1 = 8$, $n_2 = 7$, $P = 1$). For mixed broods, mean prey volume per nestling per hour (**fig. 2**) did not differ significantly between species, neither at three days of age (Wilcoxon signed-rank test $V = 28$, $n = 8$, $P = 0.1953$), or ten days of age ($V = 11$, $n = 7$, $P = 0.68$). The mean prey volume fed to ten-day old nestlings per hour was slightly higher than for younger nestlings, this difference was quite significant for great tit nestlings, (U-test: $W = 40$, $n_1 = 8$, $n_2 = 7$, $P = 0.18$), but not for pied flycatcher nestlings: (W = 34, $n_1 = 8$, $n_2 = 7$, $P = 0.53$). Mean volume given to each nestling per visit did not differ between the species

(**fig. 3**) in the three day old group (Wilcoxon signed-rank test $V = 26$, $n = 8$, $P = 0.31$), but there was a slight difference in the ten day old group ($V = 3$, $n = 7$, $P = 0.078$).

Between the age groups, there was no significance for the pied flycatcher ($W = 31$, $n_1 = 8$, $n_2 = 7$, $P = 0.77$), or for the great tit ($W = 14$, $n_1 = 8$, $n_2 = 7$, $P = 0.11$).

When comparing the sizes (i.e. volume) of different prey types fed to the two species (**fig. 4**) in the three day old group, there were no significant differences in spiders, flies or larvae. This also applied to the ten day old group ($P > 0.05$, data not shown).
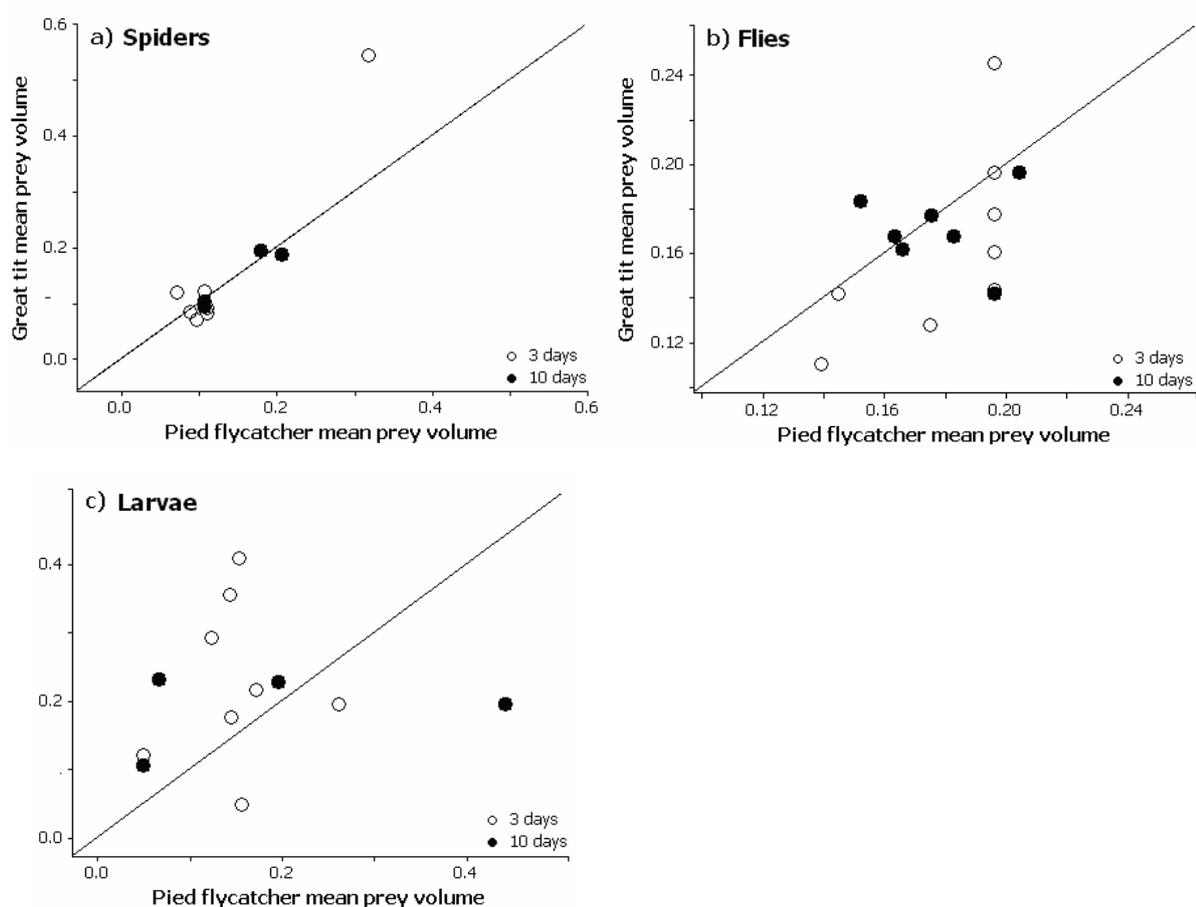

Figure 4 Mean prey volume fed to pied flycatcher nestlings and great tit nestlings in the same mixed broods, open dots represent three day old nestlings and filled dots ten day old nestlings. a) Spiders ($n_1 = 7$, $n_2 = 4$), b) flies ($n_1 = 8$, $n_2 = 7$) and c) larvae ($n_1 = 8$, $n_2 = 7$). Line equals y = x.

Swallowing time

The nestlings of the two species differed significantly in swallowing time of all prey types combined in mixed broods (**fig. 5**), both in the three-day old group (Wilcoxon signed-rank test $V = 0$, $n = 8$, $P = 0.007$), and the ten-day old group ($V = 0$, $n = 7$, $P = 0.015$).
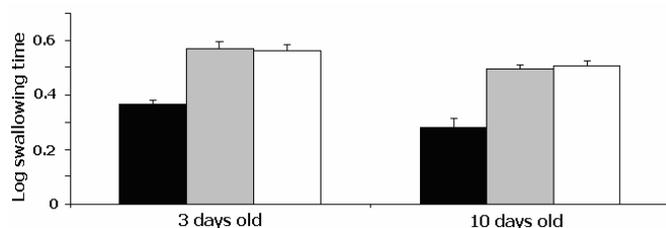

Figure 5 Log-transformed swallowing times for pied flycatcher nestlings (black columns) and great tit nestlings (grey columns) in the same mixed broods, and great tit nestlings only (white columns), at both three days ($n = 8$) and ten days ($n = 7$) of age.

Age

The swallowing times was significantly higher at three days of age when comparing to ten days of age, the difference being larger for the pied flycatcher nestlings (U-test $W = 6$, $n_1 = 8$, $n_2 = 7$, $P = 0.009$) than for the great tit nestlings ($W = 9$, $n_1 = 8$, $n_2 = 7$, $P = 0.028$) in mixed broods. There was a slight difference in the swallowing times of three day old and ten day old great tit nestlings in the sessions with great tit nestlings only, but this was not significant ($W = 13$, $n_1 = 8$, $n_2 = 7$, $P = 0.093$).

Prey type

In the matter of swallowing times for the different types of prey, it becomes clear that the great tit nestlings are slower than their counterparts, especially when it comes to spiders and flies (**fig. 6**).

There was a significant difference between the species at three days of age in the swallowing times of spiders (U-test $W = 0$, $n_1 = 7$, $n_2 = 8$, $P = 0.001$), flies (Wilcoxon signed-rank test $V = 32$, $n = 8$, $P = 0.054$), and larvae ($V = 1$, $n = 8$, $P = 0.015$).

At ten days of age, there was considerable differences both in spiders (U-test $W = 0$, $n_1 = 5$, $n_2 = 6$, $P = 0.007$), and flies (Wilcoxon signed-rank test $V = 28$, $n = 8$, $P = 0.015$, but there was no significance in the mean swallowing time of larvae (U-test $W = 7$, $n_1 = 5$, $n_2 = 6$, $P = 0.16$).

Mean swallowing times for spiders did not differ between the age groups, neither in the pied flycatcher nestlings ($W = 37$, $n_1 = 8$, $n_2 = 6$, $P = 0.10$) or the great tit nestlings ($W = 27$, $n_1 = 7$, $n_2 = 5$, $P = 0.14$). In flies, however, differences were detected in the pied flycatcher

16

nestlings ($W = 56$, $n_1 = 8$, $n_2 = 7$, $P = 0.001$), but not in the great tit nestlings ($W = 37.5$, $n_1 = 8$, $n_2 = 7$, $P = 0.29$). Swallowing times for larvae did not differ between the age groups in the pied flycatcher nestlings ($W = 26.5$, $n_1 = 8$, $n_2 = 6$, $P = 0.79$), but a result, although weak, was found for the great tit nestlings ($W = 33$, $n_1 = 8$, $n_2 = 5$, $P = 0.065$.
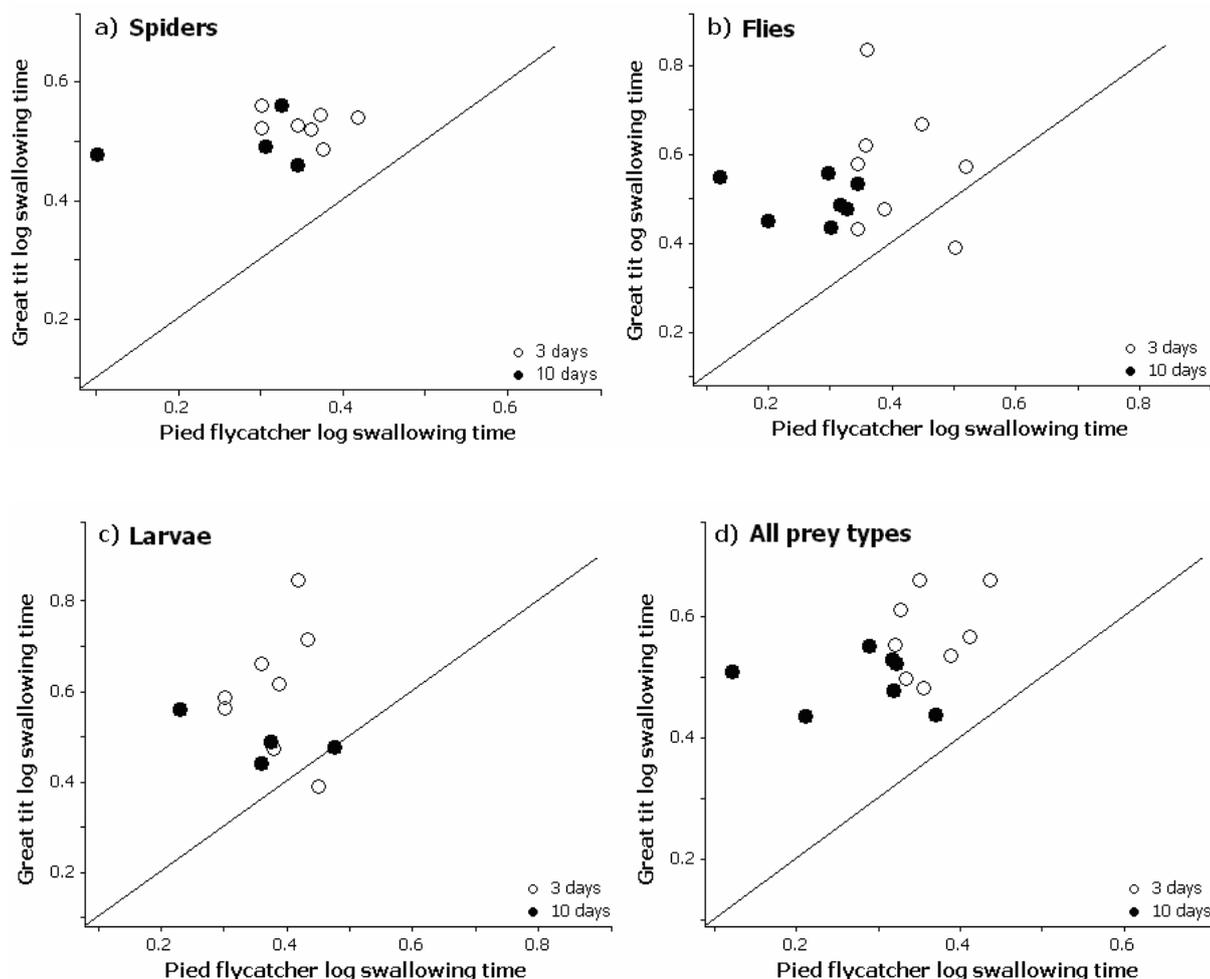

Figure 6 Mean swallowing times (log-transformed, +SE) for great tit nestlings and pied flycatcher nestlings in the same mixed broods, open dots represent three day old nestlings and filled dots ten day old nestlings, for a) spiders ($n_1 = 7$, $n_2 = 4$), b) flies ($n_1 = 7$, $n_2 = 8$), c) larvae ($n_1 = 8$, $n_2 = 4$) and d) all prey types ($n_1 = 7$, $n_2 = 8$). Line equals y = x.

There was a significant correlation (we assume that the responses are independent) between prey size and swallowing time (**fig. 7**) at three days of age, in both the pied flycatcher nestlings (Spearman's rank correlation $r_s = 0.18$, $n = 121$, $P = 0.046$), and the great tit nestlings ($r_s = 0.19$, $n = 194$, $P = 0.006$). As for the ten day old nestlings, no relationship was found in the pied flycatcher nestlings ($r_s = 0.083$, $n = 206$, $P = 0.23$), but a correlation was detectable in the great tit nestlings ($r_s = 0.14$, $n = 201$, $P = 0.044$).
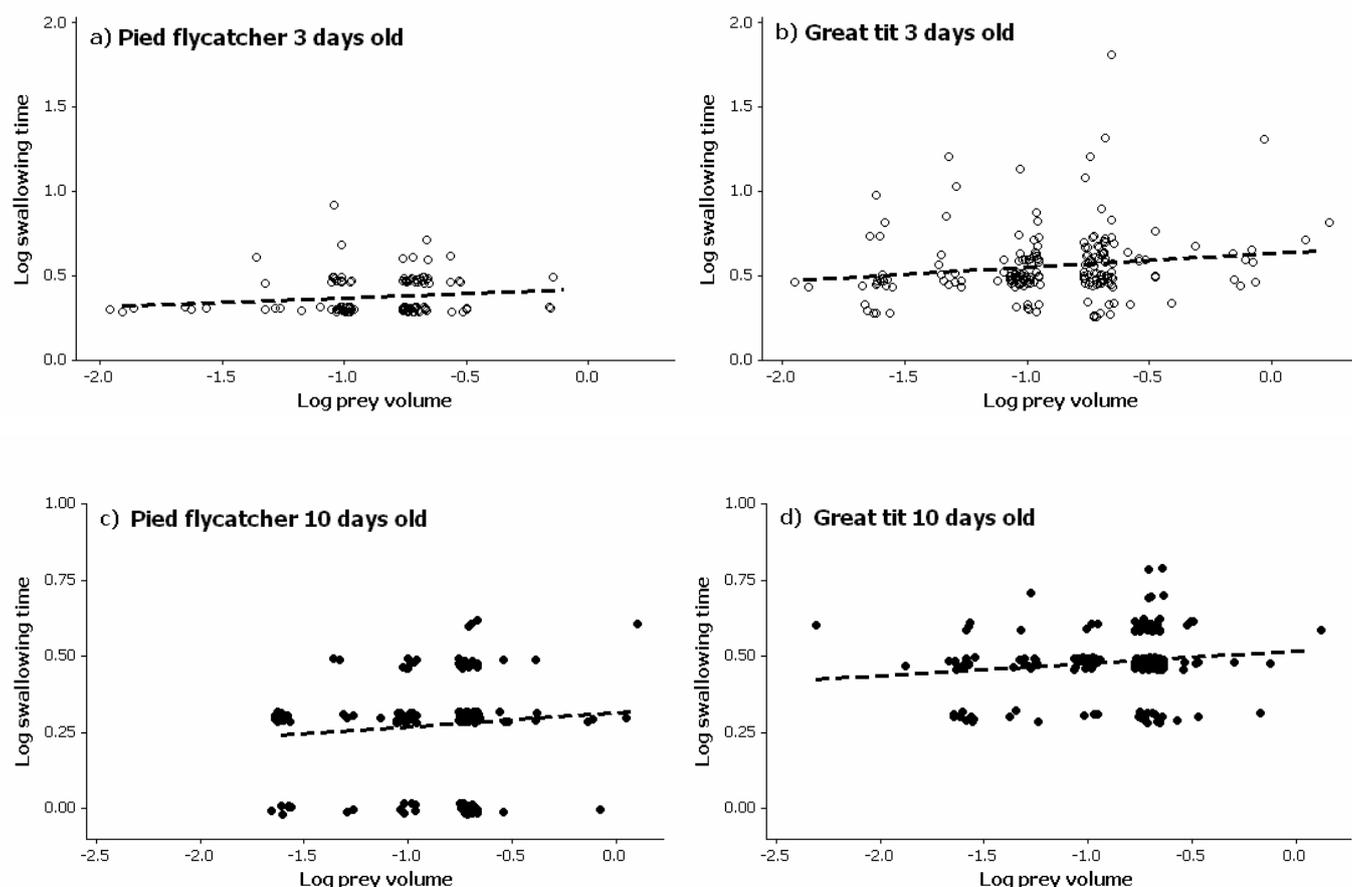
Figure 7 Correlation (with regression line) between prey volume (log transformed) and swallowing time (log transformed) for pied flycatcher nestlings and great tit nestlings in the same mixed broods. a) three days old pied flycatcher nestlings (n =121), b) three day old great tit nestlings (n =194), c) ten day old pied flycatcher nestlings (n =206) and d) ten day old great tit nestlings (n =201).

Prey preparation

The prey items showed no sign of preparation, legs and wings being intact upon feeding attempts. A substantial proportion of the prey items were still alive upon the time of the feeding attempt, 19.3 % in the 3 d group and 17.9 % in the 10 d group. No differences in swallowing time between live or dead prey items within age groups were found, however, either in the pied flycatcher or in the great tit nestlings ($P > 0.05$, data not shown).

Testing

Testing was significantly more frequent during attempted feedings to great tit nestlings than to pied flycatcher nestlings in mixed broods (**fig. 8**), especially in the three-day old group (Wilcoxon signed-rank test $V = 36$, $n = 8$, $P = 0.007$). A weaker result was found for ten day-old nestlings ($V = 21$, $n = 7$, $P = 0.036$). Comparing between age groups revealed a substantially higher frequency of testing for three days old great tit nestlings (U-test $W = 5$, n_1

= 8, n_2 = 7, P = 0.005) but there was no significant difference for pied flycatcher nestlings (W = 22, n_1 = 8, n_2 = 7, P = 0.50).
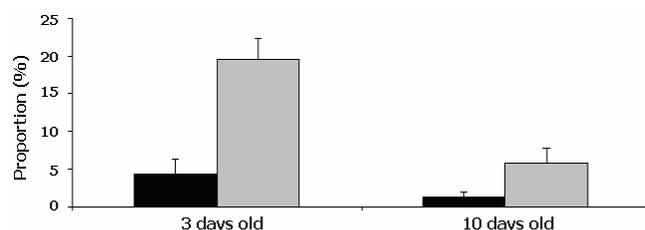

Figure 8 Mean proportion of testing incidents (+ SE) during attempted feedings to pied flycatcher nestlings (black columns) and great tit nestlings (grey columns) in the same mixed broods, at three days and ten days of age.

Testing was often done in a repetitive manner, with up to 14 attempts in a row in three day old nestlings, and a staggering 30 in the ten day old group. Even with extensive testing, some of the great tit nestlings still failed in their attempt to swallow the prey item. Of the total number of feeding attempts with testing to great tit nestlings in the 3 d group, the parents gave up and fed the prey item to a pied flycatcher nestling in 25 % of cases. As for ten day old nestlings, this occurred in 32 % of cases.

In nests with great tit nestlings only, testing was common, and occurred in 37 % of cases during feeding attempts in the three day old group (ranging up to 80 % in one of the nest boxes), and in 7 % of cases in the ten day old group.

Discrimination between species and adjustment of prey

The species differed slightly when comparing the mean percentage of nestlings gaping upon arrival of an adult at three days of age (V = 5, n = 8, P = 0.078); great tit nestlings gaping more frequently. There was no such difference in the 10 d group (V = 7, n = 7, P = 1). When comparing the mean percentage of great tit nestlings gaping open upon arrival in the mixed group to that of the group with great tit nestlings only, it turned out that while there was no difference at three days of age (V = 22, n = 8, P = 0.64), they were gaping slightly more often at ten days of age (V = 25, n = 7, P = 0.078).

Despite this, the mean number of successful feedings did not differ between the species, neither in the three day old group (Wilcoxon signed-rank test V = 23, n = 8, P = 0.54), or in the ten day old group (V = 13, n = 7, P = 0.93). The feeding rate (i.e. number of feedings to each nestling per hour (**fig. 9**)), did not differ significantly between the age groups either (great tit: U-test W = 40, n_1 = 8, n_2 = 7, P = 0.18, pied flycatcher: W = 42.5, n_1 = 8, n_2 = 7, P = 0.10). When comparing the feeding rate to great tit nestlings in mixed broods to that of the

groups containing great tit nestlings only, there was no significance in either age group (three days: $V = 15$, $n = 8$, $P = 0.74$, ten days: $V = 20$, $n = 7$, $P = 0.37$). Mean feeding rates at three days of age (pied flycatcher mix: $\bar{x} = 5$, great tit mix: $\bar{x} = 4.5$, great tit only: $\bar{x} = 4.5$) did not deviate from the normal feeding rate of pied flycatcher parents to three day old nestlings, which is usually between four to eight feedings per hour (Alatalo *et al.* 1982). The feeding rate at ten days of age, however (great tit mix: $\bar{x} = 6.8$, pied flycatcher mix: $\bar{x} = 6.7$, great tit only: $\bar{x} = 6$), deviated from the usual feeding rate which is 10-20 feedings per hour (Alatalo *et al.* 1982).
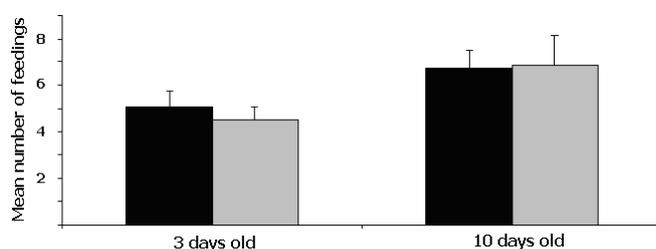

Figure 9 Mean number of successful feedings (+ SE) by pied flycatcher parents to pied flycatcher nestlings (black columns) and great tit nestlings (grey columns) in the same mixed broods per hour, at both three days ($n = 8$) and ten days ($n = 7$) of age.

When comparing the mean proportions within each prey category (**fig. 10**), there were no differences concerning the distribution of spiders between the species in the 3 d group (Wilcoxon signed-rank test $V = 13$, $n = 8$, $P = 0.67$). This also applied to flies ($V = 22$, $n = 8$, $P = 0.20$) and larvae ($V = 8$, $n = 8$, $P = 0.36$). In the matter of other prey items, however, there was a significant difference ($V = 36$, $n = 8$, $P = 0.013$). In the 10 d group there were no discrepancies within any of the prey categories ($P > 0.05$, data not shown).
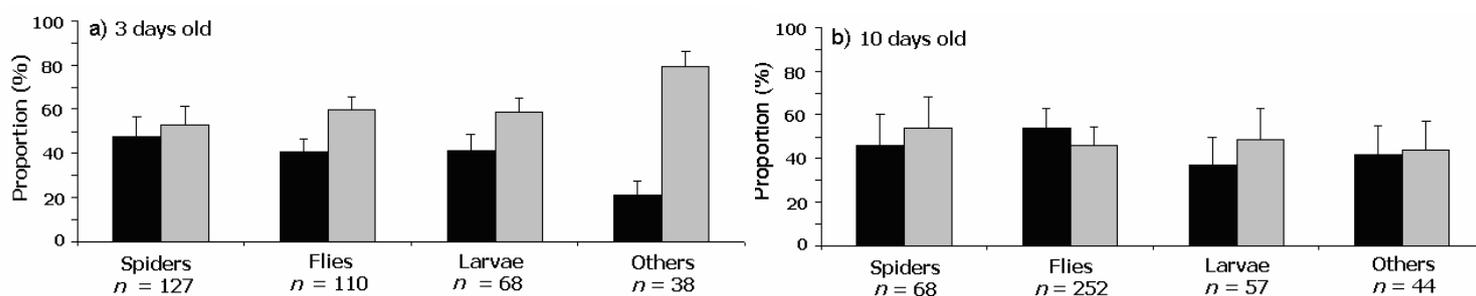

Figure 10 Mean proportion within each prey category (+SE), fed to pied flycatcher nestlings (black columns) and great tit nestlings (grey columns) in the same mixed broods at a) three days of age and b) ten days of age. Sample size is shown below bars.

Discussion

The main findings were:

Parents did not adjust prey type to great tit nestlings, thus they did not feed them prey types that would be easier for them to ingest, such as larvae.

The parents did not adjust the prey size to great tit nestlings either, feeding nestlings of the pied flycatcher and great tit prey of equal sizes.

Swallowing times differed significantly between species, especially in the 3 d group; great tits were much slower than their pied flycatcher nest mates.

The parents did not seem to prepare prey items before feeding them to nestlings, legs and wings being intact upon the time of the feeding attempt.

Testing was by far more extensive during attempted feedings to great tit nestlings than to pied flycatcher nestlings, in both age groups.

Pied flycatcher and great tit nestlings were fed equal amounts of food, both with regards to feeding rate (i.e. mean number of feedings to each nestling per hour) and the mean prey volume per nestling per hour.

One must take into account that the data collected in the present study only represents a single year, and one can not rule out the possibility that the different proportions of prey items were deviant from the usual proportions in other years. This is not likely, however, considering the conformity to other studies.

Sample size is small, partly due to ethical reasons; the great tit nestlings were obviously suffering (e.g. swallowing problems) when placed in nests of the pied flycatcher. Despite low sample size, it was still sufficient to support significant results.

When filming in nature, one must always consider the degree of disturbance inflicted on study species, because disturbance could potentially bias the results. The pied flycatcher is a fairly tolerant species, being reluctant to abandon breeding attempts after being caught and handled, and is thus considered to be amenable for field experimentation (Lundberg and Alatalo 1992). They did not seem to be disturbed by the presence of a camera, and started feeding within a matter of minutes after the camera had been switched on.

Prey type

When seeing the total prey load, pied flycatcher nestlings and great tit nestlings were fed equal proportions of spiders, flies, larvae and other prey items, with no substantial deviations in prey types. This applied to both age groups. The composition of nestling diet is not likely

to be random, seeing how it is important that nestlings receive the right amounts of proteins, fats and nutrients they need in order to grow and thrive. Potential prey items come in many shapes and sizes, from a variety of orders, and may differ in handling time, swallowing time, digestibility and nutritional value.

The great tit is known to be a caterpillar specialist and good caterpillar availability is crucial for a successful breeding in this species (van Balen 1973; Perrins 1991; Naef-Daenzer and Keller 1999; Naef-Daenzer et al. 2000). It has even been shown that great tit fledging probability is related to the proportion of larvae in their diet (Eeva 2005). The pied flycatcher is considered to be a more opportunistic forager, known to use very variable diets in different geographical locations and habitats (Cramp and Perrins 1993). For example, it has been known to eat ladybirds (Coccinellidae), whereas the great tit seems to avoid these unpalatable beetles (Eeva 2005).

There are reasons to believe that nestling birds (and offspring of other animals) may be in particular need of carotenoids, with regards to both immune responses, the neutralization of free radicals omitted as a consequence of rapid growth, and in the development of plumage colour. It has been shown that the quality and quantity of food during feather growth partly determine the expression of carotenoid-based plumage coloration (Horak et al. 2000). Lutein is the main carotenoid that great tit nestlings need to get from their diet to develop their yellow breast feathers (Partali *et al.* 1987), and caterpillars are known to be a rich source of lutein in birds (Slagsvold and Lifjeld 1985; Eeva *et al.* 1998). The pied flycatcher have a less colourful plumage than great tits, ranging from jet black to brown or greyish brown (Lundberg and Alatalo 1992), and may not be as dependent of this carotenoid. This may cause great tit nestlings to suffer more extensively to a decline in the proportion of larvae in their diet. In this study with pied flycatcher parents, it is clear that larvae was not the most numerous prey item fed to nestlings, being outnumbered by spiders and flies, and the proportion was virtually unaltered between age groups. This could be a critical factor on the path to explaining why great tit nestlings won't thrive in pied flycatcher nests.

The main types of prey fed to nestlings undoubtedly differ between bird species and habitat, and nestling diet often differs from the diet of the adult birds. This is also the case for pied flycatchers, where the stomachs of nestlings have been found to contain more spiders and butterfly and moth larvae, whereas in adults, ants were found to be more frequent (Silverin and Andersson 1984). Thus, nestlings are probably given softer prey items than those eaten by the parents (Lundberg and Alatalo 1992). The difference between adult and nestling diet may be even more pronounced when comparing small nestlings to adults, than in

the case of fully grown nestlings (Pruska 1980). This is in concordance with the present results, where the amount of flies fed to nestlings increased with age. In addition, the number of spiders, considered to be softer and easier to consume than flies, were more frequently fed to nestlings at three days of age. Similar results are evident for great tits. It has been shown that spiders were fed especially to young nestlings (van Balen 1973), and that the proportion of spiders in the diet of great tit nestlings increased steadily after hatching, and peaked at about days six or seven and then declined (Royama 1970). This trend, while strongly correlated with the age of the nestlings, occurs irrespective of season or habitat, which implies that it is not related to variations in natural spider abundance. Rather, it appears that spiders are actively selected by adults and have some special nutritional value for nestlings in the early stages of development (Ramsay and Houston 2003). Is has been hypothesized that this could be related to the development of feathers, since both feather keratin and spider proteins are rich in the sulphur-containing amino acid cysteine. Feather development starts at about day five, with a growth rate that is at its greatest between then and day nine, when the quills rupture (Gosler 1993). Spiders, however, also have vastly higher levels of the sulphur-containing amino acid taurine, and a recent study suggests that this may cause the seemingly active selection of spiders by the great tit parents. Taurine has a number of extremely important functions in postnatal developing young (Ramsay and Houston 2003), and could act as a limiting factor if not sufficiently provided through the diet.

Only having filmed feeding at three and ten days of age, it is difficult to draw conclusions from the present study as to whether or not the amount of spiders in the diet, and their amount of cysteine could act as a constraint on feather development in its crucial phase, or if the amount of taurine is sufficient for normal growth.

Pied flycatcher parents did not, at least not in this case, adjust prey type to great tit nestlings, even though they seemed to cope more easily with spiders and especially larvae, than with flies. If parents had fed the great tit nestlings more larvae, they might have been able to meet their nutritional requirements, subsequently causing them to thrive in nests of the pied flycatcher.

Prey size

Size of prey must be considered when feeding young and small nestlings, especially during the first week, due to their low capacity to be able to handle and swallow large prey items (Bengtsson and Rydén 1981). This also applies to the great tit (Gosler 1993), and the size of prey brought to great tit nestlings usually increases gradually until about day five, after which

there is no further change (Royama 1970). Betts (1955) found that, in the first five days, prey items of 15 mm or more constituted only 8 % of the nestlings' diet and 65 % were less than 10 mm. Between day six and ten, 58 % of items were larger than 15 mm, and from this age to fledging, prey of this size formed 74 % of diet, with caterpillars of up to 3 cm sometimes brought in. Due to the fact that we used beak length to assess prey length in this study, it is not instantly comparable to these measurements. The mean beak length of pied flycatchers has been measured to about 13 mm (Alatalo *et al.* 1985), making a prey item one beak length long about 13 mm. When comparing prey items given to nestlings from the different age groups in the present study, it becomes evident that prey items measuring less than half a beak length (< 6.5 mm) constituted 44 % of the diet in the 3 d group, and 26 % in the 10 d group. Prey items measuring one beak length or more (> 13 mm) constituted 57 % and 74 % of the diet to three day old and ten day old nestlings, respectively. Given these prey lengths and the fact that pied flycatcher parents, at least to a certain degree, adjust prey size to the age and size of the nestlings, makes it unlikely that length and size of prey items alone are the limiting factor for great tit nestlings in pied flycatcher nests.

The parents brought prey of similar sizes to both species, hence they did not show any sign of adjusting the size of prey items fed to the great tit nestlings. This occurred despite the fact that the great tit nestlings were struggling to swallow prey items at both three days and ten days of age. The fact that the parents do not seem to adjust size of prey may partially explain why the great tit nestlings suffer in nests of the pied flycatcher.

Swallowing time

The species differed significantly in the swallowing time of prey, especially in the three day old group. The dissimilarity between the species was linked to prey type. The species differed in the swallowing times of both spiders, flies and larvae at three days of age, but this did not apply indiscriminately to the 10 d group. Here, significant differences were found for spiders and flies, but not in larvae. Thus, larvae seemed to be easier to handle for the great tits at ten days of age, ending up with a mean swallowing time that was similar to that of the pied flycatchers, which is in concordance with our expectations.

When comparing between age groups within species, it was shown that the mean swallowing time of spiders did not differ for either species, indicating that neither species seem to improve their ability to handle spiders significantly with age. Pied flycatcher nestlings were, however, faster than their nest mates in both age groups. When comparing mean swallowing times for flies, it became apparent that pied flycatchers were improving

their ability to swallow flies as they grew older, but this did not seem to be the case for the great tit nestlings. This shows that the swallowing difficulties of the great tit nestlings are not all age-dependent; at ten days of age they are still struggling with flies brought by the pied flycatcher parents.

As for larvae, it was shown that there was no significant difference between age groups in the swallowing times for pied flycatcher nestlings, but a result was evident for great tit nestlings. This implies that even though pied flycatcher nestlings had overall shorter larvae handling times at three days of age, this difference was evened out at ten days of age, when the great tit nestlings had strongly improved swallowing abilities.

In another tit species, the blue tit, prey size and swallowing time has been shown to be correlated, ranging from larvae with the shortest swallowing times, followed by spiders and then grasshoppers (Bañbura *et al.* 1999). In the blue tit study, it was deduced that the factor of greatest importance affecting handling time of prey items was the variation between prey categories in their suitability for nestlings. The handling efficiency of particular prey types turned out to be strongly inversely related to prey diameter. It is likely that this could apply to the great tit as well. In fact, Royama (1966, 1970) reported a positive correlation between the size of prey and the time needed to feed it to great tit nestlings. In the present study, there was a positive correlation between prey sizes and swallowing time when seeing all prey items as a whole. This was particularly relevant at three days of age, but also at ten days of age, although only applying to the great tit nestlings.

It is not likely that increased swallowing time alone could be a critical factor, even though they were struggling with the prey item; they still managed to ingest it within ten seconds, in most cases. In some cases, however, swallowing time was recorded to be 40 seconds and more, and even though this might not affect the nestling directly, it could have an indirect impact on food provisioning. Parents usually stayed in the nest until the nestling had managed to ingest the prey item, and this time could have been spent foraging, thus lowering provisioning rate.

Prey preparation

Even though the great tit is principally insectivorous, thus preferring insects and other invertebrates, other food sources must be utilized during the winter, when temperatures drop and access to such prey items is scarce. With its bill, adapted for heavy duty work, the great tit can easily crack open seeds and consume the nutritious contents. Great tits also kill prey items such as caterpillars and bees, by clamping the item to a perch and striking it repeatedly and powerfully with the strong bill (Gosler 1993). The bill of the great tit has been shown to change shape during the year, being finer in summer when insects constitute the main proportion of the diet, and heavier in winter, when they turn more to seeds (Gosler 1987).

The pied flycatcher is an almost completely insectivorous species, and has a smaller and more fragile beak than the great tit. This could potentially constrain their ability to rip and tear off wings and legs effectively. Prey items fed to nestlings in this study showed no signs of preparation, legs and wings being intact upon the time of the feeding attempt. Considering the swallowing ability of the nestlings, prey preparation is not likely to be a necessity in this species. The habit of prey preparation in great tits could be related to the fact that they are usually single prey loaders (Naef-Daenzer *et al.* 2000), bringing nestlings one prey item at a time. Dealing with one prey item at a time makes it easier to prepare or kill it. The pied flycatcher is a multiple-prey loader (Haartman 1954, and present study), and this obviously makes it more difficult to prepare each prey item before feeding them to their young. One would, however, expect this to cause swallowing difficulties for great tit nestlings, especially with regards to insects with protruding legs. This proved to be the case; great tit nestlings did struggle more extensively with flies than with larvae in both age groups. It is very likely that the lacking prey preparation is a critical factor, both making the prey items more difficult to swallow for the great tit nestlings, and impeding digestion.

Testing

Testing of prey items before feeding them to nestlings has been noted in several studies and has been ascribed various functions (Slagsvold and Wiebe 2007). It has been proposed that the parents use testing to assess the amount of food already in the nestlings' crop in order to give food to the hungriest nestling (Wilson and Clark 2002), but that does not seem to the case here. The same nestling was tested in several feeding visits in a row, even after being fed. This applied both to pied flycatcher nestlings and great tit nestlings. Kaspari (1990) suggested that testing is used by parents to find a gape size large enough to handle the food load to be delivered and to avoid giving smaller nestlings food that may choke them or would be

difficult to digest. Testing was significantly more frequent during feeding attempts to great tit nestlings, especially in the 3 d group, and this could imply that the pied flycatcher parents perceived the gapes of the great tit nestlings as too small to handle the prey items they provided. Comparing mean sizes of the nestlings' bills revealed that great tit nestlings actually had a greater bill width, at both three days ($\bar{x} = 13.0$ mm) and ten days of age ($\bar{x} = 14.4$ mm) than pied flycatcher nestlings ($\bar{x} = 11.3$ mm, $\bar{x} = 12.7$ mm, respectively). Concerning the length of the nestlings' bills, no difference was found at three days of age (great tit $\bar{x} = 9.2$ mm, pied flycatcher $\bar{x} = 9.8$ mm). At ten days of age, however, pied flycatcher nestlings had longer bills ($\bar{x} = 13.6$ mm) than great tit nestlings ($\bar{x} = 12.4$ mm). Considering that great tit nestlings had wider beaks in both age groups, it is not likely that the parents would have perceived their gapes as being too small to handle prey items.

No discrimination between species and no adjustment of prey

Larger and older nestlings in the brood tend to receive priority (Hamilton and Orians 1965; Slagsvold 1997), and considering that the great tit has a higher adult body mass and is overall larger than the pied flycatcher, one might suspect that size differences between nestlings could induce some sort of discrimination. Pied flycatcher nestlings weigh about 1.5 g at hatching (Gosler 1993), whereas the great tit weighs about 1.3 g (Lundberg and Alatalo 1992), and the two species are displaying fairly similar growth curves initially. Upon reaching day four and five, the great tit starts gaining weight at a faster rate, weighing about 16.5 g at day ten, whereas the pied flycatcher weighs about 14 g (Perrins 1979; Lundberg and Alatalo 1992). This held true in the present study, where the nestlings had approximately the same body mass at three days of age (pied flycatcher $\bar{x} = 5.9$ g, great tit $\bar{x} = 6$ g). At ten days of age, great tit nestlings were significantly heavier ($\bar{x} = 15.4$ g) than the pied flycatcher nestlings ($\bar{x} = 13.8$ g). Hence great tit nestlings, though roughly equal in size to pied flycatcher nestlings in the 3 d group, may have appeared larger than their nest mates at ten days of age. In spite of this, no evidence of discrimination was found, implying that the parents fed the nestlings in mixed broods indiscriminately, not favouring either species. However, when considering that the great tit is a larger bird, it is likely that they have a higher nutritional threshold than the pied flycatcher nestlings in order to meet the requirements. Thus, even though feeding rates were equal, the amount of food could have been insufficient for great tit nestlings, not being able to maintain normal growth.

Considering the swallowing difficulties exerted by the great tit nestlings, especially at three days of age, one could imagine that the parents interpreted this as saturation, lowered the feeding rate, thus adding to the already existing food deficiency of the great tit nestlings. Nestlings were given a large amount of flies at ten days of age, unprepared with their wings intact. Considering the great tit nestlings' lacking ability to cope with these even at this age, one would guess that this would have an impact like the one described for the 3 d group, namely giving the parents the impression that the nestlings are saturated, lowering the feeding rate. This is quite plausible considering the lower than usual feeding rate at ten days of age. This explanation could also apply to nests with great tit nestlings only, given the fact that there was no significant difference in feeding rate between the two sessions.

Taking into account that the great tit nestlings tended to gape more often upon arrival of an adult at three days of age, may sustain this notion that they did receive insufficient amounts of food, since gaping could be interpreted as an indicator of hunger level of the nestlings. The great tit nestlings were gaping significantly more often in the broods with great tit nestlings only, than in mixed broods, at ten days of age. This could be caused by the increased impact of having five or six nestlings in the brood exerting swallowing difficulties as a result of a high proportion of unprepared flies in the diet, the former being interpreted by parents as saturation. This could potentially lead to a lowering of feeding rate, inducing more begging in terms of gaping.

Furthermore, the begging and gaping of the great tit nestlings may have increased the provisioning rate of the parents, leaving the pied flycatchers saturated as a cause of receiving more than their calorific requirements. This could explain their modest gaping upon arrival in the 3 d group. However, even though the provisioning rate was potentially higher than normal for pied flycatcher parents, leaving pied flycatcher nestlings saturated, this could still have been insufficient for great tit nestlings due to their higher growth rate.

The tarsus length of the nestlings could potentially affect the parents' response during a feeding attempt. Nestlings with a longer tarsus bone exert greater flexibility in terms of stretching toward the feeding parent, and could potentially receive priority. The nestlings did not differ significantly in tarsus length, thus it is not likely than any nestling would receive priority during feeding attempts.

In terms of food provisioning and discrimination, testing may have had an influence as well. As mentioned previously, parents gave up on several occasions during attempted feedings to great tit nestlings, having tested a number of times, and fed the prey item to a pied

flycatcher nestling. This would surely reduce the food provisioning to great tit nestlings, thus adding to the food deprivation.

Conclusion

There is probably a combination of several factors causing great tit nestlings to suffer in nests of the pied flycatcher. First of all, the pied flycatcher parents did not seem to adjust prey types to the nestlings. This was evident in both the mixed broods and in the broods consisting of great tits only, even though the nestlings obviously had difficulties coping with the prey items. Different prey items differ in their content of important nutrients, and it could be possible that the great tit nestlings were suffering more extensively as a result of low amounts of carotenoids in the diet than pied flycatcher nestlings.

Second, it appears that the pied flycatcher parents failed in assessing the nestlings' hunger level correctly, though possibly bringing more food than usual due to more begging, but not bringing enough food to meet the higher requirements of the great tit nestlings. These problems related to prey type and swallowing constraints may help to explain why great tits do not parasitize pied flycatcher broods.

References

Alatalo, R. V., K. Gottlander and A. Lundberg (1988). Conflict or cooperation between parents in feeding nestlings in the pied flycatcher Ficedula hypoleuca. Ornis Scandinavica **19**(1): 31-34.

Alatalo, R. V., A. Lundberg and K. Stahlbrandt (1982). Why do pied flycatcher females mate with already-mated males? Animal behaviour **30**(2): 585-593.

Alatalo, R. V., A. Lundberg and S. Ulfstrand (1985). Habitat selection in the pied flycatcher Ficedula hypoleuca

Bañbura, J., M.-M. Lambrechts, J. Blondel, P. Perret and M. Cartan-Son (1999). Food handling time of Blue Tit chicks: Constraints and adaptation to different prey types. Journal of avian biology **30**(3): 263-270.

Barba, E., J. A. Lopez and J. A. GilDelgado (1996). Prey preparation by adult Great Tits Parus major feeding nestlings. Ibis **138**(3): 532-538.

Bell, G. P. (1990). Birds and mammals on an insect diet: a primer on diet composition analysis in relation to ecological energetics. Studies in avian biology(13): 416-422.

Bengtsson, H. and O. Rydén (1981). Development of parent young interaction in asynchronously hatched broods of altricial birds. Zeitschrift für Tierpsychologie-Journal of Comparative Ethology **56**(3): 255-272.

Betts, M. M. (1955). The Food of Titmice in Oak Woodland. Journal of Animal Ecology **24**(2): 282-323.

Clutton-Brock, T. H. (1991). The evolution of parental care. Princeton, N.J., Princeton University Press

Cowie, R. J. and S. A. Hinsley (1988). Feeding Ecology of Great Tits (Parus-Major) and Blue Tits (Parus-Caeruleus), Breeding in Suburban Gardens. Journal of Animal Ecology **57**(2): 611-626.

Cramp, S. S. and C. M. Perrins (1993). Handbook of the birds of Europe, the Middle East and North Africa : the birds of the Western Palearctic Oxford, Oxford University Press.

Creutz, G. (1955). Der Trauerschnäpper (Muscicapa hypoleuca) (Pallas). Eine Populationsstudie. Journal für Ornithologie **96**(3): 241-326.

Dahlsten, R. D. and R. R. Grundel (1991). The feeding ecology of mountain chickadees (*Parus gambeli*) - Patterns of arthropod prey delivery to nestling birds. Canadian journal of zoology **69**(7): 1793-1804.

Davies, N. B. (2000). Cuckoos, cowbirds and other cheats London, T & AD Poyser.

Dornbusch, M. (1981). Die Ernahrung einiger Kleinvogelarten in Kiefernjungbestockungen. Beiträge zur Vogelkunde **27**(2): 73-99.

Eeva, T. (2005). Pollution-related changes in diets of two insectivorous passerines. Oecologia **145**(Berlin): 629-639.

Eeva, T., E. Lehikoinen and M. Ronka (1998). Air pollution fades the plumage of the Great Tit. Functional ecology **12**(4): 607-612.

Gosler, A. (1987). Pattern and process in the bill morphology of the great tit Parus major. The Ibis **129**(4): 451-476.

Gosler, A. (1993). The great tit. London, Hamlyn.

Gosler, A. G. and R. Riddington (1995). Differences in reproductive success and parental qualities between habitats in the great tit Parus major. The Ibis **137**(3): 371-378.

Graveland, J. and R. H. Drent (1997). Calcium availability limits breeding success of passerines on poor soils. Journal of Animal Ecology **66**(2): 279-288.

Hamilton, W. J. and G. H. Orians (1965). Evolution of Brood Parasitism in Altricial Birds. The Condor **67**(5): 361.

Horak, P., H. Vellau, I. Ots and A.-P. Moller (2000). Growth conditions affect carotenoid-based plumage coloration of great tit nestlings. Die Naturwissenschaften **87**(10): 460-464.

Haartman, L. v. (1954). Der Trauerfliegenschnäpper. III. Die Nährungsbiologie. Acta Zoologica Fennica **83**: 1-96.

Kaspari, M. (1990). Prey preparation and the determinants of handling time. Animal behaviour **40**: 118-126.

Kaspari, M. (1991). Prey preparation as a way that grasshopper sparrows (*Ammodramus savannarum*) increase the nutrient concentration of their prey. Behavioral ecology **2**(3): 234-241.

Lack, D. (1966). Population studies of birds. Oxford, Clarendon Press.

Lifjeld, J. T. and T. Slagsvold (1988). Effects of energy costs on the optimal diet - An experiment with pied flycatchers *Ficedula hypoleuca* feeding nestlings. Ornis Scandinavica **19**(2): 111-118.

Lundberg, A. and R. V. Alatalo (1992). The Pied Flycatcher. London, T. & A. D. Poyser.

Moreno, J., R. J. Cowie, J. J. Sanz and R. S. R. Williams (1995). Differential Response by Males and Females to Brood Manipulations in the Pied Flycatcher - Energy-Expenditure and Nestling Diet. Journal of Animal Ecology **64**(6): 721-732.

Naef-Daenzer, B. B. and B. L. Keller (1999). The foraging performance of great and blue tits (Parus major and P-caerulens) in relation to caterpillar development, and its consequences for nestling growth and fledging weight. The Journal of animal ecology **68**(4): 708-718.

Naef-Daenzer, L., B. Naef-Daenzer and R. G. Nager (2000). Prey selection and foraging performance of breeding Great Tits Parus major in relation to food availability. Journal of Avian Biology **31**(2): 206-214.

Nour, N., D. Currie, E. Matthysen, R. Van Damme and A. A. Dhondt (1998). Effects of habitat fragmentation on provisioning rates, diet and breeding success in two species of tit (great tit and blue tit). Oecologia **114**(4): 522-530.

Partali, V. V., V. S. Liaaen-Jensen, V. T. Slagsvold and V. J. Lifjeld (1987). Carotenoids in food chain studies II. The food-chain of Parus spp. monitored by carotenoid analysis. Comparative biochemistry and physiology. B. Comparative biochemistry **87**(4): 885-888.

Payne, R. B. (1977). The Ecology of Brood Parasitism in Birds. Annual review of ecology and systematics **8**(1): 1.

Perrins, C. C. (1991). Tits and their caterpillar food supply. The Ibis **133**: 49-54.

Perrins, C. M. (1965). Population Fluctuations and Clutch-Size in the Great Tit, Parus-Major L. Journal of Animal Ecology **34**(3): 601-647.

Perrins, C. M. (1979). British Tits. London, Collins.

Perrins, C. M. and R. H. McCleery (1989). Laying Dates and Clutch Size in the Great Tit. Wilson Bulletin **101**(2): 236-253.

Ponz, A., J.-A. Gil-Delgado and E. Barba (1999). Factors affecting prey preparation by adult Magpies feeding nestlings. The Condor **101**(4): 818-823.

Pruska, M. M. (1980). The diet of the nestlings of the great tit Parus major, the pied flycatcher Ficedula hypoleuca and of the redstart Phoenicurus phoenicurus in a pine wood. Bulletin of the Museum and Institute of Zoology **17**(21): 321-332.

Ramsay, S. L. and D. C. Houston (2003). Amino acid composition of some woodland arthropods and its implications for breeding tits and other passerines. Ibis **145**(2): 227-232.

Rothstein, S. I. (1990). A model system for coevolution: avian brood parasitism. Annual review of ecology and systematics **21**: 481-508.

Royama, T. (1966). Factors Governing Feeding Rate Food Requirement and Brood Size of Nestling Great Tits Parus Major. Ibis **108**(3): 313-&.

Royama, T. (1970). Factors Governing Hunting Behaviour and Selection of Food by Great Tit (Parus-Major L). Journal of Animal Ecology **39**(3): 619-&.

Schifferli, L. (1979). Warum legen Singvogel (Passeres) ihre. Eier am fruhen Morgen? Der Ornithologische Beobachter **76**(1): 33-36.

Siikamaki, P., J. Haimi, M. Hovi and O. Ratti (1998). Properties of food loads delivered to nestlings in the pied flycatcher: effects of clutch size manipulation, year, and sex. Oecologia **115**(4): 579-585.

Silverin, B. and G. Andersson (1984). Food composition of adult and nestling pied flycatchers, Ficedula hypoleuca, during the breeding period. Vår fågelvärld **43**(8): 517-524.

Slagsvold, T. (1975). Competition between the Great Tit *Parus major* and the Pied Flycatcher *Ficedula hypoleuca* in the breeding season. Ornis Scandinavica **6**: 179-190.

Slagsvold, T. (1997). Brood division in birds in relation to offspring size: sibling rivalry and parental control. Animal Behaviour **54**(6): 1357-1368.

Slagsvold, T. (1998). On the origin and rarity of interspecific nest parasitism in birds. The American naturalist **152**(2): 264-272.

Slagsvold, T. (2004). Cross-fostering of pied flycatchers (Ficedula hypoleuca) to heterospecific hosts in the wild: A study of sexual imprinting. Behaviour **141**(Part 9): 1079-1102.

Slagsvold, T., B.-T. Hansen, L.-E. Johannessen and J.-T. Lifjeld (2002). Mate choice and imprinting in birds studied by cross-fostering in the wild. Proceedings of the Royal Society B: Biological Sciences **269**(1499): 1449-1455.

Slagsvold, T. and J. T. Lifjeld (1985). Variation in plumage colour of the great tit Parus major in relation to habitat, season and food. Journal of zoology. Series A **206**(3): 321-328.

Slagsvold, T. and K. L. Wiebe (2007). Hatching asynchrony and early nestling mortality: the feeding constraint hypothesis. Animal Behaviour **73**: 691-700.

ten Cate, C. C. and C. D. Vos (1999). Sexual imprinting and evolutionary processes in birds: A reassessment. Advances in the study of behavior **28**: 1-31.

Tinbergen, J. M. (1981). Foraging decisions in starlings (Sturnus vulgaris L.). Ardea **69**(1): 1-67.

van Balen, J. H. (1973). A comparative study of breeding ecology of the Great Tit (*Parus major*) in different habitats. Ardea **61**(1-2): 1-93.

Wilson, D.-S. and A.-B. Clark (2002). Begging and cooperation: an exploratory flight. In: The Evolution of Begging. Competition, Cooperation and Communication (Ed. by Wright, Jonathan & Leonard, Marty L.). Dordrecht, Kluwer Academic Publishers.

Yom-Tov, Y. and E. Geffen (2006). On the origin of brood parasitism in altricial birds. Behavioral Ecology **17**(2): 196-205.

Zandt, H. S. (1997). Water content of prey of nestling blue tits in a Corsican habitat. Netherlands journal of zoology **47**(2): 125-131.

APPENDIX 1

Descriptive statistics for log transformed swallowing times

Table 1 Descriptive statistics for log transformed swallowing times, for all prey types combined.

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
3 days PF	8	0	0.3661	0.015	0.0423	0.3206	0.3292	0.3538	0.4068	0.4365
3 days GT	8	0	0.5707	0.0239	0.0675	0.4824	0.508	0.5594	0.6471	0.6599
3 days GT only	8	0	0.5634	0.0207	0.0586	0.4994	0.512	0.5491	0.6115	0.6682
10 days PF	7	0	0.2795	0.032	0.0846	0.122	0.2125	0.3186	0.3233	0.3715
10 days GT	7	0	0.4951	0.0171	0.0452	0.4362	0.4387	0.5092	0.5287	0.5519
10 days GT only	7	0	0.5079	0.0167	0.0442	0.4366	0.4752	0.5108	0.5527	0.5573

Table 2 Descriptive statistics for log transformed swallowing times, for different prey types (spiders, flies and larvae).

Spiders:

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
3 days PF	8	0	0.3471	0.0153	0.0434	0.301	0.301	0.353	0.3756	0.4184
3 days GT	7	0	0.52814	0.00902	0.02386	0.48509	0.51925	0.5267	0.5445	0.56034
10 days PF	6	0	0.2749	0.0364	0.0892	0.1003	0.2274	0.3039	0.3309	0.3451
10 days GT	5	0	0.4928	0.0177	0.0396	0.4582	0.4677	0.4771	0.5257	0.5604

Flies:

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
3 days PF	8	0	0.4082	0.0253	0.0714	0.3451	0.3484	0.3744	0.4882	0.5188
3 days GT	8	0	0.5711	0.0503	0.1423	0.3891	0.443	0.5754	0.6552	0.8331
10 days PF	7	0	0.2731	0.0306	0.081	0.1226	0.2007	0.301	0.3281	0.3447
10 days GT	7	0	0.4975	0.0184	0.0487	0.4348	0.4483	0.4845	0.5465	0.5573

Larvae:

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
3 days PF	8	0	0.3793	0.02	0.0565	0.301	0.3157	0.3848	0.4294	0.4515
3 days GT	8	0	0.6061	0.0499	0.141	0.3891	0.495	0.6018	0.701	0.8451
10 days PF	6	0	0.3602	0.0443	0.1084	0.23	0.2381	0.368	0.4771	0.4771
10 days GT	5	0	0.4533	0.0428	0.0956	0.301	0.3705	0.4771	0.524	0.5604