

# **ALLOCATION OF PARENTAL INVESTMENT IN BIRDS**

**PhD Thesis**

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## 1. Introduction

The amount of parental investment has a profound effect on the fitness of both parents and offspring (Allander 1997; Nur 1984a; Nur 1984b). The amount and allocation of parental investment are therefore key questions in behavioural ecology. In iteroparous species, parental investment is distributed over multiple reproductive events, thus we have to distinguish two levels of allocation: one within broods and one among subsequent broods. The quality of the young may vary considerably at both levels, thus their contribution to parental fitness may also differ. As a consequence, an even allocation of resources is not necessarily the best option for the parents, and preferential allocation is expected to evolve.

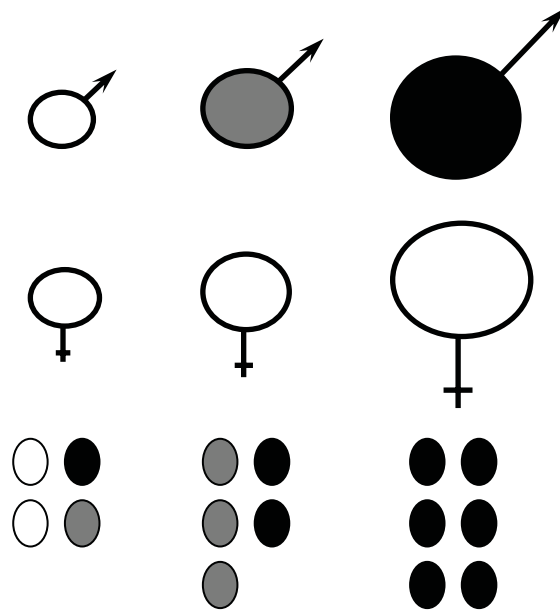
### *1.1. Between-brood differences in offspring value*

Fluctuations of weather conditions among reproductive events may result in significant variation of the food resources. Furthermore, seasonal changes in weather conditions and food supply may also constrain reproduction (Pärt 2001; Wiggins et al. 1994). Reduced food intake may result in slower growth (Birkhead et al. 1999; Boag 1987; Ohlsson and Smith 2001; Quinney et al. 1986), and delayed fledging in birds (Cucco and Malacarne 1996; Searcy et al. 2004). If nest predators are abundant, later fledging is expected to result in increased predation risk (Hussel 1972), thereby reducing the survival prospects of the young. Under poor food conditions, nestlings usually fledge with smaller reserves (in worse body condition), and smaller size (de Kogel 1997; Pettifor et al. 2001; Quinney et al. 1986; Sanz 1997) further reducing their chances to survive (de Kogel 1997; Pettifor et al. 2001). Furthermore, surviving young from bad years may be more susceptible to infections (Saino et al. 1997; Stjernman et al. 2004), perhaps due to shortage of resources during the development of their immune system. Malnutrition early in life may have long-term negative effects also on the future reproductive value of the progeny. Young animals in poor condition may delay the time of the first breeding (Arroyo 2002), and show less developed secondary sexual characters or smaller body size as adults (Boag 1987; de Kogel 1997; Nowicki et al. 2000; Ohlsson et al. 2002; Ohlsson and Smith 2001; Searcy et al. 2004). The latter effects may result in competitive disadvantage when fighting for resources (Pärt and Qvarnström 1997), reduces the chance that those individuals will be chosen by females (Gustafsson et al. 1995; Qvarnström et al. 2000), and increases the probability of losing paternity because of extra-pair copulations (Hasselquist et al. 1996; Kempnaers et al. 1992; Sheldon et al. 1997; Sheldon and Ellegren 1999). As a consequence, recruits from bad years or bad parts of the

season confer less fitness benefit to their parents, than those from good years or favorable parts of the season.

The reproductive value of an offspring is also dependent on its genetic quality. High quality young have better chance to survive. With improving quality, reproductive success is also expected to improve, but the slope of the correlation may be different for males and females. Though the number of young a female can produce varies with female quality or condition in some species (Garamszegi et al. 2004c; Newton et al. 1983; Slagsvold and Lifjeld 1990), it is strongly limited in birds and mammals. In birds, females have to invest large amount of resources in the ova, and after laying, costly incubation (Reid et al. 2000) is also exclusively their task in many species. The need for incubation may even set a physical limit to clutch size (Engstrand and Bryant 2002; Moreno et al. 1991). Furthermore, hatchlings need parental feeding and/or guarding in many species, which means further limitation to brood size (Nur 1984b). In mammals the number of embryos which can grow in the uterus is also limited and usually only females provide post-natal parental care. On the contrary, males can produce large numbers of sperm at a relatively low cost, which can be used to fertilize multiple females. As a consequence, in many species a portion of the males is enough to fertilize all the females (Höglund and Alatalo 1995). In these cases, the access to females may strongly depend on male quality. High quality males may copulate with many individuals, while low quality males may have no access to the females. Even in species with a socially monogamous reproductive system, large variation in male reproductive performance may exist due to extra-pair fertilizations (Griffith et al. 2002). Because of the difference in reproductive potential between males and females, the reproductive success of high-quality males is generally higher than that of high quality females, but low quality females may have higher reproductive output than low quality males (Figure 1). If so, female offspring will return larger benefit per unit investment for low quality parents, while male progeny will contribute more to the fitness of high quality parents (Trivers and Willard 1973). A similar relationship may apply to parental attractiveness even if it is independent of quality (e.g. survival prospects), but preferred during mate choice (e.g. due to sensory exploitation).

Finally, parental age and experience may also affect the life prospects of the progeny in two ways. First, young, less experienced parents may be less efficient in finding optimal territories (Pärt 2001) or in foraging (Brandt 1984) and may therefore provide less or lower quality resources to their offspring. Getting more experienced, their parental abilities often improve (Cichoń 2003; Lessells and Krebs 1989; Pärt 2001). Second, change in the composition of the cohorts with time may result in quality differences between young and old parents. One might



**Figure 1. The expected relationship between parental quality and reproductive success in species with extra-pair copulations.** Quality of the parents is improving from the left to the right. The reproductive success of females is indicated by the number of eggs produced, while reproductive success of males is indicated by the eggs with the colour of the father (i.e. these are the eggs fertilized by the male).

expect that in species where many juveniles can breed in their second calendar year, selection will eliminate low quality individuals after their first breeding attempt (Mauck et al. 2004; Pärt 2001). Hence, older birds should on average be of better quality and are expected to transfer their „good genes” for survival to their progeny. Under both this scenario and in the case of phenotypic improvement of parental abilities, the offspring of old individuals have higher chance to survive and reproduce. On the contrary, when breeding opportunities are strongly limited, we might expect that only good quality juveniles can breed in their second calendar year, while low quality individuals delay their reproduction to the next breeding season. As a result, young breeders (i.e. the individuals of high quality) would produce offspring with on average better prospects than old breeders (though this difference might disappear due to the low efficiency of young breeders in foraging).

### *1.2. Within-brood differences in offspring value*

In birds, survival and reproductive chances of nestlings may vary considerably also within broods due to either genetic or phenotypic differences. Since 93% of the passerine species have a socially monogamous reproductive system (Lack 1968), genetic differences between siblings might be considered to be less important. However, using molecular markers, the majority (86%) of these species turned out to be genetically polygamous (Griffith et al. 2002). A single brood may therefore contain nestlings sired by multiple males. Some evidence exist that females participate in extra-pair copulations (i.e. copulations with males other than the social mate) when they are mated to low-quality or non-attractive males, and the extra-pair father is of better quality (Hasselquist et al. 1996; Kempenaers et al. 1992; Sheldon et al. 1997; Sheldon and Ellegren 1999). Consequently, the half-siblings may have different chance to survive and/or reproduce.

However, the main source generating phenotypic differences within broods is hatching asynchrony. In many avian species, females start to incubate their eggs before clutch completion. Many hypotheses have been proposed to explain the adaptiveness of this behaviour (for a review see e.g. Nilsson 1993; Stenning 1996), but either size hierarchy is the goal (see e.g. brood reduction hypothesis, sibling rivalry hypothesis) or only a by-product of hatching asynchrony (see e.g. nest failure hypothesis, egg viability hypothesis), as a result, remarkable size differences among offspring can often be observed (Krebs 1999; Saino et al. 2003). Older, larger chicks may limit the access of their younger siblings to the food (Ostreiher 1997), or – in siblicid species – they may even kill them (Legge 2000). As a consequence, higher mortality (Krebs 1999; Krist et al. 2004), slow growth (Johnson et al. 2003; Nilsson and Svensson 1996) and smaller fledging size (Clotfelter et al. 2000; Cotton et al. 1999) of late hatched young has been reported in many species.

### *1.3. Compensate for the disadvantages or enhance the advantages?*

As a result of the considerable variation in offspring reproductive value, an even distribution of parental investment is not necessarily the best choice for the parents. On one hand, preferential investment into good-quality chicks is more likely to return benefit to the parents than investment into low quality nestlings. On the other hand, compensatory investment may eliminate the disadvantage of the young with initially worse life prospects. Not surprisingly, both types of preferential investment have been observed (see below).

However, preferential allocation should be favored by natural selection if it results in an increase of the inclusive fitness of the parents. Therefore, the key question is whether or not

additional investment into a young can increase its survival and mating prospects. Above a certain value, for example, further increase in body mass may not improve the survival of the young (Martin 1987). If so, it is not adaptive to invest additional resources into nestlings which have already reached this limit (i.e. into the good quality nestlings), since further investment would reduce the survival prospects of the parents, without enhancing that of the young. Similarly, the disadvantage of the poor quality young may be so large in some environments that further parental investment would not significantly improve their survival/mating chances. Furthermore, a compensation for the disadvantages of low quality young may divert investment from good quality chicks, thereby decreasing the fitness value of the latter. As follows from this discussion, optimal allocation of the resources among chicks or reproductive events is probably strongly dependent on the social / ecological environment.

#### *1.4. Means of preferential parental investment*

The mechanisms the parents can apply to favor certain broods are similar to those used to favor certain nestlings within the brood. In birds, preferential investment can be observed during egg laying (e.g. sex manipulation (Hasselquist and Kempenaers 2002), differential nutrient (Uller et al. 2005), hormone (Groothuis et al. 2005) and carotenoid investment (Saino et al. 2002)) and after hatching of the chicks (differential food allocation; Lessells 2002).

In the last decade, an increasing number of studies have found significant departures from 50:50 brood sex ratios (Hasselquist and Kempenaers 2002). Since in birds the female is the heterogametic sex, the possibility arises that females adjust the sex of their offspring so that they maximize their fitness benefit. As expected from the sex difference in quality dependent reproductive success (Trivers and Willard 1973, also see above), females have been found to produce male biased brood sex ratios when mated to high quality or attractive males, while female biased sex ratios in the broods of poor quality or poorly ornamented males (Burley 1986; Ellegren et al. 1996; Kölliker et al. 1999; Sheldon et al. 1999, but see Leech et al. 2001; Radford and Blakey 2000). Sex ratio adjustment in sexually size dimorphic species has also been often found. Females overproduced the larger (more costly) sex when they were in good body condition, while in poor condition they produced more offspring of the cheaper sex (Clout et al. 2002; Nager et al. 1999). Similar pattern can be expected even in sexually size monomorphic species, if the environmental sensitivity of the offspring is sex dependent (Martins 2004). In these cases, females will produce the less sensitive sex when they are less

able to provide extensive parental care (i.e. when they are in poor body condition; Bradbury and Blakey 1998; Kilner 1998).

In some species, the sex of the offspring is not random in relation to the laying order either (Arnold et al. 2001; Badyaev et al. 2003; Blanco et al. 2002; Legge et al. 2001). Such a pattern can be expected mainly in sexually size dimorphic and asynchronously hatching species. In such species, females can substantially modify the survival chances of the chicks by changing the competitive asymmetry among nestlings through laying sequence dependent sex manipulation (Badyaev et al. 2002).

Females are also able to change the amount of nutrients, the concentration of hormones and carotenoids in relation to laying order. Depending on the reproductive strategy (brood reduction or brood survival) of the species, both decreasing (testosterone: Schwabl et al. 1997; nutrients: Arnold 1989; Heeb 1994; Viñuela 1997; carotenoids: Saino et al. 2002) and increasing investment (testosterone: Lipar and Ketterson 2000; Schwabl 1993; nutrients: Cichoń 1997; Howe 1976; Reynolds et al. 2003; Royle et al. 1999; carotenoids: Török, Hargitai, Hegyi, Matus, Michl, Péczely, Rosivall and Tóth unpubl. manuscript) have been observed. Differences between broods have also been revealed in a number of studies. For example, female zebra finches laid eggs containing more testosterone when mated to attractive males (Gil et al. 1999). Since testosterone had positive effects on the nestlings in most of the species (Eising et al. 2001; Lipar and Ketterson 2000; Schwabl 1993; Schwabl 1996, but see Sockman and Schwabl 2000), this pattern can be interpreted as preferential allocation into more valuable broods. On the contrary, female barn swallows laid eggs with larger carotenoid content, when mated to non-attractive males (Saino et al. 2002), which can be considered as a compensatory investment.

After hatching of the chicks females may either continue to support the nestlings already preferred by early maternal effects, e.g. by unevenly distributing the food, or they may alter their preference according to environmental changes during the incubation period. Feeding the chicks, however, is not exclusively the task of females in most bird species. Thus, from this point, male parents also have chance to influence the survival and reproductive prospects of the young. Paternal preferences may be in agreement with the maternal preferences, but we can expect parent-parent conflict over food allocation, e.g. when the brood contains extra-pair nestlings, because extra-pair young increases maternal, but not paternal fitness.



## 2. Aims of the research

### 2.1. Between-brood patterns

Two of the papers in this thesis (*paper 1 and 2*) focused on sex ratio adjustment. Avian sex ratio adjustment is much in the highlight of recent literature in behavioural ecology. However, despite the accumulating number of studies, it is still a controversial issue. Contradictory results have often been found among and even within species (table 1). Therefore, some researchers argued that sex ratio manipulation is merely a statistical artefact and not a significant biological phenomenon (Ewen et al. 2004). Others (e.g. West and Sheldon 2002) have pointed out that some strong experimental evidence supports the existence of avian sex ratio adjustment (Komdeur et al. 1997; Nager et al. 1999; Pike 2005; Sheldon et al. 1999), and mentioned methodological differences between studies or environmental differences between populations as potential reasons for the opposing results. Thus, long-term studies and population comparisons are clearly needed. Until now, however, the number of these studies has been low, and in the case of population comparisons, the results are often hard to interpret, since studies in different populations used different estimates of e.g. paternal quality (table 1). In *paper 1* I aimed to investigate the brood sex ratio pattern in a Hungarian population of Collared Flycatchers (*Ficedula albicollis*), examining the same explanatory variables as the earlier study (Ellegren et al. 1996) on the Gotland (Sweden) population of the same species. The possibility of direct comparison was especially important because I did expect differences in the results due to other differences between the two study populations. Namely, the forehead patch size, a sexually selected trait (Michl et al. 2002; Qvarnström et al. 2000; Sheldon and Ellegren 1999), is a condition dependent „good genes” signal in the Swedish population (Gustafsson et al. 1995; Qvarnström 1999; Sheldon et al. 1997), and plays a role in sex ratio adjustment, whereas the same trait is independent of body condition (Hegyi et al. 2002) in our study population, which reduces the expected benefit of sex manipulation related to this trait (i.e. contrary to the Swedish population, male offspring of attractive fathers in our population have better reproductive prospects, but not a better chance to survive). I also investigated if male wing patch size (another sexually selected trait; Sheldon and Ellegren 1999), the size of the parents, and the course of the breeding season had an effect on brood sex ratios.

Table 1. Some examples of contradictory results in studies of brood sex ratio adjustment. Only those traits are showed, which differed between studies in the impact they had on brood sex ratios.

species	study	sample size/year	number of years	male attractiveness	male quality	female quality	laying date	male age	female age	clutch size	food regime
<i>Parus caeruleus</i>	(Svensson and Nilsson 1996)	41	1		+a	0a	0	0	0		
	(Sheldon <i>et al.</i> 1999)	41/57	1	+	+a	0a	?	?	?		
	(Leech <i>et al.</i> 2001)	51	3		0abcd	0abcd	0	0	0	0	
	(Griffith <i>et al.</i> 2003)	57	3	+	+a		0	0	0(+1)	0(-1)	
	(Lessells <i>et al.</i> 1996)	103	1		0b	0b	+	0	0	0	
<i>Parus major</i>	(Kölliker <i>et al.</i> 1999)	57	1	{+}	+c	0c	0	0	0	0	
	(Radford and Blakey 2000)	24	5	0	0c(-1)0e	0c(+1-1)0e(+1)	0(+1)	0(-1)	0		
	(Oddie and Reim 2002)	79[23]	3		0ce	0ce[+e]	0	0	0	0	
<i>Taeniopygia guttata</i>	(Kilner 1998)	33	1								-
	(Rutkowska and Cichoń 2002)	24	1								0

(Empty cells indicate traits which were not examined in the studies. 0=no effect, +/-=positive/negative correlation between the trait and sex ratio. ?, reports of significant effects without indicating the direction of the correlation. In the case of parental quality a= overwinter survival, b=body mass, c=tarsus length d=parasite load, e=condition (residual from body mass-tarsus length regression). In multiple year studies the overall result is shown. If traits were significant in certain years only, then the direction of the effect and the number of years in which it was found is indicated in parentheses. {} indicates marginally significant result. [] indicates between year comparisons for the same individuals. In Sheldon *et al.* (1999) first sample size refers to male attractiveness, while the second to all the other traits.)

Apart from the population differences in sex ratio adjustment (which was the focus of *paper 1*), methodological differences between studies, and statistically false positive results, there exists another explanation for the contradictory results, which has been neglected until now and was the topic of *paper 2*. Our knowledge of the mechanisms of avian sex ratio adjustment is poor (Pike and Petrie 2003), however the proposed mechanisms differ in their predictions concerning the level of sex ratio manipulation. Many of them suggest that, in species with large clutches, sex ratio adjustment should be restricted to the first egg (Emlen 1997). Still, most of the studies investigate only clutch sex ratios. In *paper 2* I report a simulation study examining the detectability of sex ratio adjustment at the clutch level when the manipulation is restricted to the first egg in large clutches.

In *paper 3* our goal was to investigate the possible reasons for a previously observed maternal effect. Our research group found significantly higher concentration of testosterone (Michl *et al.* 2005) and carotenoids (Török, Hargitai, Hegyi, Matus, Michl, Péczely, Rosivall and Tóth unpubl. manuscript) in the eggs of subadult (1 year old) than in those of adult (at least two years old) males. Since testosterone and carotenoids have beneficial effects on nestlings (testosterone: Eising *et al.* 2001; Lipar and Ketterson 2000; Schwabl 1993; Schwabl 1996; carotenoids: Saino *et al.* 2003), such biased egg investment could be considered as help to the nestlings of young males. This could be either due to a preference for good quality chicks or compensation for the disadvantage of the bad quality chicks depending on whether young males are of better or worse quality than adult males (see above). Therefore, we planned an experiment in which we could evaluate the potential quality differences between offspring of young and old males, separating the genetic and early maternal effects from the effects of parental rearing ability.

## 2.2. *Within-brood patterns*

Several hypotheses have been proposed to explain why hatching asynchrony is beneficial for the parents (Nilsson 1993; Stenning 1996). However, delayed hatching is generally detrimental for the late hatched young. These offspring often experience a competitive disadvantage (Ostreiher 1997; Price and Ydenberg 1995) and reduced fledging size (Clotfelter *et al.* 2000; Cotton *et al.* 1999). If hatching asynchrony has a reason other than producing competitive differences among offspring, it would be advantageous, not only for the offspring but also for the parents, to compensate for its detrimental effects, given that the physiological condition of parents allows this compensation. In some species, increased investment into later laid eggs has been reported and discussed as a compensation mechanism (Cichoń 1997),

but its effect on nestling growth and fledging size has not been examined in details. **Paper 4** investigates if the preferential maternal nutrient investment (estimated by egg size) in the late laid eggs by Collared Flycatcher females successfully reduced the disadvantage of late hatching young.

Similarly to the nutrient allocation into the eggs, food allocation among nestlings is expected to have strong impact on parental fitness. Maximizing fledging success in a favorable environment requires food distribution based on signals of offspring need (Godfray 1991). However, food limitation, differences among individual nestlings in their quality or survival prospects (e.g. due to hatching asynchrony) and variation in the costs of rearing different young may result in preferential allocation of food by parents (Kilner and Johnstone 1997; Stamps 1990). If signals of nestling quality or need change their meaning with age, parents are expected to adjust their feeding rules to these changes. In **paper 5** I examined food allocation in broods of Collared Flycatchers in two nestling ages. In a multivariate analysis, I investigated the role of sex, size, condition, position and begging intensity.

### 3. Methods

#### 3.1. Study site and species

Four out of the five studies (**paper 1 and 3-5**) were performed on a nestbox-breeding population of Collared Flycatchers (*Ficedula albicollis*) in the Pilis Mountains (47°43'N, 19°01'E), near Szentendre, Hungary. Our plots cover a part of a continuous oak-dominated woodland where about 800 nestboxes have been maintained and regularly monitored since the early 1980's (Török and Tóth 1988). The area has been free of timber harvesting since 1989. The nestboxes are occupied mainly by Collared Flycatchers and in smaller numbers by Great Tits (*Parus major*) and Blue Tits (*Parus caeruleus*). Collared Flycatcher is a small, migratory, insectivorous passerine. Males arrive at the study area in the middle of April and females a few days later. After pair formation females build the nest, and lay and incubate the eggs. The typical clutch size is 5-7 eggs. Incubation usually starts before clutch completion, resulting in a moderately asynchronous hatching ranging from 6.85 to 43.50 hours (mean  $\pm$  S.E. = 27.08  $\pm$  1.32), starting approximately 12 days after clutch completion. After hatching, only females brood the young, but both parents participate in nestling feeding. Nestlings fledge about 14-15 days after hatching.

The Collared Flycatcher has a predominantly monogamous social mating system and in our study area only ca. 6% of the males are socially polygynous (Garamszegi et al. 2004b). However, the rate of extra-pair fertilizations is quite high as ca. 30% of the broods contain extra-pair young (Garamszegi et al. 2004a). Two of the conspicuous white plumage characters of the males have been reported to be important in sexual selection. The forehead patch is important for social and/or extra-pair mate choice (Michl et al. 2002; Qvarnström et al. 2000; Sheldon et al. 1997; Sheldon and Ellegren 1999), but may signal different qualities in different populations. It has been shown to be condition-dependent in a Swedish population (Gustafsson et al. 1995; Qvarnström 1999; Sheldon et al. 1997), but not so in our Hungarian study population (Hegyi et al. 2002). The wing patch is known to be important for extra-pair mate choice in the Swedish population (Sheldon and Ellegren 1999), and it is known to be condition-dependent in our study population (Török et al. 2003), but not in Sweden (Garant et al. 2004).

### *3.2. General field procedures*

After arrival of the birds to the breeding area, we regularly checked the nestboxes for new nests and recorded the laying date of the first egg, clutch size, hatching date, number of hatched and fledged nestlings. Parents were captured in the nest when feeding their young. Morphological variables such as body mass and tarsus length were measured for both parents. For males we also recorded the forehead patch and wing patch size (for the methods see Hegyi et al. 2002 and Török et al. 2003, respectively). Males show delayed plumage maturation, thereby allowing us to distinguish between 1-year-old and older males based on the colour of their wing feathers (Svensson 1992). A drop of blood was collected from the brachial vein of both parents and chicks and stored in SET-buffer or absolute ethanol for later genetic analysis.

### *3.3. Study-specific details*

**Paper 1.** Nestlings were sexed using a PCR-based molecular method (Fridolfsson and Ellegren 1999), that I optimized for our samples. When analyzing brood sex ratios, I used generalized linear models with binomial error and logit link (Crawley 1993; Krackow and Tkadlec 2001). To be able to compare the results with those obtained in a previous study on a Swedish Collared Flycatcher population, the initial model included the same variables as in the study of Ellegren et al (1996), namely male forehead patch size, male size (tarsus length), male and female age, clutch size, and laying date. After the backward deletion of non-

significant terms from the model, two additional variables were also tested. These were female size (tarsus length) and male wing patch size. Since I expected a difference between our study population and the previously studied Swedish population in the role of male forehead patch size in sex ratio adjustment (see introduction), and I wanted to be sure that any observed difference is not the consequence of sex manipulation restricted to the first egg (see paper 2), the sex of the first egg in relation to the paternal forehead patch size was also analysed in some broods. For this, we numbered the eggs during laying, and one day before the expected hatching date we moved them into an incubator, where they hatched in separated compartments. All hatchlings were individually marked and returned to the nest, where they were blood-sampled 9-13 days after hatching.

**Paper 2** is based on a computer simulation. I created a theoretical population with 100 individuals and one normally distributed trait. I assumed that only this trait affected the sex ratio manipulation of this species, and that this manipulation is restricted to the first egg. Accordingly, the sex of the first egg was determined based on the value of the trait, while it was assigned randomly for all eggs laid later in the sequence. Keeping the trait values constant, I repeated the process 500 times. As a consequence, I had 500 „populations” with 100 individuals, in each of which females used the same sex allocation rules. The only difference was generated by the random chromosomal segregation in later laid eggs. To simulate the effect of sample size, I took random samples of 50, 25 and 13 individuals from my original population and proceeded as above. To explore the effect of clutch size on the probability of detecting sex ratio manipulation at the clutch level, I repeated the above procedures twice. First with medium sized (6-egg) clutches, then with large (12-egg) clutches. In all populations, I analysed clutch sex ratios and sex of the first egg in relation to the explanatory trait. I reported the clutch level detectability of sex ratio manipulation and compared it to the detectability in studies using the sex of the first egg as the dependent variable.

**Paper 3.** To investigate the relationship between paternal age and offspring quality, we measured nestling growth and fledging size in Collared Flycatcher broods. A special cross-fostering design allowed us to draw conclusions about the reasons for such differences. Two days after hatching we sequentially cross-fostered complete broods within trios of nests which hatched on the same. Within each trio two of the fathers were adult and one was subadult (1 year old). Thus, three experimental groups were created: subadult male rearing adult offspring (SMAO), adult male rearing subadult offspring (AMSO) and adult male rearing adult offspring (AMAO). Specific comparisons among these groups, enabled us to distinguish

between origin effects (genetic and early maternal effects) and rearing effects (the effect of parental rearing ability). In case of origin effect we expect differences between AMSO and the other two groups, while in case of rearing effects SMAO and the other two groups are expected to differ. To separate between the possible maternal and paternal rearing effects, we recorded feeding activity of the parents 4 days after hatching, using video recorders.

**Paper 4.** We numbered the eggs with a permanent marker on the day of laying. Nutrient investment into the eggs was estimated by the volume of the eggs. One day before the expected hatching date, eggs were moved into an incubator and replaced with dummy eggs. All females accepted these dummy eggs and continued the incubation. The original eggs were hatched in separated compartments of the incubator. We recorded the hatching time and hatching mass of all chicks. They were then individually marked and returned to their original nest. We measured body mass and the length of the primary wing feathers every other day from day0 (=the day of hatching) and day8, respectively. The effect of laying order (hatching asynchrony) on growth and fledging size was analysed using general linear mixed models. By analysing the effect of relative size of the last laid egg on both the growth rate difference and fledging size difference between the last hatching chick and its older siblings, we also investigated whether larger late laid eggs could decrease the disadvantage of the last chick.

**Paper 5.** Food allocation patterns were investigated using videocameras mounted in the nestboxes. Nestlings were individually marked and measured before recordings. Recordings were made at two developmental phases (4-5 days and 10-11 days after hatching). At each nest we made two types of recordings. The first time we videotaped the nests under natural conditions (i.e. with the six own nestlings allowed to move freely), whereas on the following day we controlled for nestling size and position. In this size-position manipulation we replaced the six original nestlings with four foreign offspring so that two were large and two were small. Thus in this forced test we offered two classes of well-distinguishable chicks instead of six nestlings with a continuous size spread, which could make it easier to demonstrate a possible size preference, even if it was weak. The four nestlings were separated from each other by a cross shaped wooden wall, which controlled for the position and physical competition of nestlings. The layout was turned by 90 degrees every 45 minutes during the 3-hour-long period, thus each nestling occupied each part of the nest for one period. This way the effects of size and position could be analysed independently.

## 4. Results and Discussion

### 4.1. Between-brood patterns

Our results on brood sex ratio patterns in the Hungarian population of Collared Flycatchers (*paper 1*) differed at two points from those obtained in a previous study in a Swedish population. First, contrary to the result of Ellegren et al. (1996), we did not find any relationship between brood sex ratio and the forehead patch size of the male. This result is not surprising and can probably be explained by other factors that differ between these two populations. In both populations, forehead patch size seems to be important for female social and/or extra-pair mate choice (Michl et al. 2002; Qvarnström et al. 2000; Sheldon et al. 1997; Sheldon and Ellegren 1999). However, in the Swedish population, male forehead patch size is condition-dependent (Gustafsson et al. 1995; Qvarnström 1999), while no such relationship has been found in the Hungarian population (Hegyi et al. 2002). Thus, the payoff of benefits and costs of brood sex ratio adjustment may differ between these two populations such that only in the Swedish population can females accrue benefits from male nestlings with large forehead patches that are large enough to outweigh the costs<sup>1</sup> of sex ratio manipulation (i.e. in the Swedish population male offspring in male biased broods will be not only more attractive, but also more probably to survive, thus they increase maternal fitness benefit more than in our study population).

The other difference was found in the role of laying date in sex ratio adjustment. Laying date had no effect on brood sex ratios in the Swedish population (Ellegren et al. 1996). In the Hungarian population, however, there was a seasonal shift in brood sex ratios, so that an excess of sons was produced late in the season. Since we analysed secondary brood sex ratios, this pattern could be a consequence of different survival of male and female embryos/nestlings, but this is probably not the case. First, similar patterns were observed in broods without embryo and nestling mortality (Rosivall, Török, Hasselquist & Bensch unpubl. data). Second, a Swedish study did not find any difference between the sexes in environmental sensitivity (Sheldon et al. 1998), though population differences may exist also in this factor. An alternative explanation for the seasonal shift is based on the suggestion that steroid hormones may play a role in avian sex manipulation (Petrie et al. 2001). Michl et al. (2005) found that there is a seasonal shift in maternally transferred hormones, so that egg yolks of later laid Collared Flycatcher clutches contain higher testosterone levels. According

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<sup>1</sup> Exact costs are unknown, because the mechanism of sex ratio adjustment is unknown too. They may involve for example delayed clutch completion or high maternal testosterone levels.



to the hypothesis proposed by Petrie et al. (2001), higher levels of testosterone in the egg yolk should result in male-biased broods, just as observed in the present study. Such a mechanism could be adaptive, if sons for some reason fare better than daughters when produced late in the season. E.g. the need for early fledging (Naef-Daenzer et al. 2001; Verboven and Visser 1998) may result in a preference for the faster growing sex in late broods. Indeed, in another study we found that the body mass of male nestlings increased faster than that of females (Rosivall, Szöllősi, Török & Hasselquist unpubl. results). However, the observed pattern of sex ratio bias related to a change in mean egg yolk testosterone levels could also be non-adaptive, for example if the change in testosterone level is a consequence of changing levels of female aggression (Whittingham and Schwabl 2002), e.g. caused by a change in breeding density over the season. To draw firm conclusions of the laying date effect, however, long-term studies are clearly needed, as between-year differences in seasonal brood sex ratio patterns may occur (Radford and Blakey 2000).

The absence of the seasonal shift in broods sex ratios in the Swedish Collared Flycatcher population can be the result of shorter breeding season, difference in the seasonal decline in food supply, or the low brood-level detectability of sex ratio manipulation if it is restricted to the first egg. The last possibility could also explain the absence of an effect of male attractiveness in the Hungarian population. To exclude this possibility, we also investigated the effect of paternal attractiveness (estimated by the forehead patch size) on the sex of the first egg. We found that it had no effect. Though the sample size was low ( $n=13$ ), according to the results of my simulation study (*paper 2*, see below), at this sample size we have already 100% chance to detect sex manipulation (at least with the parameters used). Further support for our negative result comes from the fact that when I repeated the analysis using data on the first egg from two different years and two different studies (resulting in a sample size of  $n=40$ ) the result was similar (Rosivall et al. unpubl. data). Thus, we can conclude that the two populations differ in the role of parental attractiveness in brood sex ratio patterns. Unfortunately, we have no information on the sex of the first egg in the Swedish population and the broods in which we have this information in the Hungarian population hatched within a few days, so we cannot draw conclusions about the extent of sex manipulation (first egg or full brood). To explore the probability that existing sex manipulation in the first egg was undetected in the Swedish population and to provide a possible explanation for the contradictory results in the literature, I performed a simulation study (see methods).

My results (*paper 2*) clearly show that contradictory results between populations might be the consequence of the low detectability of sex ratio adjustment on the brood level if sex ratio

adjustment is restricted to the first egg. In species laying six-egg clutches (i.e. the median clutch size in our Collared Flycatcher population), by sampling 50 broods one would have 47.4% chance not to detect sex ratio manipulation. Even when sample size is twice as large, only 84.4% of the „studies” would find significant effect of the examined trait on clutch sex ratios. The sample size was 57 broods in our and 79 broods in the Swedish population. In species with large clutch sizes (12 eggs), the situation is even worse. Even with large samples (n=100), significant relationship between the trait and clutch sex ratio would be detected in only 54.2% of the cases, even though there was sex ratio manipulation in all cases. However, when I analysed the effect of paternal attractiveness specifically on the sex of the first egg, the results were significant in all 500 „populations” independent of sample size. So, when only the sex of the first egg is manipulated in relation to patch size, this can be easily detected with even very small sample sizes (n=13) if we have information on the sex of the first egg. However, one would have a large chance to miss this effect if data only on clutch sex ratios is available. On the contrary, if sex ratio adjustment has a mechanism which results in a brood level sex manipulation (e.g. segregation distortion), studies on the brood level are more appropriate. However, we have no evidence for any of the proposed mechanisms so far, thus sex ratio studies at present should either combine the brood-level and first-egg approach, or increase much the sample sizes. The latter seems to be quite hopeless (especially in the case of large clutches, which require even larger sample sizes), since samples larger than 100 clutches are rare and usually yielded from multiple years, which introduce an additional noise (i.e. due to environmental differences, sex ratio adjustment might be adaptive in one year but not in another).

The results of *paper 3* revealed a significant year type x male of origin effect on nestling performance. In two out of the three study years (2002 and 2004), the young from the broods of subadult males developed slower and fledged with smaller mass and skeletal size. In the third year (2003), there was no such effect. The age of the rearing male did not affect offspring performance. Feeding rates of subadult and adult males did not differ, while females mated to adult males fed the chicks more frequently than females mated to subadult males. This feeding pattern indicates that the nonsignificant rearing effect is not due to an additional feeding effort by the mates of subadult males as a compensation for the poor paternal performance, or a compensatory feeding by any member of the pair in response to the low quality of the territory. It has to be noted that we cannot rule out male age differences in the quality of prey brought to nestlings. However, to explain why the feeding pattern of females lead to no rearing effect on nestling development, would require higher rather than lower

quality food brought by subadult males in comparison to adults. We therefore conclude that there is no evidence for worse parental ability or lower territory quality of subadult males in our population. The origin effect on nestling growth and fledging size could be explained by either early maternal effects or parental genetic quality (Eising et al. 2001; Schwabl 1996; Sheldon et al. 1997). Although some of the measured female traits differed among treatment groups, the origin effect was probably not the result of female quality differences, since inclusion of those traits to the analysis did not alter our main results. Furthermore, incubation behaviour of females, which may have long-term effects on offspring performance (Gorman and Nager 2004), did not differ, as investigated by the analysis of incubation length, hatching success, and the degree of hatching asynchrony. Preferential maternal investment into the broods of adult males may also not explain the faster nestling growth in this group, because there was no effect of male age on egg macronutrients, as reflected by egg size (Hargitai et al. 2005), and egg testosterone content, which was reported to promote postembryonic development in passerine birds (Eising et al. 2001; Schwabl 1996), was higher in the broods of subadult males (Michl et al. 2005). Another alternative explanation to the male age effect on nestling growth would be a year-specific sex ratio bias in relation to paternal age, accompanied by sex-specific growth trajectories, but brood sex ratio was unrelated to male age in 2002 (paper 1), i.e. in one of the years when male age affected nestling development. Thus, paternal age specific growth most probably reflects a paternal genetic quality effect, subadult males being on average of lower quality. As mass growth was in general steeper and levelled off earlier in 2003 than in the other two years, we can consider 2003 as a „good year” and the year type x male of origin interaction as environment x genetic quality interaction. The fact that the growth disadvantage in broods of subadult males was present only in less favourable years may be due to the increased importance of genetic quality in limiting environmental conditions (David et al. 2000). The higher egg testosterone levels in those broods (Michl et al. 2005) may also have contributed to the lack of growth difference according to „male of origin” in the good year. Slower growth and small body size in less favourable years has probably severe impact on the survival prospect and future reproduction of the nestlings (Birkhead et al. 1999; Blount et al. 2003; Lindén et al. 1992; Merilä and Wiggins 1997; Morgan and Metcalfe 2001; Stjernman et al. 2004) thus higher testosterone and carotenoid content may enhance the reproductive value of these nestlings, even if they can not completely eliminate the effect of low quality.

#### 4.2. *Within-brood patterns*

Similarly to earlier results on the species (Cichoń 1997), we found that Collared Flycatcher females increased the egg size throughout the laying sequence (*paper 4*). As a consequence, chicks from the last laid egg hatched with significantly larger body mass. However, they hatched on average 27.08 hours later than their siblings, which resulted in a slower body mass growth, and shorter feathers on day 14 (just before fledging), which are often reported disadvantages of late hatching young (Johnson et al. 2003; Nilsson and Gårdmark 2001; Nilsson and Svensson 1996). Both of these disadvantages increased with increasing hatching asynchrony, but were partially counterbalanced by the larger egg size (i.e. the larger the last egg the smaller the disadvantage). The growth of the primaries was not related to laying order, probably because synchronized fledging is important for the survival of the nestlings, and therefore, they allocate more energy into growing their wing feathers at the cost of lower body mass increase (Nilsson and Gårdmark 2001; Nilsson and Svensson 1996). Still, the relative size of the last egg tended to correlate positively with the relative feather growth rate of the last chicks. Body mass on day 14 was not affected by laying order either, which might be an effect of the logistic nature of the growth curve (i.e. older siblings finished their growth a few days before fledging, thus the last chicks had time to catch up), or the joint effect of logistic mass growth and larger last eggs. The fact that independent of the relative egg size most of the last chicks reached their maximum body mass on day 14 at the latest, might indicate that larger egg size is not needed to reach the same size as their older siblings. However, it is hard to draw conclusions on this aspect, because we do not know how they would have grown if they had hatched from smaller eggs. Reduced growth or bad body condition early in life has been reported to have negative impact, for example, on immune responsiveness, resistance to parasite infection, adult condition, time of sexual maturation and long-term survival in vertebrates, even if young could catch up in terms of size (Birkhead et al. 1999; Blount et al. 2003; Morgan and Metcalfe 2001; Stjernman et al. 2004). Late or asynchronous fledging due to shorter feathers, may also be disadvantageous. For instance, parents preferentially fed fledged chicks if fledglings and nestlings were begging simultaneously in the great tit *Parus major* (Lemel 1989). Thus, we argue that even the partial compensation for the adverse effects of hatching asynchrony by laying larger final eggs probably increased the survival prospects of the last chicks and thus the fitness of the parents. The compensatory maternal investment pattern also indicates that, at least in this species, the goal of hatching asynchrony is not to create size hierarchy among nestlings as suggested by some of the hypotheses (e.g. the brood reduction hypothesis and sibling rivalry hypothesis;

Forbes et al. 1997; Mock and Ploger 1987; Ricklefs 1965). Our data rather suggest that the size hierarchy is a cost of hatching asynchrony, which is adaptive for other reasons than sibling size asymmetry (e.g. reduced nest failure or better egg viability; Arnold et al. 1987; Hussell 1972; Veiga 1992).

In the light of the above results, one would expect that Collared Flycatcher parents preferentially allocate food to the small nestlings, at least when they are young, because this could further help late hatched chicks to catch up with their nestmates (Stamps et al. 1985), thereby increasing the fitness of the parents. However, just before fledging, smaller size and worse condition may not only indicate later hatching, but also slower development due to lower genetic quality (Sheldon et al. 1997) and/or infections (Fair et al. 1999). Therefore, preference for chicks in poor condition might not be advantageous even if food is abundant. This age dependent role of nestling size and condition is not supported by our results (*paper 5*), because nestling size and condition did not affect food distribution of male and female parents in any of the two nestling age categories (4-5 and 10-11 day old chicks). Nor did the sex of the offspring affect food allocation. Sex preference can be expected when the benefit of rearing male and female offspring differs (Gowaty and Droge 1990; Stamps 1990). The lack of general feeding sex preference is not surprising, since in species with sexually size-monomorphic fledglings, offspring sex-dependent survival was found only in case of food shortage (Martins 2004). Furthermore, in a Swedish population of the Collared Flycatcher such difference was not found even in enlarged broods (Sheldon et al. 1998). However, preference for a certain sex in relation to male quality/attractiveness could still be advantageous for the parents, because the reproductive performance of males is strongly affected by their attractiveness (Sheldon et al. 1997; Sheldon and Ellegren 1999). The lack of this preference may suggest that in this species either nestlings of the two sexes are not distinguishable or sex-specific differences in their future reproductive values are too small to favor sex-biased food allocation. Indeed, only two factors, begging intensity and the position of the nestlings affected food allocation. Begging intensity has been suggested by several studies to be a reliable signal of offspring need (Delman 1998; Godfray 1991; Hussell 1990; Kilner and Johnstone 1997; Kölliker et al. 1998; Leonard and Horn 2001; Lotem 1998; Price et al. 1996; Price and Ydenberg 1995; Roulin et al. 2000; Saino et al. 2000). Our results show that in Collared Flycatchers, both parents preferred to allocate food to the more intensively begging and presumably needier offspring in both age categories. Nestling position in the nest has also been shown to affect food allocation in other species (Gottlander 1987; Kilner 1995; Kölliker et al. 1998; Leonard and Horn 1996; Porkert and Špinka 2004). In Collared

Flycatchers, male parents did not show clear position preference with young nestlings, but they did have preferred positions with old nestlings. Female parents showed position preference in both age categories. When feeding old nestlings, the preferred positions of male and female parents differed, still there were positions in which nestlings had larger chance to obtain food than the average of the brood. This might be the consequence of the overlapping position preference of the parents. Anyway, this raises the possibility that nestlings compete for those positions, though the level of competition is probably moderate due to the difference between the preferred positions of the parents. Similarly to our results, position and begging behaviour of nestlings affected food allocation in many species (Gottlander 1987; Kölliker et al. 1998; Leonard and Horn 1996; Porkert and Špinka 2004; Whittingham et al. 2003). However, it is hard to determine the relative importance of these two factors. The relative role of active parental decision versus nestling competition in determining access to food may depend on, for example, food abundance or the ability of parents to cover the need of their chicks. It is supported by the results of Porkert and Špinka (2004), who found more pronounced position preference in larger broods (i.e. in broods where we might expect stronger competition). In the present study, begging intensity seems to have a more important role than position, since univariate within-brood analyses (our unpublished results) more often gave significant results for begging intensity than for nestling position.

## **5. Final remarks and perspectives**

The results presented in this thesis (*paper 3 and 4*) together with other data on parental investment from our study population that were not included in the thesis (Hargitai et al. 2005; Michl et al. 2005; Török, Hargitai, Hegyi, Matus, Michl, Péczely, Rosivall and Tóth unpubl. manuscript), suggest that Collared Flycatchers follow a compensatory strategy, rather than provide further supplement to the good quality young. Preferential investment into good quality young was suggested by two hypotheses. The first argue that parents should increase their investment into the young if the expected fitness return is high. This hypothesis is often incorrectly referred to as the differential allocation hypothesis (Limbourg et al. 2004; Uller et al. 2005). The second, Nancy Burley's differential allocation hypothesis (Burley 1988) says that when the mate of the focal individual is of better quality (or more attractive to the opposite sex) than the focal animal itself, then the latter should invest more in reproduction, thereby enhancing its mate-acquisition or mate-retention ability and reducing the partner's

workload. As a consequence, Burley argued, the mates of attractive individuals will on average invest more into reproduction than mates of unattractive individuals. This is, however, true only when there is no assortative mating. Furthermore, in species where early parental investments (nest building, egg laying and incubation) are all the exclusive task of the female, like in the Collared Flycatcher, this hypothesis seems less applicable (i.e. the extra investment may indicate that the individual will take larger share of feedings later, but will not alter the actual workload of the partner). Finally, this hypothesis can not explain within-brood preferences. The applicability of the other hypothesis, concerning the probability and the amount of the fitness return, is also limited. Extra investment into the good quality offspring is beneficial only if it further improves the survival or reproductive prospects of the young, so that the resulting fitness benefit is larger than the loss by the withdrawal of investment from other nestlings (or future broods). The same amount of compensatory investment, on the other hand, is more likely to improve the survival and reproductive prospects of the low quality young.

It is apparent that a compensatory investment does not necessarily eliminate completely the disadvantage of handicapped young. For example, larger late laid eggs reduced the growth and size disadvantages of the late hatching young but these were still apparent. This raises the question why females did not invest more heavily in those eggs. The absence of the relationship between laying order and egg size in low quality years (Hargitai et al. 2005) may indicate that the incomplete compensation is explained by physiological constraints, because the poor development of young in asynchronously hatching enlarged broods suggests that maternal compensation would have been adaptive even in low quality years (Szöllösi, Rosivall, Török unpubl. manuscript). The long-term effects of the observed limited compensation on survival and reproductive success has to be investigated in the future.

Interestingly, the size of the nestlings, which is strongly determined by the hatching asynchrony, played no role in the food allocation of the parents, though females preferred the late hatching young through preferential nutrient investment into the egg. Since egg size could not completely eliminate the disadvantage of the last hatched young, preference for those chicks during food allocation could have further helped them to catch up with their nestmates. The absence of such a pattern may indicate that parents are not able to evaluate size differences among nestlings, or, as Kölliker et al. (1998) suggested, it would perhaps be too time-consuming to evaluate size, therefore parents use more simple cues. It is also possible that begging intensity, which was the major determinant of food allocation in our study, reliably estimates both short-term need (hunger) and long-term need (backlog in

development) (Price et al. 1996), therefore it was not necessary for the parents to use size as an additional cue. However, the food was abundant in the study years. In case of food shortage, both late hatching and older young are expected to be malnourished and to show intensive begging signals. In this case larger nestlings, which more probably return fitness benefit to the parents, should be favored, because an extra investment can increase their survival prospects, and as a consequence, size might be used as a cue in food allocation. Also, nestling competition for positions preferred by the parents is expected to be stronger under such circumstances (Kilner 1995; Kölliker et al. 1998), resulting in an indirect preference for the larger nestlings. Further studies are needed to investigate such an environment-dependent change in the food allocation rule of bird parents.

Sex ratio adjustment is hard to fit to the „compensatory-supplementary” framework mentioned above, but considering the sex related costs and benefits of rearing an offspring, it can also be an efficient tool to improve maternal fitness. Experimental manipulations in different bird species revealed that brood sex ratios can be substantially altered by the females (Komdeur et al. 1997; Nager et al. 1999; Pike 2005; Sheldon et al. 1999), and the observed departures from unity were in the expected adaptive direction (West and Sheldon 2002). However, similarly to our study, contradictory results were found between populations living in different environments (see table 1). To better understand the reasons for that, further comparative studies are needed. Furthermore, long-term studies with large sample sizes should be used to investigate the changes between years within populations. The investigation of the underlying mechanism of sex ratio adjustment would be of exceptionally high importance, since at present we do not even know if studies should focus on the sex of the first egg or on brood sex ratios.

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## 8. Published papers and manuscripts included in the thesis

- paper 1: Rosivall B**, Török J, Hasselquist D, Bensch S (2004) Brood sex ratio adjustment in collared flycatchers (*Ficedula albicollis*): results differ between populations. *Behav Ecol Sociobiol* 56:346-351
- paper 2: Rosivall B** (under review) The importance of the first egg in avian sex ratio studies. *Biol Lett*
- paper 3:** Hegyi G, **Rosivall B**, Török J (in press) Paternal age and offspring growth: separating the intrinsic quality of young from rearing effects. *Behav Ecol Sociobiol*
- paper 4: Rosivall B**, Szöllösi E, Török J (2005) Maternal compensation for hatching asynchrony in the Collared Flycatcher *Ficedula albicollis*. *J Avian Biol* 36:531-537
- paper 5: Rosivall B**, Török J, Szöllösi E (2005) Food allocation in collared flycatcher (*Ficedula albicollis*) broods: Do rules change with the age of nestlings? *Auk* 122:1112-1122

## 9. Other publications

- Hegyi G, Török J, Tóth L, Garamszegi LZs, **Rosivall B** (in press) Rapid temporal change in expression and age-related information content of a sexually selected trait. *J Evol Biol*
- Hargitai R, Török J, Tóth L, Hegyi G, **Rosivall B**, Szigeti B, Szöllösi E (2005) Effects of environmental conditions and paternal quality on the inter- and intraclutch egg size variation in the Collared Flycatcher (*Ficedula albicollis*). *Auk* 122:509-522
- Herényi M, Török J, Garamszegi LZs, Hargitai R, Hegyi G, Michl G, **Rosivall B**, Szigeti B, Szöllösi E (2004). Másodlagos nemi jellegek és utódszám kapcsolata a hím örvös légykapónál. {in Hungarian} *Állatt Közl* 89:31-41
- Török J, Garamszegi LZs, Hegyi G, Hettyey A, Michl G, **Rosivall B** (2003): Szigalizáció és szexuális szelekció. {in Hungarian} *Magyar Tud* (8):976-983

# Paper 1





Balázs Rosivall · János Török · Dennis Hasselquist · Staffan Bensch

## Brood sex ratio adjustment in collared flycatchers (*Ficedula albicollis*): results differ between populations

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**Abstract** Recently, a number of studies have found adaptive brood sex ratio (BSR) manipulation in birds. The reason for such manipulations is thought to be the different reproductive value of male and female nestlings. Several studies have found that parental quality and food supply can affect BSR, however results are sometimes inconsistent between species and populations. We investigated BSR patterns in a Hungarian population of Collared Flycatchers (*Ficedula albicollis*) and compared the results with those obtained in a previous study of the same species in Sweden. We found two significant differences. First, the male forehead patch size, a heritable, sexually selected trait, affected the brood sex ratio in the Swedish population, but not in our Hungarian study population. This difference might be a consequence of the different information content of the forehead patch size in the two populations. Second, a seasonal shift in BSR (more sons late in the season) was observed in the Hungarian, but not in the Swedish population.

**Keywords** *Ficedula albicollis* · Geographic variation · Male attractiveness · Seasonal patterns · Sex allocation

### Introduction

In the last decade an increasing number of studies have found that manipulation of brood sex ratio is an existing phenomenon in birds and that it may be adaptive for the parents (Bensch 1999; Hasselquist and Kempenaers 2002; Komdeur and Pen 2002). The adaptive value of this behaviour may be the potential for higher future reproductive success of nestlings of one sex given the current circumstances. For example, heritable parental quality/attractiveness may affect the future reproductive success of males more than that of females, if females show preference for high quality or attractive males. Hence, male-biased broods increase the fitness of females mated to preferred males more than female-biased or unbiased broods (Burley 1981; Ellegren et al. 1996; Sheldon et al. 1999).

Laying date has also been reported to affect the future reproductive success (FRS) differently for male and female nestlings. For example if only one sex starts to reproduce as yearlings, laying date has a higher impact on the FRS of this sex. In the crimson rosella (*Platycercus elegans*), females often breed as yearlings, while males do not, and it is therefore advantageous to produce female-biased broods early in the season (Krebs et al. 2002). Laying date effects, however, can also be the consequence of a seasonal change in food supply, because environmental sensitivity of the two genders may be different, especially in the case of sexually dimorphic species (Nishiumi 1998; Teather and Weatherhead 1989; Westerdahl et al. 2000). Thus, in several species brood sex ratio (BSR) adjustment has been observed in relation to laying date or food supply (see Table 1 in Hasselquist and Kempenaers 2002).

Brood sex ratio manipulation in birds is still a controversial issue, because results are often contradictory. In some cases, such contradictory results may be the consequence of different measurements of the same variable (e.g. male quality: Leech et al. 2001; Sheldon et al. 1999), while in other cases they may reveal actual differences between populations [in blue tits (*Parus caeruleus*):

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Leech et al. 2001; Svensson and Nilsson 1996; and in great tits (*Parus major*): Kölliker et al. 1999, Radford and Blakey 2000]. Comparable studies on different populations are therefore important, but so far scarce.

The mechanism of the sex ratio adjustment can also be responsible for differences between populations. Though this mechanism is not yet known (Krackow 1999; Oddie 1998), it may affect the probability of detecting brood sex ratio manipulations. This is because post-ovulatory mechanisms, such as the selective reabsorption of an ovulated egg, could result in a delayed completion of the clutch, resulting in an increased predation risk and possibly reduced egg viability (Emlen 1997). Hence, if sex is manipulated after ovulation, species with multiple-egg clutches should restrict sex ratio manipulation to the first egg of the clutch. If so, the probability of detecting sex ratio adjustment at the brood level would decrease and, as a consequence, the probability of yielding contradictory results should increase. This potentially confounding effect can be evaluated by investigating the sex of the first laid egg.

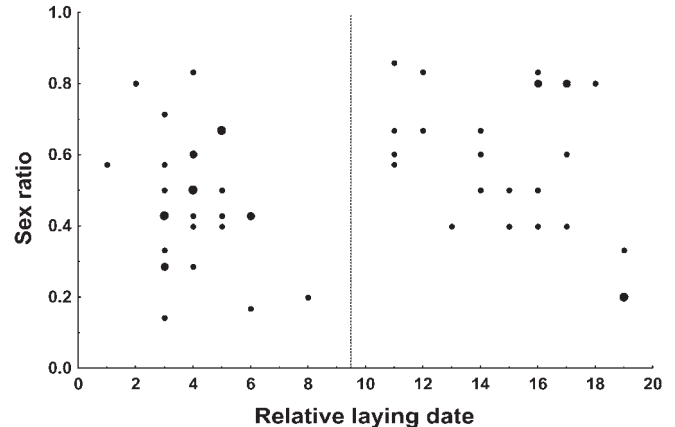
In the present study, we investigated brood sex ratios in a Hungarian population of Collared Flycatchers (*Ficedula albicollis*). In this species, we can expect adaptive modification of brood sex ratios, because two secondary sexual characters (i.e. the forehead patch and wing patch size of the males) have been shown to be heritable (Hegyi et al. 2002; Qvarnström 1999; Török et al. 2003) and play a role in social and/or extra-pair mate choice (Michl et al. 2002; Qvarnström et al. 2000; Sheldon et al. 1997; Sheldon and Ellegren 1999). Therefore, we predict that females mated to males with large patches will produce male-biased broods, an effect which has already been observed in relation to the forehead patch size in a Swedish population of this species (Ellegren et al. 1996). We therefore investigated the forehead and wing patch size, as well as a number of other traits (male size, female size, clutch size, and laying date), to compare BSR patterns between two populations of the same species. Because the mechanism of sex ratio adjustment is not yet known, we also investigated whether sex ratio adjustment is detectable only on the first egg, which is predicted in the case of post-ovulation mechanisms, or at the brood level (which is expected if sex ratio adjustment occurs before ovulation).

## Methods

### Study site and species

This study was conducted in an artificial nest box plot in the Pilis Mountains, Hungary, in 2002. The Collared Flycatcher is a small hole-nesting, long-distance migratory passerine bird. It has a predominantly monogamous social mating system and in our study area only ca. 8% of the males are socially polygynous (Török et al. 1999). However, the rate of extra-pair fertilizations is quite high as ca. 30% of the broods contain extra-pair young (Garamszegi et al. 2004).

The breeding season usually starts in mid-April, but its length is variable dependent on the arrival of the birds. In 2002, birds arrived in two separated migratory waves (Fig. 1.) and, as a consequence,



**Fig. 1** Secondary sex ratio of Collared Flycatcher (*Ficedula albicollis*) broods early and late in the season. Dot size indicates the number of cases (1, 2, 3)

the breeding season was long (the range of start of egg laying was 19 days for the broods included in the present study). Females usually lay 5–7 eggs.

Two of the conspicuous white plumage characters of the males have been reported to be important in sexual selection. The forehead patch is important for social and/or extra-pair mate choice (Michl et al. 2002; Qvarnström et al. 2000; Sheldon et al. 1997; Sheldon and Ellegren 1999), but may signal different qualities in different populations. It has been shown to be condition-dependent in a Swedish population (Gustafsson et al. 1995; Qvarnström 1999), but not so in our Hungarian study population (Hegyi et al. 2002). The wing patch is known to be important for extra-pair mate choice in the Swedish population (Sheldon and Ellegren 1999), and it is known to be condition-dependent in our study population (Török et al. 2003).

### Sex determination

Blood samples were collected from 9 to 13-day-old nestlings. Parents were captured on the same day as their young. Some of the adult samples were used as controls during molecular sexings. These adult samples were always correctly sexed ( $n=32$ ). PCR reactions were performed in 10- $\mu$ l volumes on a 9700 Thermal Cycler (Applied Biosystem) including 25 ng DNA, 0.5 units of Taq DNA polymerase, 0.4  $\mu$ M of each primer [2550F and 2718R (Fridolfsson and Ellegren 1999)], 1 $\times$ PCR buffer, 0.125 mM of each nucleotide and 3.0 mM  $MgCl_2$ . Our thermal profile differed from the original protocol (Fridolfsson and Ellegren 1999) in that we used 10 $^\circ$ C and 5 $^\circ$ C lower annealing temperatures for the “touch down” and the following cycles respectively. PCR products were run in 2% agarose gels, pre-stained with ethidium-bromide, and detected in a FluorImager (Vistra).

In some of the samples, DNA was partially degraded, preventing us from sexing these offspring with the previous protocol. In these cases, we used a special asymmetric nested PCR protocol. The tests were performed separately for the male and the female band. For the male band, we used a PCR with the 3007 and 3112 primers (Ellegren and Fridolfsson 1997) and a thermal profile starting with ten “touch down” cycles from 50 to 40 $^\circ$ C annealing temperature with 1 $^\circ$ C decrease in each cycle followed by ten cycles at 45 $^\circ$ C annealing temperature. One  $\mu$ l of this reaction was used in a second PCR with the 3007 primer together with a new primer, FalbZ on the CHD-Z intron (5'-TACAAAGATTTTGTATCTTA-3'). This time, the touch down thermal profile started at an annealing temperature of 55 $^\circ$ C, followed by ten cycles at 50 $^\circ$ C. Otherwise the conditions were the same as for the 2550F and 2718R primers. For the female band, we used the same thermal profiles, with the 2987 and 3112 primers in the first, and the 2987

and FalbW (5'-TTTACTTACATAACTCCTTA-3') primers in the second PCR.

#### Analysis of sex of the first egg

In 21 nests, eggs were numbered consecutively during egg laying. One day before hatching, we placed the eggs into an incubator (PL Machine SK75) and used dummy eggs to temporarily replace the clutch in the nest box. Nestlings were hatched at 37.2°C and 70–80% humidity. Hatching success was 100% for fertile eggs. After hatching, nestlings were marked individually with permanent ink on their stomach and delivered back to their nests immediately. Color marking was randomized in relation to hatching order. Nestlings were sampled for sexing 12 days after hatching. Because of infertile eggs, degraded samples and widowed females, we have data on the size of the paternal forehead patch and the sex of first egg in only 13 broods.

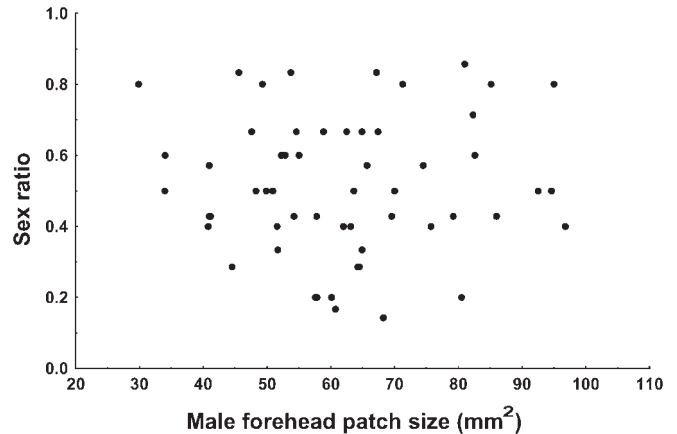
#### Statistical analysis

Throughout this study we used generalized linear models with binomial errors and logit link (Crawley 1993). To be able to compare the results with those obtained in a previous study on a Swedish population of Collared Flycatchers, the initial model of secondary BSR patterns included the same variables as in the study by Ellegren et al. (1996). After including the main effects, a step-wise backward deletion of factors was performed. Interactions were not included in the initial model, as including more than  $n/3$  ( $n$  = sample size) parameter is not recommended (Crawley 1993), but were instead tested after the first model simplification. Finally, we also tested two variables that were not included in the study by Ellegren et al. (1996), i.e. female body size and male wing patch size.

The variables included in the initial model were: the size of the male forehead patch, male body size (tarsus length), male age (subadult or adult: Svensson 1992), female minimum age (females not ringed as chicks were treated as 1 year old in the first year they were captured), clutch size and laying date. In 2002, birds arrived in two migratory waves resulting in a non-normal distribution of laying dates, we therefore assigned each brood to one of two laying date groups, i.e. either an early or a late brood (Fig. 1).

To the minimal model (model including the significant terms only) of the previous variables we added two new variables: female size (tarsus length) and the size of the male's wing patch. As the size of the male wing patch increases significantly from subadult to adult, the age and age  $\times$  wing patch interaction was also included in this model.

In cases where males were socially polygynous, we included only one nest from each male in the analyses (in four cases out of the five it was the primary brood of the male). In ten nests we failed to sex one nestling, because DNA was degraded. These nests were included in the analysis of secondary BSR, as we have no reason to believe that DNA degradation would occur non-randomly in respect to offspring sex. Moreover, exclusion of these nests did not change any of the results. For all nests ( $n=57$ ), we used the number of sexed nestlings when calculating the sex ratios. We used the SAS 8.02 GENMOD module (SAS 1990) for the statistical analyses. Because all generalized linear models of brood sex ratios had a scale parameter that was less than 1.0, and thus the samples were not over-dispersed, we tested the significance of the parameters using  $\chi^2$ -values. When we analyzed the sex of the first egg, the dispersion parameter was larger than 1.0 and we tested significance of parameters with  $F$ -tests (Crawley 1993; Krackow and Tkadlec 2001; the dscale option was used, SAS 1990). For calculating the 95% confidence limit of the deviance ratio we used a jackknife procedure (Sokal and Rohlf 1981).



**Fig. 2** Secondary sex ratio of Collared Flycatcher broods in relation to paternal forehead patch size

**Table 1** Effect of parental traits and laying date on secondary sex ratios in Collared Flycatchers (*Ficedula albicollis*) with parameter estimates and likelihood-ratio statistics from single-variate generalized linear models

Variable	Sample size	Estimate	$\chi^2$	$P$
Laying date	57	-0.4510	4.11	0.04
Male age	57	0.3237	1.97	0.16
Male tarsus	57	-0.0196	0.50	0.48
Forehead patch size	57	0.0006	0.01	0.93
Male wing patch size	54	-0.0003	0.18	0.67
Clutch size	57	-0.1310	0.70	0.40
Female age	56	-0.0883	1.00	0.32
Female tarsus	57	0.0317	2.62	0.11

## Results

In the initial model we included all the variables that were included in a previous study by Ellegren et al. (1996) on a Swedish population of Collared Flycatchers. After removing the non-significant factors, the single significant variable that remained was laying date ( $\chi^2=4.11$ ,  $df=1$ ,  $P=0.043$ ). The secondary sex ratio showed a shift to male-biased clutches late in the season (Fig. 1). Contrary to the results in the Swedish population, the brood sex ratio was not affected by male forehead patch size ( $\chi^2=0.01$ ,  $df=1$ ,  $P=0.933$ , deviance ratio = 0.0001, 95% CI =  $\pm 0.0012$ ; Fig. 2). Moreover, the brood sex ratio was not related to male body size, age, or wing patch size, nor to clutch size or female body size (for the results of single-variate analyses see Table 1).

Post-ovulation mechanisms of sex ratio adjustment predict that females laying multiple-egg clutches may restrict the sex ratio manipulation to the first egg of a clutch (Emlen 1997) and would reduce the probability of detecting sex ratio manipulation at the brood level. However, we found no effect of the forehead patch size of the father, i.e. the character found to be related to brood sex ratios in a previous study (Ellegren et al. 1996), on the sex of the first laid egg ( $F_{1,11}=0.70$ ,  $P=0.42$ ). Thus, even

when we assumed a sex manipulation mechanism that mainly affects the first laid egg, we found no evidence for an offspring sex-bias related to the forehead patch size of the father.

## Discussion

Our study revealed that the brood sex ratio in the Hungarian population of Collared Flycatchers was affected by different factors than in a Swedish population of the same species (Ellegren et al. 1996). There are so far very few species in which brood sex ratio patterns have been investigated in different populations. However, in some species inconsistencies between populations have been found (e.g. in great tit: Kölliker et al. 1999; Lessells et al. 1996; Oddie and Reim 2002; Radford and Blakey 2000). In blue tits, inconsistent BSR patterns in relation to male appearance quality may be explained by the fact that different measurements of male quality was used. In a Swedish population, the UV coloration of male blue tits was used as an indicator of male quality and it was found to affect the brood sex ratio (Sheldon et al. 1999), whereas when male size was used as an indicator of quality in an English population, it did not affect the brood sex ratio (Leech et al. 2001). However, when the same factor, i.e. male survival, was investigated in two different blue tit population there was no consistent effect on brood sex ratios (Leech et al. 2001; Svensson and Nilsson 1996). Because so few studies of BSR between populations or closely related species have been conducted, it is hard to draw any conclusions. So far, however, data suggest that BSR patterns may differ between populations of the same species. This has been used as an argument for the lack of BSR manipulation in birds, and that observed patterns are rather statistical artifacts than true biological effects (Krackow 1999). However, given the recent evidence for the occurrence of adaptive BSR manipulation in birds (Badyaev et al. 2002, 2003; Komdeur 1998; Sheldon et al. 1999), the other possibility is that BSR manipulation is a flexible mechanism that is affected by many different social and environmental factors and therefore prone to differ between populations and species.

One important difference between the studied populations of Collared Flycatchers is that, contrary to the result found in a Swedish population (Ellegren et al. 1996), in Hungary we did not find any relationship between brood sex ratio and the forehead patch size of the male, and this was true also when restricting the analysis to the sex of the first egg. In the analysis of the effect of forehead patch size on BSR, the deviance ratio (corresponding to  $r^2$ ) published by Ellegren et al. (1996) was 0.083, which is far outside the 95% confidence interval of our deviance ratio ( $0.0001 \pm 0.0012$ ). Hence the probability of obtaining these different patterns between the two population by chance is very low. Instead this between-population difference may be explained by other factors that differ between these two populations. In both popu-

lations, forehead patch size seems to be important for female social and/or extra-pair mate choice (Michl et al. 2002; Qvarnström et al. 2000; Sheldon et al. 1997; Sheldon and Ellegren 1999). However, in the Swedish population, male forehead patch size is condition-dependent (Gustafsson et al. 1995; Qvarnström 1999), while there is no such relationship in the Hungarian population (Hegyí et al. 2002). Thus, the payoff of benefits and costs of BSR adjustment may differ between these two populations such that only in the Swedish population can females accrue benefits from males with large forehead patches that are large enough to outweigh the costs of sex ratio manipulation.

In the Hungarian population, we found a significant relationship between laying date and BSR, so that an excess of sons was produced late in the season. If sex differences in nestling mortality rates are related to the food resources, either seasonal change in food supply (laying date effect) or seasonal changes in parental quality (i.e. low quality individuals arrive later: Hasselquist 1998; Møller et al. 2003) may result in a seasonal change of the secondary brood sex ratios in a way that nestlings of the less sensitive sex will be over-represented in late broods. This pattern can be the result of an adaptive primary sex ratio adjustment or sex-biased nestling mortality. However, a study of Swedish Collared Flycatchers does not support this idea, as males and females did not differ in environmental sensitivity (Sheldon et al. 1998). On the other hand, population differences may exist also in this factor, and given the quite large number of broods with some embryo or nestling mortality (42% in our dataset) further investigations are needed. Another explanation for the seasonal shift in BSR is based on the suggestion that steroid hormones may play a role in avian sex determination (Petrie et al. 2001). We have found that there is a seasonal shift in maternally transferred hormones, so that egg yolk of later laid clutches contain higher testosterone levels (Michl et al., unpublished data). According to the hypothesis proposed by Petrie et al. (2001), higher levels of testosterone in the egg yolk should result in male-biased broods, just as observed in the present study. Such a mechanism could be adaptive, if sons for some reason fare better when produced late in the season, but we have no direct evidence for this. However, the observed pattern of sex ratio bias related to a change in mean egg yolk testosterone levels could be non-adaptive, if testosterone adjustment is to induce rapid nestling growth in late hatched broods (Schwabl 1996), or if it is a consequence of changing levels of female aggression (Whittingham and Schwabl 2002), e.g. caused by a change in breeding density over the season. To draw firm conclusions of the laying date effect, however, long-term studies are clearly needed, as between year differences in seasonal BSR patterns may occur (Radford and Blakey 2000).

In the present study, we found a different pattern of BSR manipulation compared to a Swedish population of Collared Flycatchers. There was no relationship between BSR and male forehead patch size in our Hungarian study population whereas such a relationship was found in the

Swedish population. Instead, in Hungary we found that broods became more male-biased later in the season, a pattern that may have both adaptive and non-adaptive explanations. To be able to investigate the occurrence of and circumstances for adaptive brood sex ratio manipulation in birds, more studies of avian BSR patterns between years and populations, of the same species or closely related species, are clearly needed.

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# Paper 2





# The importance of the first egg in avian sex ratio studies

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**In sex ratio studies results often differ between species and between populations within species. Some researchers argued that positive results are simply statistical artefacts and sex ratio adjustment does not exist. However, many of the proposed mechanisms of sex ratio adjustment result in costly laying gaps. In these cases, females laying large clutches may restrict the sex manipulation to the first egg of the clutch. I conducted a simulation study to examine the detectability of sex ratio adjustment on the level of the clutch when the adjustment is restricted to the first egg. Sex ratio adjustment in large samples (n=100) was not detected in 16 and 46 percent of the cases in 6-egg and 12-egg clutches respectively. These data suggest that, unless the exact mechanism of sex ratio adjustment is known, no conclusion can be drawn from nonsignificant results for clutch sex ratio without information on laying order and the sex of the first egg.**

**Keywords:** sex allocation, sex determination, laying order, population differences

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## 1. INTRODUCTION

After molecular methods for sexing birds had been developed, the number of studies published on avian sex allocation increased rapidly. Still, sex ratio adjustment is a controversial issue. Results often differ between populations of the same species (table 1) and even two recent studies using a meta-analytic approach came to opposite conclusions. West & Sheldon (2002) performed their analyses on studies with clear predictions (i.e. females mated to more attractive males should produce more sons, and in cooperatively breeding

species females without helper should bias their sex ratio toward the helping sex). They found that the observed brood sex ratio patterns from various taxa were in agreement with these predictions. They argued that 3 to 18% of the variance in sex ratio among families may be explained by facultative sex ratio adjustment. On the contrary, Ewen and his colleagues (2004) claimed that, excluding some exceptional cases, avian sex ratio adjustment is rather a statistical artefact. They found that biological (e.g. parental traits, territory quality) and temporal traits (e.g. season) had strong effect on sex ratios mainly in studies with moderate sample sizes, while in very large datasets the effect size was close to zero. They argued that the disappearance of significant patterns with increasing sample size suggests that the significances were due to type I error rather than real biological phenomena. However, if large sample sizes are achieved using data from multiple years (which is often the case), the overall small effect size might be the consequence of different optima of sex allocation in different years. This is because females face different environments across years and populations, and the costs and benefits of sex ratio adjustment might therefore also change. Selection should favour the evolution of preferential sex allocation only in cases where the benefits overcome the costs, or more probably selection should favour the evolution of reaction norms so that females adjust the sex ratio only when it is beneficial to do so. In addition, conflicting sex allocation patterns between populations may also be explained by methodological differences. Such differences may involve e.g. different breeding conditions like in the case of captive zebra finch (*Taeniopygia guttata*) studies (Burley 1986; Zann & Runcimen 2003; Rutstein *et al.* 2005). Furthermore, as I discuss below, we may find contradictory results if we investigate sex allocation at the clutch rather than individual egg level.

Potential mechanisms of sex ratio adjustment (for a review see Pike & Petrie 2003) can be divided into two major groups, in which sex is manipulated either with or without gaps in the egg laying sequence. Since follicles are growing in a hierarchical way and secondary follicle is in a developmental delay compared to the primary follicle, mechanisms acting after meiosis and requiring the resorption or elimination of the primary follicle or ova are expected to result in laying gaps. On the other hand, mechanisms affecting the outcome of the chromosomal segregation can lead to biased sex ratios without

Table 1. Some examples of contradictory results in studies of sex ratio adjustment. Only those traits are showed, which differed between studies in the impact they had on sex ratio.

species	study	sample size/year	number of years	male attractiveness	male quality	female quality	laying date	male age	female age	clutch size	food regime
<i>Parus caeruleus</i>	(Svensson & Nilsson 1996)	41	1		+a	0a	0	0	0		
	(Sheldon <i>et al.</i> 1999)	41/57	1	+	+a	0a	?	?	?		
	(Leech <i>et al.</i> 2001)	51	3		0abcd	0abcd	0	0	0	0	
	(Griffith <i>et al.</i> 2003)	57	3	+	+a		0	0	0(+1)	0(-1)	
<i>Parus major</i>	(Lessells <i>et al.</i> 1996)	103	1		0b	0b	+	0	0	0	
	(Kölliker <i>et al.</i> 1999)	57	1	{+}	+c	0c	0	0	0	0	
	(Radford & Blakey 2000)	24	5	0	0c(-1)0e	0c(+1-1)0e(+1)	0(+1)	0(-1)	0		
	(Oddie & Reim 2002)	79[23]	3		0ce	0ce[+e]	0	0	0	0	
<i>Ficedula albicollis</i>	(Ellegren <i>et al.</i> 1996)	79	1	+	0c		0	0	0	0	
	(Rosivall <i>et al.</i> 2004)	57	1	0	0c	0c	+	0	0	0	
<i>Taeniopygia guttata</i>	(Kilner 1998)	33	1								-
	(Rutkowska & Cichoń 2002)	24	1								0

(Empty cells indicate traits which were not examined in the studies. 0=no effect, +/-=positive/negative correlation between the trait and sex ratio. ?, reports of significant effects without indicating the direction of the correlation. In the case of parental quality a= overwinter survival, b=body mass, c=tarsus length d=parasite load, e=condition (residual from body mass-tarsus length regression). In multiple year studies the overall result is shown. If traits were significant in certain years only, then the direction of the effect and the number of years in which it was found is indicated in parentheses. {} indicates marginally significant result. [] indicates between year comparisons for the same individuals. In Sheldon *et al.* (1999) first sample size refer to male attractiveness, while the second to all the other traits.)

laying gaps. Our expectations are substantially different in these two cases. If the mechanism results in laying gaps, birds with large clutches may have to pay severe costs, such as increased predation risk and decreased viability of eggs, due to delayed clutch completion (Emlen 1997). Thus, sex ratio manipulation should be restricted to the first egg(s) in such species. In species laying small clutches (2-3 eggs), there is probably no egg viability cost, so they are expected to adjust the sex of each egg. On the other hand, if sex ratio adjustment act via mechanisms which do not lead to laying gaps, females might manipulate the sex of their offspring throughout the laying sequence independent of clutch size. For large clutches the effect sizes at the clutch level are expected to differ under the two scenarios. Since we have no clear evidence so far for any of the possible mechanisms of sex ratio manipulation, and not necessarily the same mechanism has evolved in different species, it is important to adjust the methods of analysis so that sex ratio patterns can be reliably detected.

Here I report a simulation study examining the detectability of sex ratio adjustment at the clutch level when the manipulation is restricted to the first egg in large clutches. According to earlier findings on brood sex ratios (Burley 1986; Sheldon *et al.* 1999), I assume in the model that females produce male offspring in the first egg for attractive males and females for unattractive fathers, while the sex of later laid eggs is determined randomly. I create 500 simulated populations (or studies), and report the proportion in which sex ratio manipulation in relation to male attractiveness is detected on the

clutch level. I then compare this clutch-level detectability to „studies” investigating specifically the sex of the first egg in relation to male attractiveness.

## 2. METHODS

### (a) Data generation

First, I created a theoretical population with 100 individuals. I assumed that only one phenotypic trait, paternal ornament size, plays a role in the sex ratio manipulation of this species, and this trait ranges from 30 to 100 and is normally distributed (mean±S.E.= 65.0±17.5). Then, using the cumulative probability values of finding individuals with a certain or smaller trait size, I calculated how many individuals should have the trait size in question (I used only integer trait values). In the resulting model population the mean (±S.E.) trait size was 64.79±16.32. I assumed that females can manipulate the sex of the first laid egg, but not that of the later laid eggs, and that sex manipulation has some cost. Since benefits should overcome the costs for a behaviour to be adaptive, I also assumed that females manipulated the sex of the first egg only in cases when their mate had a particularly large or small ornament, and otherwise the sex of the first egg would result from random segregation. Thus I sorted the males by their ornament size. The first egg of highly ornamented males (one third of the population) was always male, the first eggs of poorly ornamented males (one third of the population) were always female and the first egg of males with medium sized ornaments was decided using a random number generator (in Microsoft Excel 2000). For all eggs laid later in the sequence sex was assigned randomly. Keeping ornament size constant, I repeated the process 500 times. As a

consequence, I had 500 „populations” with 100 individuals, in each of which females use the same sex allocation rules. The only difference is generated by the random chromosomal segregation in later laid eggs.

To simulate the effect of different sample sizes, I took random samples of 50, 25 and 13 individuals from my original population and assigned sex to the eggs as above. I had 500 repeats for all sample sizes. Note that individuals (ornament sizes) did not change between repeats, but the sex of the eggs did.

To explore the effect of clutch size on the probability of detecting sex ratio manipulation at the clutch level, I repeated the above procedures twice. I first used medium sized clutches with 6 eggs (typical to many popular study species such as Collared Flycatcher (Török *et al.* 2004) and House Wren (Albrecht & Johnson 2002)), and then large clutches with 12 eggs (typical to e.g. Blue Tit; Török & Tóth 1999).

#### **(b) Statistical analysis**

Throughout this study, I used generalized linear models with binomial errors and logit link (Crawley 1993) in the GENMOD module of SAS 8.2 (SAS Institute Inc., Cary, NC, USA). Sex of the first egg or clutch sex ratio were the binomial response variables and patch size was the continuous predictor variable. Analyses were performed separately for each study „population”. Since the dispersion parameter was larger than 1.0 in some of these „populations”, I tested the significance of the parameters with F-tests ((Crawley 1993); dscale option in GENMOD).

### **3. RESULTS**

The simulations show that even in species with medium sized clutches (6 eggs) the detection of sex ratio manipulation at the clutch level becomes extremely difficult, if sex ratio manipulation is restricted to the first egg (figure 1a-1d). When 50 clutches were sampled (the average sample size/year was 54.5 for the clutches in table 1), the existing sex ratio manipulation was detected only in 52.6% of the cases. Even when sample size was twice as large, only the 84.4% of the „studies” found significant effect of the examined trait on clutch sex ratios.

In species with large clutch sizes (12 eggs), the situation is even worse (figure 1e-1h). Even with large samples (n=100), I found significant relationship between the trait and clutch sex ratio in only approximately half of the cases (54.2%), though there was sex ratio manipulation in all cases.

However, when I analysed the effect of paternal patch size on the sex of the first egg, the results were significant in all 500 „populations” independent of sample size. So, when only the sex of the first egg is manipulated in relation to patch size, this can be easily detected with even very small sample sizes (n=13) if we have information on the sex of the first egg, but we will probably miss this effect if we have data only on clutch sex ratios.

### **4. DISCUSSION**

Many previous studies on sex ratio adjustment found contradictory results among populations (table 1). My simulation study shows, however, that contradictory patterns may easily arise even if females follow the same decision rule in all populations. When females manipulate the sex in the first egg but not in later laid eggs, and we consider clutch sex ratio as the object of our investigation, random segregation of the sex chromosomes in later laid eggs will often mask the effect of maternal manipulation. The probability of making such a mistake is unknown, since our knowledge about the underlying mechanisms of sex ratio adjustment is rather poor. A number of pathways have been proposed (reviewed e.g. in Pike & Petrie 2003), but there is very little support for any of them. Two major groups of mechanisms could be separated in field studies according to whether or not sex ratio manipulation leads to laying gaps between subsequent eggs. For example, Komdeur *et al.* (2002) found strong sex ratio bias in both the first and the second laid eggs of Seychelles Warblers without any laying gap in between. Thus, they argued that skewed sex ratios are probably the result of segregation distortion during meiosis. However, in his recent study, Pike (2005) argued that selective resorption of the ova, a mechanism which was thought to result in laying gaps, may occur without laying gaps in two-egg clutches of pigeons. Thus, to separate between the two alternative mechanism types, species with larger clutches should be examined. In those species, sex ratio adjustment via selective resorption,

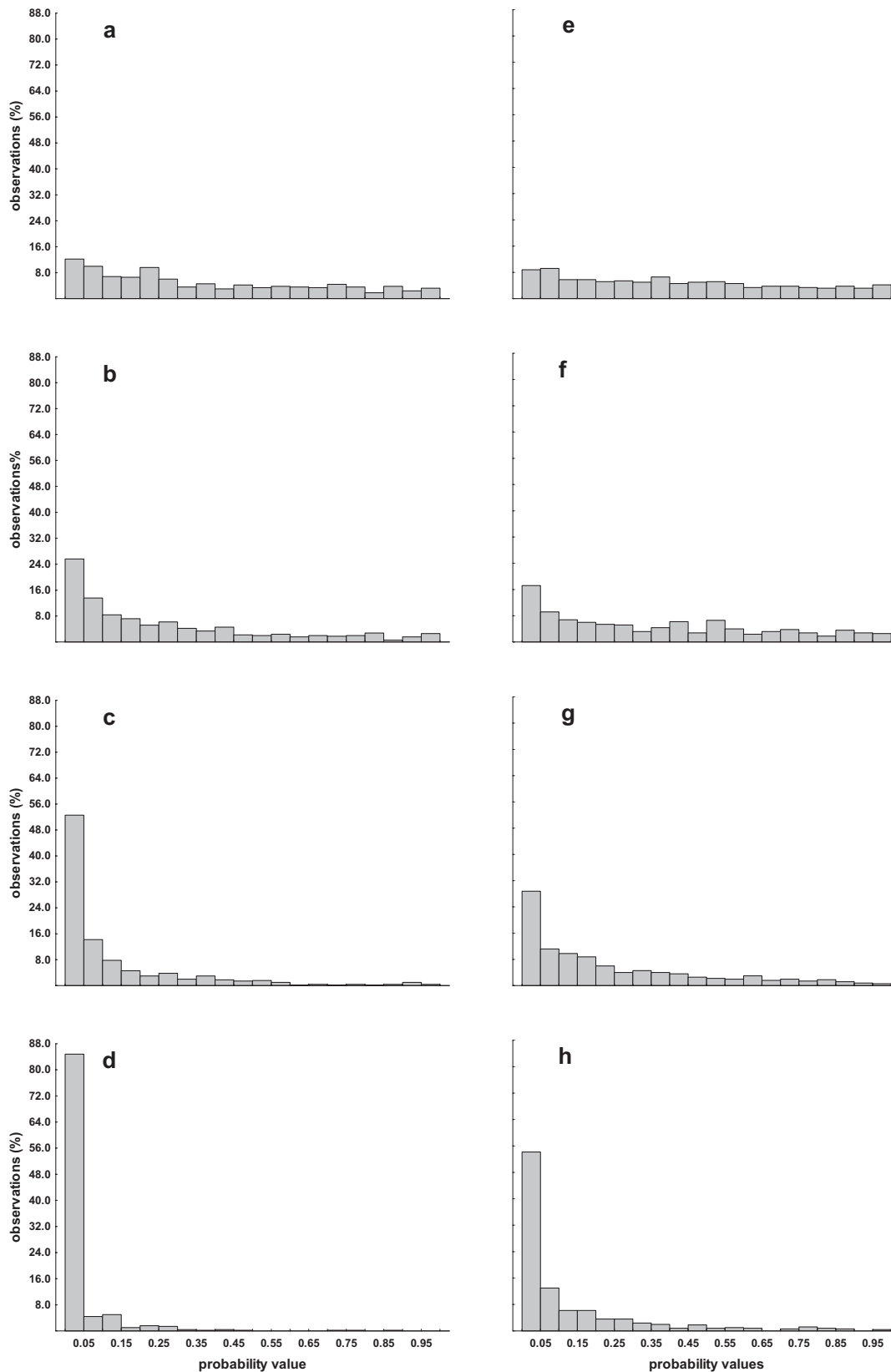


Figure 1. Detectability of sex ratio adjustment at the clutch level, when the adjustment is restricted to the first egg. The figure shows the distribution of the p-values from 500 different populations when females follow the same sex allocation rule (i.e. bias the sex of the first egg in relation to a paternal trait). Results are significant when  $p < 0.05$ . The clutch size is 6 (a-d) or 12 (e-h). The sample size within each population is 13 (a and e), 25 (b and f), 50 (c and g) or 100 clutches (d & h).

but not segregation distortion, would result in laying gaps if applied throughout the laying sequence. However, in case of selective resorption, evolution may favour biased sex allocation only in the first egg, to avoid the costs of late clutch completion because of laying gaps (Emlen 1997). Interestingly, strong sex bias among the first chicks has been found in a couple of species, while there was no bias in younger sibs (Arnold *et al.* 2001; Blanco *et al.* 2002). Furthermore, the strongest bias in brood sex ratios was found in species with small clutches (Heinsohn *et al.* 1997; Komdeur *et al.* 2002; Pike 2005). Still, my aim is not to favor selective resorption or post-ovulation mechanisms over the others, especially given that segregation distortion might have larger adaptive value than the alternative mechanisms (i.e. by extending the beneficial adjustment to more eggs, and preventing the waste of egg material), and hormones seems to affect clutch sex ratios without causing laying gaps (Rutkowska & Cichoń 2005). Instead, the goal of this study was to demonstrate how sex ratio adjustment restricted to the first egg affects the detectability of sex ratio manipulation in datasets of realistic sizes. My results show that contradictory or negative results based on clutch sex ratios has to be interpreted carefully and should not been used as an argument against the existence of sex ratio adjustment. It is important to have data not only on clutch sex ratios, but also on laying order, to enable the separate examination of sex patterns in the first egg. If it is impossible to determine laying order, very large samples may be needed to reliably detect differential sex allocation, and the required sample size increases with the clutch size of the species.

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# Paper 3





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## Paternal age and offspring growth: separating the intrinsic quality of young from rearing effects

**Abstract** Younger individuals are often less successful in reproduction than older ones. This might be because of improving breeding skills with age, or because the genetic quality of young or early maternal effects on them vary with parental age. However, no attempt has been made to experimentally separate these processes in vertebrates. We conducted a cross-fostering experiment in collared flycatchers (*Ficedula albicollis*) in three breeding seasons to disentangle origin- and rearing-related effects of paternal age on chick growth, while controlling for date-specific environmental conditions as well as differences in clutch and brood size. The age of the male at the nest of origin, but not that of the rearing male, had a year-dependent effect on nestling body mass and tarsus length. In two seasons, young of subadult males grew slower in the early linear phase of growth than young of adult males. There was no compensatory growth in the final asymptotic phase, so both body mass and tarsus length before fledging reflected the differential early development. In the remaining year, the age of the male at the nest of origin had no significant effect on chick growth. The environment-dependent origin effect we detected was unexplained by incubation times, hatching asynchrony, chick masses at swapping or previously described age-dependent egg quality patterns. Our results therefore suggest a genotype x environment interaction on the relative development of offspring sired by subadult and adult males. Our results also raise the possibility that female birds may gain genetic benefits by mating with older males. Further studies should identify general patterns of male age dependent female mate choice and offspring quality in different environmental conditions.

**Keywords:** Parental age • Nestling growth • Parental quality • Genetic quality • Subadult plumage

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### Introduction

Reproductive performance is age-dependent in a variety of taxa (Clutton-Brock 1988). In insects, for example, age has been found to affect the mating success (Jones et al. 2000) and mating tactics of males (Engqvist and Sauer 2002, Kemp 2002), as well as the quality of offspring produced by females (Fox et al. 2003). In a population of sockeye salmon *Oncorhynchus nerka*, early and late breeding females represent genetically diverged alternative strategies with contrasting levels of investment to egg production versus survival to defend the nest (Hendry et al. 2004). In the sand lizard, *Lacerta agilis*, the growth of body size appears to largely explain age-related changes in reproductive behaviour and success in both sexes (Olsson and Shine 1996). In red deer *Cervus elaphus*, sex-dependence in the relative allocation to somatic maintenance versus reproduction reflects the age-dependence of reproductive prospects of males versus females (Carranza et al. 2004). Age-dependent reproduction, however, has been most thoroughly studied in birds.

The reproductive performance of birds shows a characteristic pattern across age categories. Young breeders usually raise fewer or poorer quality young than older birds (Røskaft et al. 1983; Perrins and McCleery 1985; Lessells and Krebs 1989; Sydeman et al. 1991; Hepp and Kennamer 1993; Blomqvist et al. 1997; Hipfner and Gaston 2002), while individuals exceeding a certain age threshold often show a decline in breeding success (Ollason and Dunnet 1978; Dhondt 1989; Hamer and Furness 1991; Ratcliffe et al. 1998; Pyle et al. 2001).

Due to the unequal roles or investment of the two sexes during breeding, the age of females is more important to success than that of males in some species, such as blackbirds *Turdus merula* (Desrochers and Magrath 1993), nuthatches *Sitta europaea* (Enoksson 1993) and red-billed choughs *Pyrrhonorax pyrrhonorax* (Reid et al. 2003), while the reverse is true in other species, for example Tengmalm's owls *Aegolius funereus* (Korpimäki 1988), brown thornbills *Acanthiza pusilla* (Green 2001) and lapwings *Vanellus vanellus* (Parish et al. 2001). However, for historical and practical reasons, most studies focused on females.

The poor success of young parents has two distinct, but not mutually exclusive classes of explanations. First, the performance of individual birds may improve with age because parenting skills improve (Brandt 1984;

Desrochers 1992a; Weimerskirch 1992; Cichon 2003), or because of an increase in reproductive effort (Pugesek 1981, 1984; Pärt et al. 1992) or territory quality (Major et al. 1999; Pärt 2001a). Second, the success differences between young and old breeders may result from processes operating before or during egg production, for example, differences in genetic or long-lasting phenotypic quality of parents that cause differential survival (Nol and Smith 1987; Bradley et al. 1989; Smith 1993; Cam and Monnat 2000; Mauck et al. 2004), or male age related differences in egg investment by females (Michl et al. 2005).

If the relationship between the age and reproductive success of parents is due to genetic quality or early maternal effects on young, we expect to find differences in offspring quality among parental age classes irrespective of the rearing environment provided by the parents (Brooks and Kemp 2001). Improving parenting skills, in contrast, will lead to differences in the rearing environment, irrespective of the genetic or long-lasting phenotypic quality of young. The few published experiments on age-related reproductive success were designed to disentangle age and experience (Pärt 1995; Cichon 2003), age and territory quality (Pärt 2001a), or breeding date and parental quality effects (Desrochers 1992b; DeForest and Gaston 1996; Daunt et al. 1999, 2001). No experimental study in any vertebrate species addressed the question of whether there are intrinsic differences in quality between the offspring of young and older parents.

A straightforward way to assess the quality of young would be to measure nestling growth. However, the overwhelming majority of studies considering nestling size or condition only reported single morphological measurements taken before fledging or independence (Naef-Daenzer et al. 2001; Tremblay et al. 2003). This is unfortunate for several reasons. First, the importance of several early environmental effects decreases towards fledging (Smith et al. 1995; Schwabl 1996), which implies that fledgling data may often lead to false negative conclusions on the importance of environmental factors to chicks. Second, there is a considerable degree of flexibility in chick growth, which may itself be a signature of individual quality (Lepczyk and Karasov 2000; Bize et al. 2003). Third, developmental fluctuations may have long-term effects on the fate of young even if they do not lead to differences in final size (Metcalf and Monaghan 2001). Studies of chick growth in relation to parental age are rare, and almost completely restricted to seabirds (e.g. Sætre et al. 1995; DeForest and Gaston 1996; Galbraith et al. 1999). In some of these studies, results obtained for growth versus final size were consistent with each other (Pugesek 1993; Wendeln and Becker 1999), but there was a disagreement between them in other studies (Berrow et al. 2000).

Some of the existing evidence for parental age effects comes from work on Old World flycatchers. However, the majority of studies on age-related reproduction in these species has been restricted to females (e.g. Pärt and

Gustafsson 1990; Pärt et al. 1992; Cichon 2003). Relatively little is known on patterns with male age. In collared flycatchers (*Ficedula albicollis*), the age of males significantly predicted seasonal reproductive success even after correcting for breeding date and female age (Qvarnström et al. 2003). However, this study focused on the costs of polygyny, and no specific details were provided on the age effect, i.e. its direction or interactions with other variables were not shown. In the sister species, the pied flycatcher (*Ficedula hypoleuca*), chicks grew better in the broods of old, colourful males than in those of young, dull males, when the female parents were temporarily removed (Sætre et al. 1995). An earlier descriptive study by Harvey et al. (1985) showed that yearling males which subsequently survived to breed again bred earlier in the current season than those which did not survive. This pattern suggested that at least part of the variation in success between yearlings and adults could be attributed to differences in genetic or long-lasting phenotypic quality.

Here we report on a cross-fostering experiment in a population of collared flycatchers where we partitioned the difference in offspring growth between the nests of subadult and adult males to rearing- versus origin-related components. The experimental design corrected for confounding date-related environmental differences between male age classes, for example, timing relative to the caterpillar availability peak (Nager and van Noordwijk 1995), and also controlled for most of the differences in territory quality, as reflected by settlement and pairing order (Alatalo et al. 1986; Pärt 1991). If genetic or egg quality effects in relation to male age are important determinants of nestling growth, we predict a significant 'male of origin' effect. If, on the other hand, male parental quality (Sundberg and Larsson 1994), female parental care (Limboung et al. 2004) or territory quality (Pärt 2001a) in relation to male age are important, we expect to find a significant rearing effect. Both the origin- and the rearing-effect have several alternative explanations. To distinguish between the alternatives, we refer to previous results in our population on aspects of egg composition as early maternal effects (Hargitai et al. 2005; Michl et al. 2005), and report the feeding rates of both parents at the experimental broods.

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## Materials and methods

### Study species and area

The collared flycatcher is a small (ca. 13g), insectivorous, hole-nesting passerine (Gustafsson 1989). The species is predominantly monogamous, with approximately ten percent of males attracting two females (Garamszegi et al. 2004). The study was conducted in Pilis Mountains, near Szentendre, Hungary (for more information on the study area, see Török and Tóth 1988). Males arrive at our nestbox plots in the

middle of April, and females a few days later. After pair formation, females build the nest, lay and incubate the eggs, while the two parents share nestling feeding approximately equally. Male participation in provisioning the brood is important to reproductive success (Garamszegi et al. 2004). The median clutch size is six (range: 2-9). The incubation period is 12-13 days, and the nestlings fledge from 14 days after hatch. Males show delayed plumage maturation, with second-year (subadult) males having brown remiges and a small white wing patch, and older males (adults) having black remiges and a much larger wing patch (Török et al. 2003). Subadult males constitute approximately 30 percent of the breeding population each year (mean $\pm$ se from 1990 to 2004: 0.298 $\pm$ 0.036). Both the body mass and the tarsus length of fledglings show positive linear relationships with recruitment probability (J. Török, G. Hegyi, L. Tóth and R. Könczey, unpublished data; also see Lindén et al. 1992, Török et al. 2004).

### Experimental procedures

The experiment was done in the breeding seasons of 2002 to 2004. We presented dummy males at all nests on the experimental plots during the late nest-building or early egg-laying period, and observed the attacking resident with binoculars. The age of the resident male (subadult or adult) was determined by the colour of the remiges (Svensson 1992) and wing patch size (Török et al. 2003). Nests were monitored daily around the expected date of hatching. We identified trios consisting of the nests of one subadult and two adult males, matched for hatch date and brood size. Two days after hatching, we swapped whole broods in these trios of nests, creating three experimental foster categories: subadult males rearing offspring of adult males (SMAO), adult males rearing offspring of subadult males (AMSO), and adult males rearing offspring of adult males (AMAO). Our basic question was whether differences in nestling development between the paternal age classes were due to different rearing environments or origin-related effects. The number of nests tended by subadult males was insufficient for a second control group, with subadult males and subadult offspring, which would be needed to test the interaction between origin and rearing effects. However, because we did not detect a rearing effect in any year of this study (see Results) adding a second control group would have been unlikely to have revealed more complex patterns. To see whether differential incubation confounded the origin-effect (Gorman and Nager 2004), we compared incubation times across treatment groups. Incubation time was defined as the time between the laying of the sixth egg and hatching.

The number of trios swapped was 8 in 2002, 9 in 2003 and 12 in 2004, involving a total of 87 nests. Eight trios were dropped from the dataset because of predation (n=3 in 2002, n=1 in 2003), misidentification of male age (n=1 in each year), or the lack of the male parent (n=1 in 2004). One male was detected in the same experimental

treatment in two years, and we omitted its second observation from the analyses. Five females also occurred twice, in the same (n=2) or different experimental treatments (n=3), which necessitated the statistical control of female identity. One experimental nest in each year was identified as polygynous secondary based on laying date and male identity, all belonging to the AMSO category. Since it was confirmed that these males fed the young, and omitting the three trios including their nests did not affect the conclusions, we retained them in the analyses, but statistically corrected for their presence. The analyses presented here are thus based on a sample of 20 trios (4 in 2002, 7 in 2003 and 9 in 2004), with a total of 60 nests.

In 2002 and 2003, feeding rates by the parents were recorded by videocameras in 1h sessions when broods were four days old. The recordings of a given trio were run at the same time, with a maximum difference of 45 minutes. All young received standard numbered aluminium rings at the age of 6d. Body mass was measured to the nearest 0.1 g with Pesola spring balance every other day from the day of swapping to the age of 12d. Tarsus length was measured with a caliper to the nearest 0.1 mm at the ages of 8, 10 and 12d. Nestlings are usually considered fully grown at the age of 13d. We captured the parents in the nestbox when chicks were 8 to 10 days of age, and measured their body mass, tarsus length, and white plumage ornaments (forehead patch size, Hegyi et al. 2005; wing patch size, Török et al. 2003).

### Statistical analyses

To simplify the models and to facilitate the interpretation of the results, we pooled data from 2002 and 2004, and compared them with 2003 as a separate group. Here we show the results using these two year categories. Preliminary analyses with year as a factor showed four significant or marginal year by male of origin interactions on nestling development. In pairwise tests between years, all of the four year x origin interactions were far from significance for 2002 and 2004, while seven of the eight were significant or marginal for 2003 and the other two years. As mass gain was steeper and levelled off earlier in 2003 than in 2002 or 2004 (see Results), 2003 was termed 'good year', while 2002 and 2004 'average years'.

Growth measurements of nestlings that died in the nest were dropped from the analyses. Mortality was rare (one chick in n=8 nests and two chicks in n=3 nests), and all except one of the nestlings that died were runts originating from pronounced hatching asynchrony. The number of nests with dead nestlings did not differ among year types (6 of 39 nests in average years, 5 of 21 nests in the good year; Fisher exact p=0.493), or treatments (1 of 20 SMAO nests, 3 of 20 AMSO nests, 7 of 20 AMAO nests; Fisher exact tests SMAO-AMSO p=0.605, SMAO-AMAO p=0.043, AMSO-AMAO p=0.273, all ns after Bonferroni correction). However, as nestling mortality may enhance the growth of the remaining

Table 1. Comparison of morphology and ornamentation of males and females among treatments and year types. Linear mixed models with year type and treatment (three groups) as fixed factors and trio and female as random factors.

	Year type		Treatment		Interaction	
	df	F	df	F	df	F
Female tarsus	1,19.1	7.92*	2,34.3	0.54	2,29.7	3.79*
Female mass	1,2.27	0.42	2,9.55	1.83	2,6.46	6.32*
Female condition <sup>a</sup>	1,2.79	0.29	2,1	1.14	2,3.12	1.61
Female wing patch size	1,16.3	1.66	2,35.1	0.96	2,35.2	3.10
Female minimum age <sup>b</sup>	1,15.6	0.86	2,31.2	0.14	2,27.5	1.39
Male tarsus	1,18	1.44	2,34.2	1.92	2,34.2	0.84
Male mass	1,5	0.18	2,5	1.39	2,5	0.23
Male condition <sup>a</sup>	1,48	0.30	2,48	1.47	2,48	0.42
Male forehead patch size	1,52	0.00	2,52	3.05	2,52	0.07
Male wing patch size <sup>c</sup>	1,33	4.90*	1,33	0.19	1,33	4.57*
Male minimum age <sup>bc</sup>	1,34	0.14	1,34	0.00	1,34	0.62

<sup>a</sup>Body condition values are residuals from a regression of tarsus length on body weight. <sup>b</sup>Log (2) transformed. Female age was known for recruits and estimated as one year at first capture for an unringed adult. For male age determination see Török et al. (2003). <sup>c</sup>Only the adult males are compared here, because subadult males have very small wing patches, and their age is obvious. \* p<0.05, \*\* p<0.01.

young, we tried to correct for the small difference by entering mortality into the analyses of growth in two ways: as a binary factor, and as a continuous covariate (the number of dead nestlings). The main effect of mortality was nonsignificant in all cases (all p>0.12), and the results remained almost quantitatively identical, with no loss or gain of significance. It seems, therefore, that mortality did not confound the nestling growth patterns in our case. We therefore excluded mortality from the models presented here.

Hatching asynchrony may cause growth retardation in late-hatched nestlings, which may influence the brood-level growth data. We did not directly follow the patterns of hatching in the experimental nests. However, hatching asynchrony obviously affects size variation within broods after hatching (E. Szöllösi, B. Rosivall and J. Török, unpublished data). We therefore calculated the within-brood standard deviations of 2d chick masses, and compared them among treatment groups (see Results). In addition to male age, some of the male and female traits we measured differed among treatments (Table 1). However, when adding these traits one by one to the models, most had no significant effect (only one significance in 27 models), and none affected any of our conclusions, so we removed them from the models.

Male and female feeding rates were expressed as feedings per nestling per hour. Nestling growth rates were assessed in two phases. For the early, linear phase, only mass data were available. We calculated the slope of regression lines of brood means from 4 to 8 days of age. For the late, decreasing phase of growth, both mass and tarsus growth were quantified by two variables: changes from 8 to 10d and from 10 to 12d, with original size measures (i.e. 8 and 10d mass or tarsus, respectively) entered as covariates. Entering original size as a covariate is an attempt to correct for the decline of growth: the larger the nestling, the smaller the remaining growth (also see Results). This also implies that late growth rates are deviations from the growth expected

with the current size. Mass and tarsus growth rates for the 8-10d and 10-12d periods were only weakly correlated (mixed models with female and trio as random factors, changes corrected for original mass or tarsus; mass  $F_{1,53.9}=1.82$ ,  $p=0.182$ ; tarsus  $F_{1,53.8}=4.41$ ,  $p=0.040$ ) so they were treated separately.

To control for the occurrence of polygyny (see above), we entered it as a binary factor into the analyses. We used general linear mixed models with Satterthwaite correction, calculated in the MIXED procedure of SAS 8.02 (SAS Institute, Inc.). We treated brood means as data points, year type, age of the male of origin (hereafter: origin), age of the male of rearing (hereafter: rearing) and polygyny as fixed factors, and trio and female as random factors. Backward stepwise model selection was applied in all tests. As we used three measures of mass and two measures of tarsus growth, we corrected the critical p to 0.05/3 and 0.05/2, respectively, in the statistical tests of these variables.

## Results

### Potential background variables and confounds

Neither incubation times, nor body mass of nestlings at swapping or brood-level standard deviations of 2d mass differed across treatments (Table 2). There was no indication of differential feeding by males in relation to male age treatments. Females, in contrast, showed reduced feeding rates when paired to subadult males (significant rearing effect, Table 2, Fig. 1). None of the patterns of incubation times, original chick masses, their standard deviations, or parental feeding rates with treatment was influenced by environmental conditions, as reflected by the interactions between treatments and year type (Table 2).

Table 2. Relationship of male age at the nest of origin ('origin') and rearing ('rearing') and year type with incubation time, chick mass at two days of age, the standard deviation (SD) of mass at 2d of age, parental feeding rates at 4d of age, the mass and tarsus growth rates of chicks in different phases, and their mass and tarsus length before fledging. Linear mixed models, see Methods for details.

	Year type		Origin		Year type* origin		Rearing		Year type* rearing	
	Df	F	df	F	df	F	df	F	df	F
Incubation time	1,14.7	5.12*	1,34.9	2.56	1,33.9	0.94	1,32.5	0.36	1,32.7	0.08
2d mass	1,18.2	0.38	1,38.1	0.07	1,37.4	2.68	1,36.6	2.37	1,35.6	0.01
2d mass SD	1,14.8	1.41	1, 33.3	0.60	1,18.9	2.43	1,12.6	1.16	1,16.5	0.12
Male feeding rate	1,26	2.46	1,26	0.04	1,24	0.33	1,26	0.48	1,24	0.24
Female feeding rate	1,10.5	4.14	1,9.85	1.25	1,3.57	0.25	1,9.23	8.16*	1,4.29	1.40
Mass growth 4-8d <sup>a</sup>	1,51	32.47***	1,51	1.35	1,51	8.98**	1,5	0.30	1,49	0.14
Mass growth 8-10d	1,22.2	20.82***	1,36.7	0.15	1,38	4.41*†	1,31.6	0.11	1,31.2	1.47
Mass growth 10-12d	1,17.7	0.01	1,37	0.01	1,37.2	0.83	1,34.9	0.40	1,33.7	0.11
Tarsus growth 8-10d <sup>a</sup>	1,14.7	1.31	1,33.5	0.10	1,35.4	2.17	1,32	1.96	1,31.2	1.83
Tarsus growth 10-12d <sup>a</sup>	1,17.3	1.93	1,34.3	0.36	1,35.2	0.41	1,33.2	0.48	1,33.1	0.10
8d tarsus <sup>a</sup>	1,14.5	2.57	1,36.9	0.83	1,39.5	17.67***	1,39.8	0.12	1,39.8	0.76
12d mass	1,13.4	0.03	1,34.6	1.08	1,26.4	5.00*	1,20.1	0.10	1,13.7	0.06
12d tarsus	1,15.3	2.44	1,34.2	0.83	1,33.6	7.64**	1,30.5	0.00	1,46	0.03

\* p<0.05, \*\* p<0.01, \*\*\* p<0.001, † not significant after Bonferroni correction. <sup>a</sup>Significant effect of polygyny.

#### Experimental analysis of nestling growth rates

There was no overall origin effect on the 12d mass or the tarsus length of nestlings, and the growth of these traits also showed only weak effects (Table 2). There was, however, a significant year type by origin interaction for mass growth in the linear phase and from 8 to 10d of age. Tarsus length at 8d of age, as well as pre-fledging mass and tarsus values, also showed a similar interaction. Tarsus growth in the whole asymptotic phase, as well as 10-12d mass growth, followed the trajectory expected from original mass or size, with no treatment effect or interaction (Table 2) Rearing had neither effect on growth, nor interaction with year type (Table 2). Restricting the analysis to year types showed no significant origin effect on any trait in the good year (Table 3, Fig. 2). In the average years, however, there

were significant differences related to origin for all variables showing year type by origin interactions, except 8-10d mass growth (Table 3, Fig. 2). The early mass gain was poor among young of subadult males (hereafter YSM), which lead to a low mass at 8d of age relative to young of adult males (hereafter YAM) (Fig. 2). The origin-related growth difference disappeared after 8d of age, but the mass disadvantage of YSM was still present before fledging. Tarsus length could only be measured from 8d of age. The 8d tarsus length of YSM was significantly smaller than that of YAM, which suggests a slower growth in the early phase (Table 3, Fig. 2). The size difference between YSM and YAM persisted through the rest of the nestling period. Nestlings of subadult males reached a considerably smaller tarsus length before fledging than those of adult males.

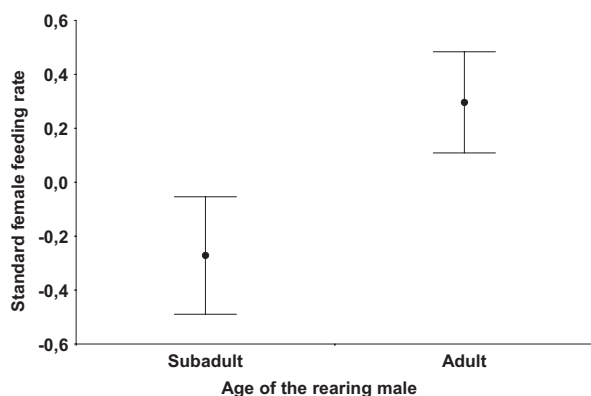


Figure 1. Feeding rates of female collared flycatchers in relation to the age of the rearing male. Feeding rates are expressed per nestling per hour, and standardized for trio (mean=0 and SD=1 for each trio).

Table 3. Effect of the age of male at the nest of origin in the two year types on the offspring traits that showed significant year type by origin interactions. Linear mixed models, see Methods for details.

	Good year		Average years	
	df	F	df	F
Mass growth 4-8d <sup>a</sup>	1,11.4	4.74	1,21.6	5.17*
Mass growth 8-10d	1,16	2.46	1,24.7	0.16
8d tarsus <sup>a</sup>	1,11	0.51	1,23	12.09**
12d mass <sup>a</sup>	1,17	0.73	1,23.5	8.50**
12d tarsus <sup>a</sup>	1,17	0.71	1,20.3	12.27**

\* p<0.05, \*\* p<0.01, \*\*\* p<0.001. <sup>a</sup>Effect of polygyny significant in average years (ns for all variables in the good year).

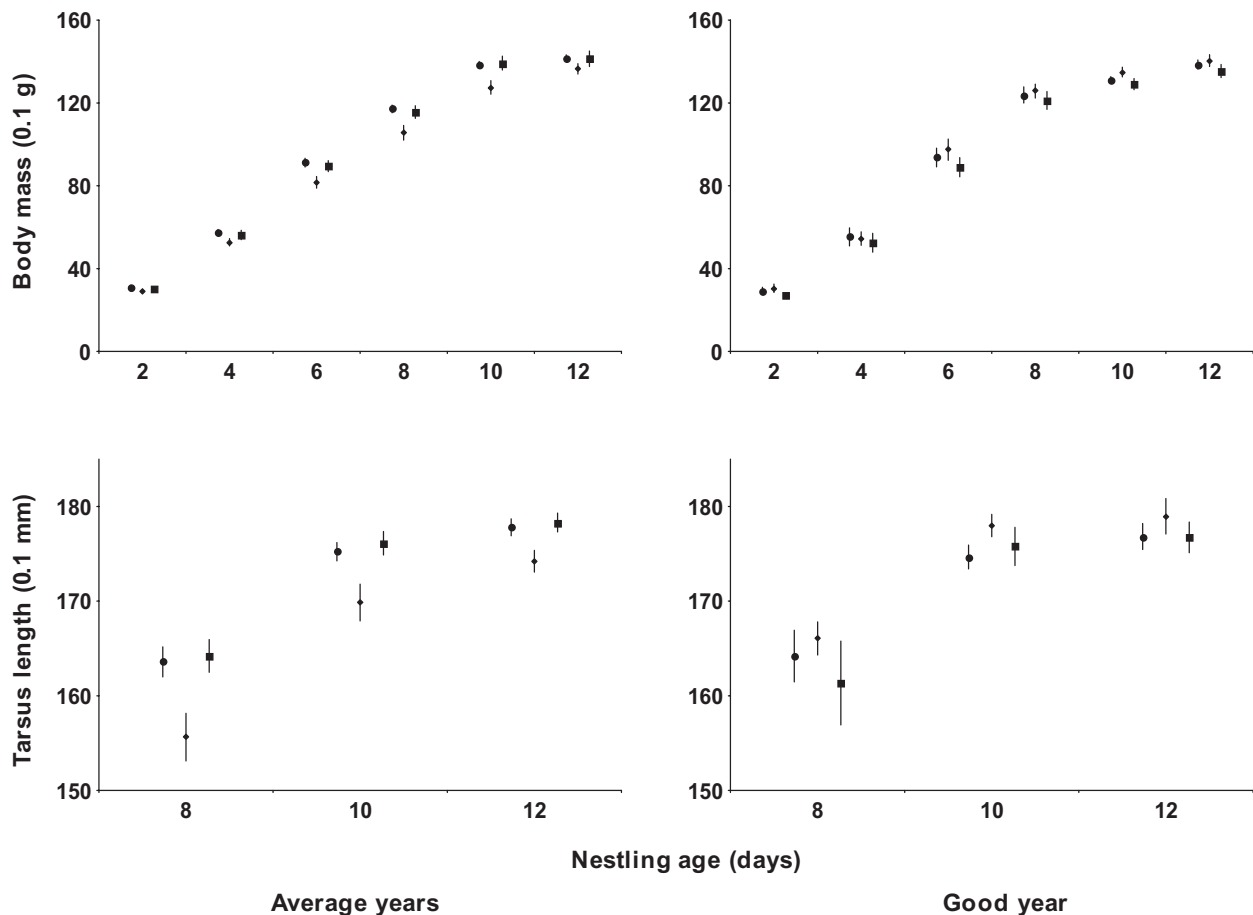


Figure 2. Age-specific body masses and tarsus lengths (means $\pm$ SE) of collared flycatcher chicks in relation to year type and treatment. Circles: subadult males rearing young of adult males, diamonds: adult males rearing young of subadult males, squares: adult males rearing young of adult males.

## Discussion

### Experimental design and confounding factors

We have presented results from an experiment aimed to separate the origin- and rearing-related components of the relationship between nestling quality and paternal age. The high breeding density of the study population (similar to that observed in unmanaged forests, van Balen et al. 1982; Török and Tóth 1988), and the synchronous breeding season of this long-distance migratory species allowed us to foster broods among trios of nests with different male age, but the same hatching date and brood size. This design made it possible to control for date-related environmental factors. Male morphology, age differences between the adult males, female age or female morphology apparently did not confound our conclusions (see Methods). The experiment allowed, on one hand, a comparison between chicks of similar origin, reared by males of different age. A significant effect of male age in this treatment pair would suggest that male parental quality or investment, female parental investment, or territory quality were responsible for male age dependent

nestling growth. On the other hand, the comparison between chicks sired by males of different age but reared in similar environments largely excludes these phenotypic effects, and reflects either genetic or early maternal effects. We detected no rearing-related effect, but significant year-type by origin interactions.

It could be argued that including only surviving chicks in the analysis reduced variance and obscured the treatment effect. The few cases of chick mortality were almost exclusively due to late hatching, and probably did not confound our results (see Methods). Moreover, the similarity of treatment groups in within-brood mass variation at swapping suggests that the patterns of growth we detected among nests were not influenced by hatching asynchrony. Laying date may represent another source of bias. The first subadult males arrive on average later than adults, and this is reflected by the date of laying (Gustafsson 1989; Potti 1998). However, there was an almost complete overlap between the breeding dates of the two age categories in the study years. If either phenotypic or genetic quality of males was correlated with their arrival and/or mating order, cross-fostering took place between subadult males of relatively high quality and adult males of relatively poor quality.

This makes our conclusions regarding genetic or parental quality conservative. In light of this, any rearing effect we detect should be viewed with caution.

#### Rearing effect

We found no difference in growth between young reared by subadult versus adult males. Possible differences in territory quality or parental feeding rates need to be considered when interpreting this result. Observations and experiments on site fidelity and settlement patterns of collared flycatchers indicate a learning process during which birds take the success of their own breeding attempt and that of their neighbours into account in future breeding attempts (Doligez et al. 1999, 2002). In contrast to adults, young and necessarily inexperienced males may lack the ability to discriminate between good and poor breeding sites, so that settlement order may not reflect territory quality differences in this age class (Pärt 2001b). This process may lead to differences in the quality of breeding sites even in our present comparisons between synchronously breeding pairs of subadult and adult males. However, it is possible that the feeding activity of parents compensated for the differences in territory quality. Feeding rates provide a reliable measure of energy expenditure and parental investment in our study species (Pärt et al. 1992). There was no age-related feeding by males, and females paired to adult males fed more frequently than those mated to subadult males, which may indicate preferential investment in the breeding attempt with an old, presumably high-quality male (Limbourg et al. 2004). The feeding pattern indicates that the nonsignificant rearing effect is not due to additional feeding effort by the mates of subadult males as a compensation for the poor parental performance of the male, or a compensatory feeding by any member of the pair in response to the low quality of the territory. It has to be noted that we cannot rule out male age differences in the quality of prey collected (Siikamäki et al. 1998). However, to explain why the feeding pattern of females lead to no rearing effect on nestling development, would require higher rather than lower quality food brought by subadult males in comparison to adults.

Our main conclusion is therefore that there is little evidence for lower parental or territory quality of subadult males in our population. The only comparable study was conducted in a Norwegian population of the sister species pied flycatcher, where females prefer old males with contrasting coloration to young, dull males (Sætre et al. 1994). In a female-removal experiment, even the short-term growth of chicks showed a significant effect of male age, with young reared by subadult males growing slower than those of adult males (Sætre et al. 1995). At first sight, this result suggests differences in parental or territory quality between male age classes. However, the experiment of Sætre et al. (1995) did not control for the potential intrinsic differences in the quality of young reared, which may

thus provide an alternative explanation to their results. The fact that the pattern was only found in the absence of the female implies that it was either due to the poor parental quality of yearling males, compensated for in the presence of females, or due to quality differences between chicks, only revealed under stressful food conditions.

#### Origin effect

The most important result of our study is the year type specific difference in the development of young of subadult versus adult males when both groups were reared by synchronously breeding adult males. Chick mass at swapping did not differ between treatments. The similarity of original mass is unlikely to reflect the balance of poor offspring quality and compensatory female investment, since females in our population were found to deposit macronutrients in the eggs at the same level irrespective of male age (Hargitai et al. 2005). In the average years of our experiment, young of subadult males (YSM) showed a poor early growth and no late compensatory growth, and therefore ended up smaller and lighter than young of adult males (YAM). This 'male of origin' effect was absent in the good year. Small body size may limit fasting endurance during the critical postfledging period (Weathers and Sullivan 1989; Merilä and Wiggins 1997), and thus affect survival, in concert with fledging mass (Lindén et al. 1992). Thus, YSM may have poor survival prospects compared to YAM in average years.

Although some of the measured female traits differed among treatment groups, the origin effect was not likely the result of female quality differences (including female age, see Methods). The pattern could still be confounded by differential incubation by females (Gorman and Nager 2004), or differential egg investment which promotes postembryonic growth (Schwabl 1996; Lipar and Ketterson 2000). There was no difference in the length of the incubation period or in hatching success between the groups. Similarly, egg macronutrients, as reflected by egg size, did not vary with male age in a very large sample of broods from our population (Hargitai et al. 2005). Moreover, females of subadult males tended to lay eggs with higher yolk volumes, which is opposite to the difference in nestling development we detected (Michl et al. 2005). Finally, females in our population deposited more steroid into eggs if paired to subadult males than if paired to adult males (Michl et al. 2005), again suggesting that maternal effects may reduce rather than explain the origin effects we found.

Another explanation for the male age effect on nestling growth would be a year-specific sex ratio bias in relation to paternal age, accompanied by sex-specific growth trajectories. We cannot completely exclude this possibility, but brood sex ratio was unrelated to male age in a study of this population conducted in 2002, one of the average years of our experiment (Rosivall et al.

2004). In conclusion, the patterns of incubation, egg investment and sex ratio do not seem to explain the poor early growth of YSM. The fact that the growth disadvantage of YSM was present only in average years may be due to the increased importance of genetic quality in limiting environmental conditions (David et al. 2000). Alternatively, the compensatory steroid investment by females (Michl et al. 2005) may also have contributed to the lack of growth difference according to 'male of origin' in the good year.

#### Male age and genetic quality?

Our results are consistent with the notion that the offspring of subadult males are genetically inferior compared to those of adult males. The resulting growth difference was not offset by any late compensatory growth in average years, but it was completely absent in a good year. None of the male traits we measured explained the origin effect, which implies that other, unmeasured age-related determinants of male quality (for example, immunocompetence, Fair et al. 1999), may have caused the pattern. A genetic quality difference between subadult and adult males is controversial at first sight, as any subadult male is expected to become an adult male soon. However, several different approaches have shown that survival after the first breeding attempt may not be random with respect to individual quality. For example, in a Swedish population of marsh tits (*Parus palustris*), first year laying date and subsequent survival of females were negatively correlated, and there was no within-individual advancement in laying date. Thus, the observed 'improvement' of laying date among age categories was entirely the result of selection (Smith 1993). In Leach's storm petrels (*Oceanodroma leucorhoa*), Mauck et al. (2004) found a positive correlation between the early breeding performance and longevity of individuals. Finally, in willow warblers (*Phylloscopus trochilus*, Gil et al. 2001) and collared flycatchers (Török et al. 2003), there were age-related differences in condition-dependent, sexually selected male traits among but not within individuals, suggesting viability selection for high genetic quality at certain age transitions. In our case, the relationship between male age and genetic quality may be complicated by the fact that probably not all males breed in their first year of life, as judged from the low number of subadult breeders. It is impossible, however, to separate nonbreeding males from those breeding outside our study plots. Breeding outside the plots may occur because of either problems with habitat assessment (Pärt 2001b) or subordination in territorial competition (Slagsvold and Sætre 1991). In any case, the most probable outcome would be that only males of high quality breed as subadults on our plots, and these sire high quality young, a pattern opposite to that found in the present study.

Brooks and Kemp (2001) reviewed theoretical and empirical studies on age-related mate choice and offspring quality, and came to the surprising conclusion

that the only experiments suggesting a relationship between paternal age and genetic advantages to offspring came from insects (e.g. Price and Hansen 1998; Jones et al. 2000). This statement holds to the present day, with our study being the first attempt to investigate intrinsic offspring quality in relation to male age in a vertebrate. A link between male age and offspring quality has important implications for studies of nonrandom mating by females in relation to partner age. Evidence from several bird species indicates that females are reluctant to mate with young males. This seems to generally apply to social mate choice (e.g. Komers and Dhindsa 1989; Enstrom 1993; Sorenson and Derrickson 1994), which is easily explained by direct benefits such as parental or territory quality (see Introduction). Results for extra-pair mating in relation to male age are equivocal, with clear preference in some species (Wetton et al. 1995; Richardson and Burke 1999; Dickinson 2001; Karubian 2002), but apparently no preference in others (Stutchbury et al. 1997; Yezerinac and Weatherhead 1997; Krokene et al. 1998; Veiga and Boto 2000). Nonrandom extra-pair mating only rarely confers direct benefits to females (Gray 1997), and is frequently explained by the genetic quality of extra-pair young (Kempnaers et al. 1997; Sheldon et al. 1997).

Our results suggest that choice of an adult as compared to a subadult mate in either a social or an extra-pair context may confer genetic benefits such that their offspring grow better in average food conditions, but these benefits may not be apparent in a favourable environment. Whether females use male age as a mate choice cue is unclear. No study investigated social mate choice in relation to male age in the collared flycatcher, but in the sister species pied flycatcher Sætre et al. (1994) detected significant preference for an ornament very closely related to age. In a single-year study, Sheldon and Ellegren (1999) showed no effect of male age on extra-pair paternity, but more data are needed to clarify this point. Studies are required that explore the relationships between male age, mating preferences and offspring quality in a range of environmental conditions. Further investigations should also explore the physiological mechanism behind the parental age – offspring quality relationship.

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# Paper 4



## Maternal compensation for hatching asynchrony in the collared flycatcher *Ficedula albicollis*

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Several hypotheses have been proposed to explain the adaptiveness of hatching asynchrony for the parents, but delayed hatching is generally detrimental for the late hatched young. These offspring often experience competitive disadvantage and delayed development. If hatching asynchrony has a reason other than producing competitive differences among offspring, it would be advantageous, not only for the offspring but even for the parents, to compensate for its detrimental effects. In some species, increasing investment into later laid eggs has been reported and discussed as a compensation mechanism, but its effect on nestling growth and fledging size has not been examined in details. In this study we investigated nestling growth and size at fledging in terms of body mass and length of primaries in relation to the accurate laying and hatching order in collared flycatcher *Ficedula albicollis* broods. We found that females laid larger eggs at the end of the laying sequence, and this helped to decrease the disadvantages for the last offspring. The last offspring had lower body mass growth rate and fledged with shorter feathers, but in both cases the larger the last egg was, the smaller the lag of the offspring was. We conclude, that even if females were not able to fully compensate for the detrimental effects of hatching asynchrony, larger eggs may improve the survival prospects of late hatched nestlings.

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In many bird species females start to incubate their eggs before the whole clutch has been laid, thus the last laid eggs hatch asynchronously. Hatching asynchrony (HA) may provide various benefits to the parent birds (Stenning 1996). For example, by starting to incubate their clutches before completion, females can reduce predation risk to their brood, because some of their offspring start to develop and fledge earlier, so the average time spent in the nest will be shorter (nest failure hypothesis, Hussell 1972). Moreover, earlier onset of incubation may also result in higher hatching success, because the viability of eggs, if they are not incubated, declines with time (egg viability hypothesis, Arnold et al. 1987, Veiga 1992).

The brood reduction hypothesis (Ricklefs 1965, Lack 1968, Forbes et al. 1997) links parental benefit from HA to environmental unpredictability. According to this hy-

pothesis, the last hatched offspring is a surplus that may die if the food is scarce, but confers extra benefit to their parents if the food is abundant. For further hypotheses on HA see e.g. Nilsson (1993) and Stenning (1996).

For the last offspring, these hypotheses predict reduced survival probability (Oddie 2000), because delayed hatching results in competitive disadvantage (Price and Ydenberg 1995, Ostreiher 1996) and reduced fledging weight (Cotton et al. 1999, Clotfelter et al. 2003). If the brood reduction hypothesis is true, parents have no interest in compensating for this disadvantage, while in case of the egg viability and nest failure hypotheses, parents benefit from compensation. The most obvious way to achieve such a compensation is preferential feeding of the later hatched offspring by one or both of the parents (Gottlander 1987). However, compensation may also act through preferential

maternal investment into the later laid eggs (Howe 1976, Schwabl 1993, Schwabl 1996, Cichoń 1997, Lipar and Ketterson 2000, Eising et al. 2001). This differential investment may be manifested in an elevated level of testosterone to increase the competitive ability (Schwabl 1993, Lipar and Ketterson 2000) and the development of nestlings (Schwabl 1996, Eising et al. 2001). Females may enhance the survival prospects of their nestlings also through preferential nutrient investment into the eggs (Howe 1976, Cichoń 1997, Royle et al. 1999, Reynolds et al. 2003). On the other hand, species with a brood reduction strategy may reduce the investment into the last laid eggs (testosterone: Schwabl et al. 1997; nutrients: Arnold 1989, Heeb 1994, Viñuela 1997), thereby exaggerating the competitive disadvantage of the surplus offspring.

Egg size is known to correlate with the nutrient investment. Several studies have reported that egg size reflected the lipid and/or protein content of the eggs (Meathrel and Ryder 1987, Williams 1994, Hill et al. 1995, Royle et al. 1999, Jager et al. 2000, Badzinski et al. 2002, Reynolds et al. 2003), providing an indirect measure of maternal favouritism through egg nutrients.

In our study we investigated whether collared flycatcher *Ficedula albicollis* females compensated for or aggravated the disadvantageous effect of HA on the later hatched nestlings. In this species, parents do not discriminate between nestlings of different size when they allocate food (Rosivall et al. 2005), thus parental compensation for HA is possible only during the egg-laying period. To assess nutrient investment into the eggs and its effect on nestling development, we measured egg size and nestling growth. Some earlier studies have already attempted to investigate the effect of laying order on the growth and fledgling condition of nestling passerines, but very few studies linked the growth of individual nestlings to the exact laying and hatching order (Badyaev et al. 2002), and these studies did not focus on possible maternal compensation for the detrimental effects of HA.

## Methods

### Study species and field methods

This study was conducted in an artificial nest box plot in the Pilis Mountains, Hungary (47°43'N, 19°01'E) in 2002 and 2003. The study plot is a part of a continuous, unmanaged, oak-dominated woodland, a protected area of Duna-Ipoly National Park. For a more detailed description of the study site see Török and Tóth (1988). The collared flycatcher is a small hole-nesting, long-distance migratory passerine bird. The breeding season starts in mid April. Females usually lay 5–7 eggs. The eggs hatch approximately 12 days after the last egg was laid. Nestlings usually fledge 14–15 days after hatching.

We studied altogether 45 broods (22 in 2002 and 23 in 2003). In order to exclude possible confounding seasonal effects on egg size and nestling growth, these broods were selected so that the first egg was laid within a 6 and 4 day interval (in 2002 and 2003, respectively). All eggs were numbered with a permanent marker on the day of laying. The length and breadth of eggs were measured to the nearest 0.1 mm using a calliper. All clutches were placed into an incubator (PL Machine SK75) one day before the expected hatching date and replaced with dummy eggs of approximately equal size. All females accepted these dummy eggs as their own and continued the incubation. The original eggs were hatched in separated compartments at 37.2°C and 70–80% humidity. All embryos, which were still alive when placed into the incubator, hatched successfully. We checked hatching every hour from 4:15 am to 9:00 pm. For eggs hatching during the night we assumed that they hatched halfway between the last and the first checking.

Each hatchling was weighed to the nearest 0.01 g with an electronic balance (Mettler PM4800), marked individually on their breast with a permanent non-toxic pen and returned to their nest immediately, or early the following morning if they hatched during the night. Colour marking was randomized in relation to hatching order. We followed body mass increase of the nestlings in all broods from the day when the first chick(s) in a brood hatched (day 0) and the growth of the wing feathers in 34 broods from day 8 until fledging. Body mass and the length of the third outer primary were measured every second day to the nearest 0.1 g and 0.5 mm respectively. On day 14, body mass was measured in all broods, and the length of the third outer primary was measured in all but one brood.

### Data analysis

In this study we used 45 clutches consisting of 6 or 7 eggs. Because it may be different being laid sixth in a clutch of 6 or 7 eggs, we ranked each egg into one of the following five categories: first, second, middle, penultimate and last laid egg. See e.g. Magrath et al. (2003) for a similar grouping.

Egg volume ( $V$ ) was calculated according to the formula  $V = -0.042 + 0.4976 \times L \times W^2$ , where  $L$  = egg length and  $W$  = egg width, described by Ojanen et al. (1978) for a sibling species, the pied flycatcher *Ficedula hypoleuca*. Place of a chick in the hatching order was described by the hatching time, which was calculated as the time elapsed between the first hatching in a brood and the hatching of the chick in question. Hatching time was therefore 0.0 h for the chicks hatched first in a brood. Mass was found to increase logistically with time, so we calculated the rate of mass increase ( $K$ ) for each individual using the following logistic growth model:



$W = A/(1 + \exp(-K \times (t - t_i)))$  (Starck and Ricklefs 1998) where  $t$  was the accurate age of the focal individual, calculated from the hatching time. The growth of the primaries was linear so we used the slope of a linear regression to describe feather growth rate (Nilsson and Svensson 1996) and we did not control for the accurate hatching time.

Unless stated otherwise, analyses were performed using general linear mixed models with laying order as repeated measure factor and year and brood size as factors. The covariance structure of the model was selected on the basis of Schwarz's Bayesian Information Criterion (Littell et al. 1996). For all but one of the dependent variables the best fit was achieved by using first order autoregressive covariance, therefore we used this covariance structure throughout the analyses. This covariance structure assumes that measurements closer in the repeated measure sequence are more similar to each other, which is our expectation if eggs are laid sequentially. Denominator degrees of freedoms are obtained by Satterthwaite approximation and are therefore not integers. Post hoc comparisons were performed contrasting the groups in question in the Test Subcommand of the SPSS. Statistical analyses were performed using SPSS 11.0 and Statistica for Windows 4.5.

Sample sizes varied among dependent variables. Five broods (2 and 3 in 2002 and 2003, respectively) were not included in the nestling growth analyses, because females reared their young alone. Inclusion of these broods in the investigation of egg size patterns did not change the result, therefore they were kept in this analysis. Feather growth was measure in 34 broods only, therefore this analysis based on a smaller dataset. Because of unhatched, died or not measured nestlings some data were occasionally missing. However, hatching success and subsequent nestling survival (95.8% and 98.8%, respectively) were not related to laying order ( $\chi^2$ -test were used to compare the distribution of hatched/survived chicks to the number of observations throughout the laying order; hatching success:  $\chi^2 = 0.615$ ,  $df = 4$ ,  $P = 0.961$ ; survival:  $\chi^2 = 0.080$ ,  $df = 4$ ,  $P = 0.999$ ) and therefore they are not expected to affect the outcome of our analyses.

## Results

We found that in collared flycatcher clutches, egg volume increased with laying order (Table 1, Fig. 1). Hatching mass showed a similar increase (Table 1), and it was the consequence of increasing egg volume, because when the residuals of hatching mass on egg volume were entered into the mixed model, laying order was no longer significant ( $F = 0.40$ ,  $df = 4$ ,  $115.85$ ,  $P = 0.81$ ).

The significant overall effect of laying order on hatching time indicates that broods hatched asynchronously, but there was a difference between the two study

Table 1. The effect of laying order (first, second, middle, penultimate, last), brood size (6, 7) and study year (2002, 2003) on egg volume, hatching time (time since the first nestling in the focal brood hatched), nestling growth (K of the logistic mass growth curve and the slope of the linear feather growth) and fledging size (body mass and length of the third outer primary on day 14). Displayed are F values with degrees of freedom in parentheses. Asterisks indicate the level of significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.005$ ).

	Egg volume	Hatching mass	Hatching time	Mass growth	Feather growth	14 d mass	14 d feather
Brood size	1.06 (1, 41.90)	1.17 (1, 36.19)	10.76 (1, 51.43)***	0.25 (1, 43.15)	0.02 (1, 34.63)	0.05 (1, 40.83)	0.03 (1, 38.81)
Year	1.02 (1, 41.90)	0.03 (1, 36.19)	2.35 (1, 51.43)	0.23 (1, 43.15)	0.33 (1, 34.63)	0.99 (1, 40.83)	22.99 (1, 38.81)***
Laying order	15.65 (4, 154.69)***	5.14 (4, 123.08)***	135.13 (4, 114.13)***	7.55 (4, 121.07)***	1.52 (4, 88.90)	0.24 (4, 124.35)	15.71 (4, 111.24)***
Brood size $\times$ year	1.29 (1, 41.90)	2.29 (1, 36.19)	0.64 (1, 51.43)	1.31 (1, 43.15)	0.17 (1, 34.63)	2.32 (1, 40.83)	1.82 (1, 38.81)
Brood size $\times$ laying order	1.08 (4, 154.69)	0.34 (4, 123.08)	1.85 (4, 114.13)	1.30 (4, 121.07)	1.68 (4, 88.90)	0.75 (4, 124.35)	1.62 (4, 111.24)
Year $\times$ laying order	1.18 (4, 154.69)	0.28 (4, 123.08)	2.02 (4, 114.13)	2.27 (4, 121.07)	0.62 (4, 88.90)	1.40 (4, 124.35)	0.33 (4, 111.24)
Brood size $\times$ year $\times$ laying order	0.68 (4, 154.69)	0.87 (4, 123.08)	2.49 (4, 114.13)*	0.72 (4, 121.07)	3.27 (4, 88.90)*	0.22 (4, 124.35)	0.18 (4, 111.24)

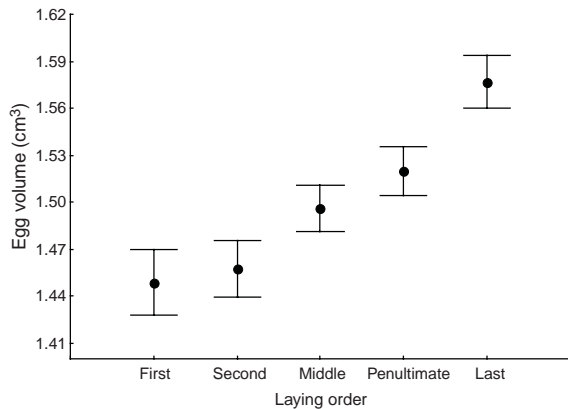


Fig. 1. Egg volume (mean  $\pm$  SE) in relation to laying order in collared flycatcher broods.

years (Table 1, Fig. 2). In 2002 the last egg hatched later in clutches with 6 eggs ( $P < 0.001$ ), while the last two in clutches with 7 eggs (both for last-earlier laid and

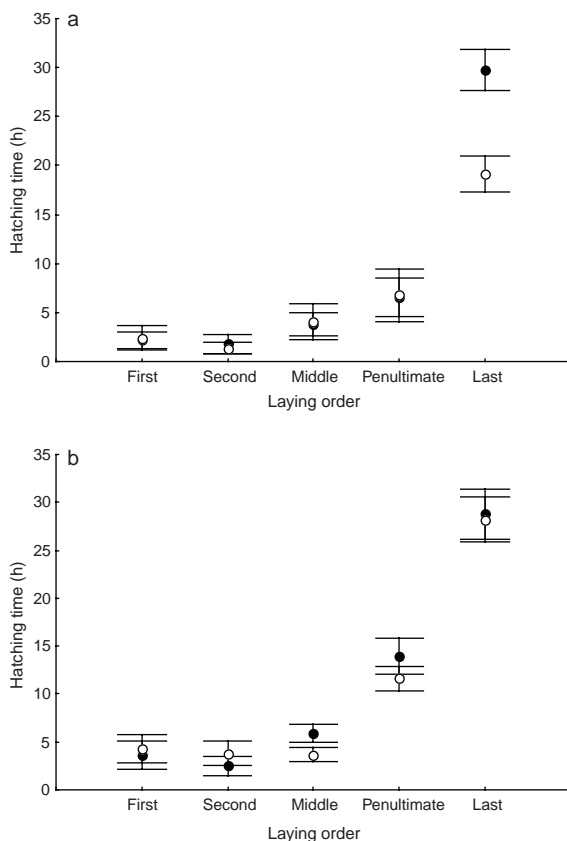


Fig. 2. Hatching time (mean  $\pm$  SE) in relation to laying order in collared flycatcher broods of six (a) and seven (b) eggs. Hatching time is the time elapsed between the first hatching in a brood and the hatching of the chick in question. Hatching time was therefore 0.0 h for the chicks that hatched first in a brood. Open circles: 2002; filled circles: 2003.

penultimate-earlier laid comparison  $P < 0.001$ ). In 2003 the last two eggs hatched later independent of the brood size (all  $P < 0.007$ ). When we entered the hatching asynchrony (the time difference between the first and last hatching) to an ANOVA with brood size and year as factors, the overall year effect was significant ( $F = 5.62$ ,  $df = 1, 33$ ,  $P = 0.024$ ). In 2003 hatching asynchrony was more pronounced (mean  $\pm$  SE was  $25.20 \pm 1.78$  and  $29.50 \pm 1.65$  in 2002 and 2003, respectively). The difference was more pronounced for the 6-egg clutches (year  $\times$  brood size:  $F = 4.12$   $df = 1, 33$ ,  $P = 0.051$ ).

Though the laying order  $\times$  brood size  $\times$  year interaction was significant, there was no overall effect of laying order on the growth of primaries (Table 1). However, laying order affected the mass increase of the nestlings (Table 1, Fig. 3). Nestlings hatched from last laid eggs experienced lower growth rate (K). In spite of the lower mass increase, there was no relationship between laying order and body mass before fledging (day 14; see Table 1). However, the primaries of the chicks hatched from the last egg were shorter than those of their nestmates (Table 1, Fig. 4). These results were consistent across years, even though in 2003 the length of the primaries was overall significantly shorter than in 2002.

The compensatory effect of larger last egg, can be tested by correlating the relative egg size of the last chicks (compared to their sibs) with their relative growth rate or fledging size. In the case of fledging mass, correlation with relative egg size is not expected, because in our broods, most chicks reached their maximal body mass on day 14 at the latest. However, the advantage of larger egg size was apparent, when we analysed its effect on body mass growth, while controlling for the effect of hatching asynchrony. In a multiple regression, relative hatching time (i.e. the difference between the hatching time of the last chick and the mean of its siblings) negatively affected the relative body mass growth rate

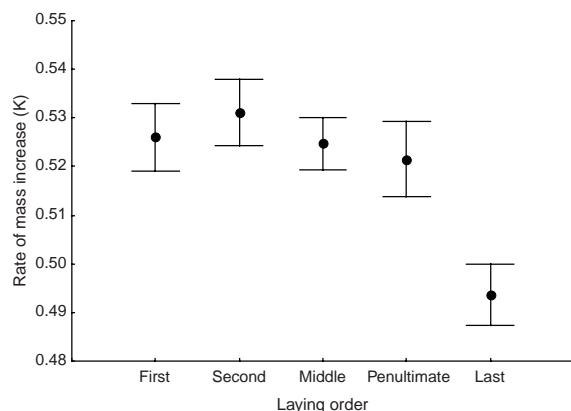


Fig. 3. Mass growth in relation to laying order in collared flycatcher broods. K is the growth rate from the logistic growth function  $W = A/(1 + \exp(-K \times (t - t_i)))$ , means  $\pm$  SE are shown.

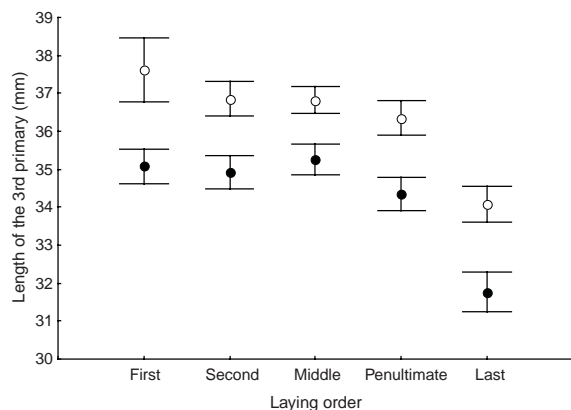


Fig. 4. Length of the third primary (mean  $\pm$  SE) before fledging (14 d) in relation to laying order in collared flycatcher broods. Open circles: 2002; filled circles: 2003.

of the last chick ( $\beta = -0.37$ ,  $F = 6.11$ ,  $df = 1$ ,  $32$ ,  $P = 0.019$ ), while relative egg size had a significant positive effect ( $\beta = 0.37$ ,  $F = 6.19$ ,  $df = 1$ ,  $32$ ,  $P = 0.018$ ). Relative egg size also tended to affect the feather growth rate ( $\beta = 0.35$ ,  $F = 3.58$ ,  $df = 1$ ,  $27$ ,  $P = 0.069$ ), though it was not affected by the relative hatching time ( $F = 1.14$ ,  $df = 1$ ,  $27$ ,  $P = 0.29$ ). Finally, the lag of the last chick before fledging, in terms of feather length, was affected by both relative hatching time ( $\beta = -0.41$ ,  $F = 7.68$ ,  $df = 1$ ,  $32$ ,  $P = 0.009$ ) and relative egg size ( $\beta = 0.32$ ,  $F = 4.73$ ,  $df = 1$ ,  $32$ ,  $P = 0.037$ ). The later the nestling hatched compared to its siblings, the larger its disadvantage was, while this disadvantage was decreasing with increasing egg size.

## Discussion

Here we aimed to investigate whether collared flycatchers compensate for, or magnify the detrimental effects of hatching asynchrony (HA) on the last hatched offspring by egg size differences. From this viewpoint, hypotheses concerning HA (reviewed in Stenning 1996) can be divided into two groups. Some of them (e.g. brood reduction hypothesis, sibling rivalry hypothesis) assume that parents start to incubate before clutch completion in order to aggravate the competitive differences among nestlings (Lack 1968), while others (e.g. nest failure hypothesis, egg viability hypothesis) assume that competitive differences are only the cost of the earlier onset of incubation which is adaptive for other reasons than sibling size asymmetry (Hussell 1972, Clark and Wilson 1981, Arnold et al. 1987, Veiga 1992). If females start to incubate before the clutch is complete to enhance the competitive differences, they are not expected to compensate for the disadvantage of the last chick, they may even reduce the investment into the later laid eggs (Heeb 1994, Schwabl et al. 1997, Viñuela 1997). In other cases

compensation for the disadvantage of the last hatched chick may increase the fitness of the females. In species with no conspicuous physical competition among nestlings, but with pronounced HA, most hypotheses predict that establishing competitive differences and reducing investment into later laid eggs has probably no adaptive value (but see insurance egg hypothesis, Clifford and Anderson 2001). Indeed, in collared flycatchers where there is no direct aggression between the nestlings (pers. obs.), we found that females increase the egg size with laying order, thus probably providing nutritional help for the later hatching young.

Some previous studies have also found similar egg size increase in relation to laying order (Howe 1976, Cichoń 1997) and hypothesized that it might adaptively reduce the detrimental effects of HA. In this paper, we investigated the growth and fledging size of individual nestlings. We found, that body mass before fledging was not related to laying order, which might be the effect of the logistic nature of the growth curve (i.e. older siblings finished their growth a few days before fledging, thus the last chicks had time to catch up), or the joint effect of logistic mass growth and larger last eggs. The fact that independent of the relative egg size most of the last chicks reached their maximum body mass on day 14 at the latest, might indicate that larger egg size is not needed to reach the same size as their older siblings, but it is hard to draw conclusions, because we do not exactly know how they would have grown if they had hatched from smaller eggs. However, nestlings from last laid eggs experienced slower body mass growth, and had shorter primaries before fledging than their siblings. These disadvantages were increasing with increasing HA, but were partially counterbalanced by the larger egg size. The growth of the primaries was not related to laying order, probably because synchronized fledging is important for the survival prospects of the nestlings, and therefore, they allocate more energy into growing their wing feathers at the cost of lower body mass increase (Nilsson and Svensson 1996, Nilsson and Gårdmark 2001). Still relative size of the last egg tended to correlate positively with the relative feather growth rate of the last chicks.

Reduced growth or bad body condition early in the life was reported to have negative impact, for example, on immune responsiveness, intensity of parasite infection, adult condition, time of sexual maturation and long-term survival in vertebrates, even if they could catch up later in size (Birkhead et al. 1999, Morgan and Metcalfe 2001, Blount et al. 2003, Stjernman et al. 2004). Late or asynchronous fledging due to shorter feathers, may also be disadvantageous. For instance, parents preferentially fed fledged chicks if fledglings and nestlings were begging simultaneously in the great tit *Parus major* (Lemel 1989). Thus, we argue that even the partial compensation for these adverse effects of HA by laying

larger final eggs, probably increased the survival prospects of the last chicks and thus the fitness of the parents. Though maternal compensation is possible not only through egg size, which is known to correlate with the amount of macronutrients, but also through biomolecules (Schwabl 1993, Lipar and Ketterson 2000, Eising et al. 2001), no preferential testosterone investment in relation to laying order has been found in this species (Michl et al. 2005).

The HA and nestling development patterns within broods were consistent across years in collared flycatchers. The only difference between the two subsequent years was that HA was higher and penultimate eggs hatched relatively later in 2003 (Fig. 2), which probably means that in this year females started to incubate their broods earlier. This phenomenon is presumably the consequence of the higher mean temperature in 2003. The daily maximum temperature exceeded 25°C some days and 20°C every day during the egg-laying period of the studied broods (Török et al. unpubl. data). Such temperatures may cause lower egg viability if eggs are not incubated (von Schalkwyk et al. 1999, Viñuela 2000, Sahan et al. 2003). Thus it may explain why females started to incubate relatively earlier in this year. Alternatively, higher mean temperatures may result in better food supply, which may cause increased HA (Nilsson 1993).

In conclusion, we found that last hatched nestlings experienced slower body mass growth, and fledged with shorter wing feathers than their older siblings, but both were partially compensated by larger last eggs. Since relatively larger wing feathers may minimize the flying ability handicap of the late-hatched nestlings after fledging, and relatively faster growth early in life may increase the survival prospects of these chicks, the increase of egg size with laying order may increase the probability that all nestlings from the focal brood survive. Thus the observed egg size pattern is beneficial for both the last chick and the parents.

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# Paper 5







## FOOD ALLOCATION IN COLLARED FLYCATCHER (*FICEDULA ALBICOLLIS*) BROODS: DO RULES CHANGE WITH THE AGE OF NESTLINGS?

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**ABSTRACT.**—Food allocation among nestlings has a strong influence on parental fitness. Maximizing fledging success in a favorable environment requires food distribution based on offspring need signals. However, food limitation, differences among individual nestlings in their quality, or variation in the costs of rearing different young may result in preferential allocation of food by parents. If signals of nestling quality or need change in meaning with age, parents are expected to adjust their feeding rules to those changes. We examined food allocation in broods of Collared Flycatchers (*Ficedula albicollis*), a sexually size-monomorphic passerine. In a multivariate analysis, we investigated the role of sex, size, condition, position, and begging intensity in two nestling ages. Size, condition, and sex of nestlings did not affect parental decisions. Begging intensity and nestling position, however, had a role in food allocation in both age categories. Both parents preferred the more intensely begging nestlings. Males did not show clear position preference in the “young” age category, but had preferred positions with older nestlings. Female position preference was observed in both age categories. Preferred positions of male and female parents differed; still, we observed overall position preference. Received 2 February 2004, accepted 11 March 2005.

Key words: begging behavior, Collared Flycatcher, *Ficedula albicollis*, food allocation, parental care.

### Asignación de Alimentos en Nidadas de *Ficedula albicollis*: ¿Cambian las Reglas con la Edad de los Pichones?

**RESUMEN.**—La asignación de alimentos entre los pichones tiene una influencia fuerte sobre la adecuación biológica de los padres. La maximización del éxito de emplumamiento en un ambiente favorable requiere que la distribución de la comida se base en las señales de necesidad emitidas por la progenie. Sin embargo, la limitación en los alimentos, las diferencias en la calidad entre los pichones o la variación en los costos de criar a distintos jóvenes puede conllevar a la asignación preferencial de alimentos por parte de los padres. Si las señales de calidad o de necesidad de los pichones cambian de significado con la edad, se espera que los padres ajusten sus reglas de provisión de alimentos en relación con esos cambios. Examinamos la asignación de alimentos en nidadas de *Ficedula albicollis*, una especie paserina en la que el tamaño de ambos sexos es igual. En un análisis multivariado, investigamos el rol del sexo, el tamaño, la condición, la posición y la intensidad de reclamos para dos edades de pichones. El tamaño, la condición y el sexo de los pichones no afectaron las decisiones de los padres. Sin embargo, la intensidad del reclamo y la posición de los pichones afectaron la asignación de los alimentos en ambas categorías de edad.

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Ambos padres priorizaron a los pichones que reclamaron con mayor intensidad. Los machos no mostraron preferencia clara por una posición dada de los pichones de edad "joven", pero prefirieron determinadas posiciones en los pichones más viejos. La preferencia por parte de las hembras de una posición dada fue observada en ambas categorías de edad. Las posiciones preferidas de los padres y las madres difirieron; aún así, observamos una preferencia de posición generalizada.

FOOD ALLOCATION PATTERNS may result from nestling competition (Mock and Parker 1986, Ostreiher 1996, Viñuela 1999), active parental decisions (Gottlander 1987, Lyon et al. 1994, Kilner 1997, Kölliker et al. 1998, Krebs et al. 1999, Loughheed and Anderson 1999, Budden and Wright 2001, Leonard and Horn 2001b), or both. The importance of the two factors may vary considerably among species; but if parents have any control over food allocation, they are expected to base their decision on the need of young (Godfray 1991), while also taking into account the possible costs and benefits of rearing an individual offspring (Stamps 1990, Kilner and Johnstone 1997).

Begging behavior is known to correlate with offspring need in several species, and parents apparently use this behavior as a signal when distributing food (Hussell 1991; Price and Ydenberg 1995; Price et al. 1996; Kilner and Johnstone 1997; Delman 1998; Kölliker et al. 1998; Lotem 1998; Roulin et al. 2000; Saino et al. 2000; Leonard and Horn 2001a, b; Porkert and Špinka 2004). If nestlings do not cheat and parents are able to cover the needs of all young, food allocation based on begging intensity is optimal for parents (Godfray 1991). However, when food is scarce and provisioning cannot cover the needs of all young, we might expect parents to use cues other than begging in such a way that the nestlings with better survival prospects are preferred. Investment in those chicks is more likely to result in fitness benefit than investment in their nestmates.

For example, preference for large nestlings has been found in several species (see Teather 1992, Smiseth et al. 1998, Saino et al. 2000). We hypothesize, however, that size differences may not have the same meaning just before fledging as they do when nestlings are young. Even in cases where all young hatch within 24 h, the last-hatched chick is distinguishably smaller for a few days after hatching. During those first days, size differences presumably do not reflect quality differences among nestlings, so

neglecting smaller young may not be adaptive. Favoring those young may even give them a chance to catch up with their nestmates, so that they can fledge with a similar weight (Stamps et al. 1985). Just before fledging, by contrast, size differences more likely result from differential growth rate attributable to individual quality (Sheldon et al. 1997) or diseases (Fair et al. 1999) or both. If so, less-developed, smaller nestlings are of lower quality, and therefore return smaller benefit to their parents. Discrimination against such nestlings can thus be adaptive even when food is abundant.

Because they differ in expected reproductive success, male and female offspring may also differ in the benefit they confer to parents. Whereas high-quality or more attractive males sire significantly more offspring than low-quality males (Sheldon et al. 1997, Sheldon and Ellegren 1999), such differences are smaller among females. Hence, the expected fitness of low-quality female offspring may trump that of low-quality male offspring. Among low-quality offspring, then, female nestlings would confer greater benefit to their parents than males; but among high-quality offspring, males would return greater benefit than females. As a result, if discrimination between male and female nestlings is possible (e.g. on the basis of vocal or behavioral differences), parents are expected to favor the sex with the higher future reproductive value under the prevailing circumstances.

The future reproductive value of nestlings, however, depends not only on their genetic quality, but also on their rearing environment. Martins (2004) found that female Zebra Finch (*Taeniopygia guttata*) chicks were more sensitive to changes in food supply than males. Malnourished females had lower growth rates and suffered higher mortality than males, but there was no such difference when food was plentiful. Thus, we might expect preference for the less sensitive sex in poor environmental conditions, even if nestlings are sexually size-

monomorphic. However, discrimination for offspring sex in food allocation has been tested only in a few sexually dimorphic passerines (Teather 1992, Whittingham et al. 2003), but not in monomorphic species.

The benefit returned by a certain offspring is not necessarily the same for the two parents. For instance, in species with extrapair matings, female parents are equally related to all young; thus, they gain fitness benefit from all offspring, whereas extrapair young do not increase the fitness of male parents. It has been hypothesized that in case of changing probability of extrapair mating during the female's fertile period, males may use nestling size as a predictor of their paternity (Slagsvold 1997). In Collared Flycatcher (*Ficedula albicollis*), extrapair young are expected from the third- and later-laid eggs (Michl et al. 2002). Thus, uncertainty of paternity may result in male preference for larger chicks (i.e. preference for those more probably sired by themselves), whereas females are expected to prefer small young (i.e. extrapair young with higher reproductive potential) or have no preference.

The aim of our study was to investigate, with multivariate statistical models, how individual characters (size, body condition, sex), position, and begging intensity of nestlings affected food allocation in Collared Flycatcher broods. To our knowledge, no previous study has tested so many variables simultaneously and, in particular, there are no data on the role of sex in species with size-monomorphic nestlings. As discussed above, the meaning of some of these nestling attributes may change with nestling development. If so, we predicted that the allocation rules would also change. Therefore, we performed our study on two nestling ages. Constraints on and previous investment by the two parents may also differ; hence, we investigated whether their food allocation rules differed.

#### METHODS

*Study site and species.*—The study was conducted in a nest-box plot in the Pilis Mountains, Hungary, between 19 May and 6 July in 1999 and 2000. The Collared Flycatcher is a small, hole-nesting, socially monogamous passerine. Females usually lay five to seven eggs. Both parents provision young. Fledglings leave the nest 14–15 days after hatching. Males with

large forehead patches are preferred in both social and extrapair mate choice; hence, they have higher reproductive success (Sheldon et al. 1997, Sheldon and Ellegren 1999, Qvarnström et al. 2000, Michl et al. 2002).

*Experimental design.*—Feeding rates (feeding per hour per offspring) did not differ between the two years ( $t = -0.42$ ,  $df = 10$ ,  $P = 0.757$ ), so we pooled the data. Median brood size was six in both years, with hatching of all young completed within 24–48 hours. We chose synchronously hatched (i.e. within 24 h) broods with six nestlings. Synchrony was important so that nestlings were in a similar developmental phase, with the same begging ability and behavioral repertoire (Muller and Smith 1978, Khayutin 1985).

At the experimental nests, we replaced the nest boxes with special ones that had the same inner dimensions but had a back chamber, hidden from the parents, in which we mounted a small videocamera. The camera was directed at a mirror at the top of the fore chamber and therefore viewed the brood from above. The mirror did not cause any behavioral anomalies during feeding (B. Rosivall pers. obs.). The changes were made one day before the experiment, so that the parents could get accustomed to the new nest boxes.

We marked nestlings individually with black marks on white paint patches on the tops of their heads when the nest boxes were changed. By using markings of the same color, we precluded the possibility of parents preferring young according to colors. We also randomized the marks among nestlings.

Recordings were made at two developmental phases (4–5 days and 10–11 days after hatching). Each recording was 3 h long. At each nest, we made two types of recordings. The first time, we videotaped the nest under natural conditions (i.e. with the six nestlings allowed to move freely). On the following day, we controlled for nestling size and position. In this size–position manipulation, we replaced the nest's six offspring with four foreign nestlings. The introduced nestlings matched the original ones in age (the difference was not more than one day), but two were "large" and two "small." We had selected the chicks so that mass difference between those two size categories was as great as that in asynchronously hatched broods. Thus, we offered two clearly distinguishable classes of chicks, in place of the six nestlings with a continuous size spread, to

facilitate detection of a size preference, even if it was weak. To avoid possible effects of (1) parents preferring their own nestlings over foreign ones and (2) behavioral differences of nestlings in native versus foreign broods (Teather 1992), we used foreign offspring exclusively. Each nestling was used in only one experiment.

In the size–position manipulation, nestlings were separated from each other by a cross-shaped wooden wall, which was placed in the nest 105–120 min before videotaping began so that parents could get used to the changes. The wall was high enough to prevent nestlings from changing their position but small enough to allow brooding in the ectothermic phase. This experimental design controlled the physical competition of nestlings, because they could not jostle. Nestlings of the same size were placed diagonally opposite to each other. The layout was turned 90° every 45 min during the 3-h period; each nestling occupied each part of the nest for one period. Thus, the effects of size and position could be analyzed independently.

*Data and statistical analysis.*—When analyzing the recordings, we considered the first feeding attempt with each food item as the decision of a parent, irrespective of whether the offspring swallowed the food; we refer to such events as “feedings.” Cases when only one nestling begged for food were omitted from analysis, because we wanted to know what rules parents use in deciding between hungry chicks. Morphological parameters (body mass, tarsus) of the young, measured just before the observation, were treated as constants. Sex of nestlings was determined by Molecular Diagnostic Services (Durban, South Africa) from blood samples collected after the last recording had been completed. Position and begging intensity of nestlings were scored just before the parental decision (unambiguous movement toward a nestling). Begging intensity was scored according to body posture and movements, as follows: 0 = no begging; 1 = beak opened, head down; 2 = beak opened, neck stretched; 3 = beak opened, neck stretched, standing; 4 = beak opened, neck stretched, standing, and wings flapping. Coding of position was different in natural and experimental recordings (see below).

To avoid any bias in the data, all recordings were processed by one person without any knowledge of the individual nestling characters. Feeding events of the two parents were

processed independently from each other. Statistical analyses were performed with SAS, version 8.2 (SAS Institute, Cary, North Carolina) and STATISTICA for Windows, version 4.5 (StatSoft, Tulsa, Oklahoma).

*Natural conditions.*—We examined the effects of offspring condition, sex, begging intensity, and position in the nest. Condition was assessed as the residual of tarsus–weight linear regression (4 days after hatching:  $r^2 = 0.881$ ,  $F = 520.20$ ,  $df = 1$  and  $70$ ,  $P < 0.001$ ; 10 days after hatching:  $r^2 = 0.709$ ,  $F = 126.99$ ,  $df = 1$  and  $52$ ,  $P < 0.001$ ) and then standardized by calculating the departure from the brood mean. During analysis of the recordings, we scored nestling position using a  $3 \times 3$  grid on the screen, but, because of statistical constraints (i.e. to ensure sufficient number of observations in each position), we used four positions during statistical analysis (i.e. front = the row closest to the nest hole; left, middle, and right = the two rows in the back divided into three equal parts).

To control for possible correlations between explanatory variables, we used multivariate tests. Analyses were performed using the GLIMMIX macro of SAS. In the mixed models, we used binomial error distribution and logit-link to deal with the binomial response variable (fed and nonfed). Nestling condition was used as a continuous predictor; nestling sex, begging intensity, and position were used as factors. Nestling position was nested within broods, because we wanted to know if position preference existed, and not if a certain position was preferred. Sex of the parent and its interaction with the above-mentioned predictors were also included in the model, because the food allocation rules of the two parents might differ. Brood identity was included as a random factor to address the nonindependence of observations from the same brood. Denominator degrees of freedom were obtained by Satterthwaite approximation. Having analyzed our initial model for begging chicks, we performed a stepwise backward deletion of nonsignificant terms. If the resulting minimal model showed significant interaction between an offspring trait and sex of the parent, the analyses were performed also for the two parents separately. Analyses were performed on seven broods with young nestlings, but after removal of offspring sex and its interaction with parental sex from the model (those were nonsignificant terms),

we included two additional broods, for which we had no data on offspring sex. Thus, our final model is based on the data of nine broods. With old nestlings, all analyses were performed on eight broods (all nestlings were sexed).

With young nestlings, we were not able to include nestling position in our mixed model, probably because of the low feeding activity of males (and some females) and the lower number of begging offspring, which resulted in low numbers of observations in each position. Thus, the effect of nestling position was analyzed in chi-square tests. We compared the distribution of feedings among positions with the expected distribution of feedings based on the number of nestlings that begged in each position. Tests were performed for each brood and parent separately, the resulting chi-square values and degrees of freedom were summed, and a combined  $P$  value was calculated (Haccou and Meelis 1994).

The models described above are suitable only for finding general rules that do not differ between nests; any observed heterogeneity among nests remains unexplained. Therefore, we used other statistics for testing the male attractiveness-dependent sex preference. First, we calculated the success of chicks in obtaining food when they begged; then we obtained a mean for male versus female nestlings (we included only those feeding events in which at least one nestling of each sex begged). Then, the success ratio of the two sexes (male:female) was correlated with the attractiveness of males, using Spearman rank correlation. Attractiveness of males was quantified by forehead patch size.

*Size-position manipulation.*—Number of begging events and number of feedings during the four experimental sessions (i.e. observations of four different chicks) were summed for each position; then the distribution of the expected number of feedings was compared with the observed number of feedings as described for young nestlings under natural conditions.

When we tested the effect of nestling size on food allocation, we restricted the analysis to feeding events in which nestlings of different sizes begged for food. We calculated the average success ratio of small and large chicks (i.e.  $100 \times$  total number of feedings divided by total number of begging events for the two chicks of the same size during the four experimental sessions) and used paired  $t$ -tests to compare those data

pairs. The number of broods included varied among analyses. In the "young" age category, we had 10 experimental nests; in 3 of those, the number of male feedings was too low for chi-square tests; thus, the sample size was 7. One male was excluded from analysis of nestling-size effect on food allocation, because it distributed food only twice; thus, the resulting sample size was nine. Sample size for females, in both position and size, was 10. In the "old" age category for all analyses, sample size was 12. In cases where we had no data for all experimental sessions because of technical failures (three in the "young" category and two in the "old"), statistics were calculated for the remaining sessions.

## RESULTS

*Young nestlings.*—Our starting model included begging intensity and sex of nestlings as factors and relative condition of nestlings as a covariate; the interactions of those variables with parental sex were also included. After the backward deletion of nonsignificant terms, only begging intensity was retained in the model. Nestlings with higher begging-intensity scores were preferred by both parents (Table 1 and Fig. 1).

In the above models, we could only test the general rules that did not differ between nests, so the dependence of sex preference on parental attractiveness (forehead patch size) was tested in a single-parameter test. Our results showed no such preference (males: Spearman rank correlation,  $R_s = 0.39$ ,  $P = 0.383$ ,  $n = 7$ ; females:  $R_s = -0.64$ ,  $P = 0.119$ ,  $n = 7$ ).

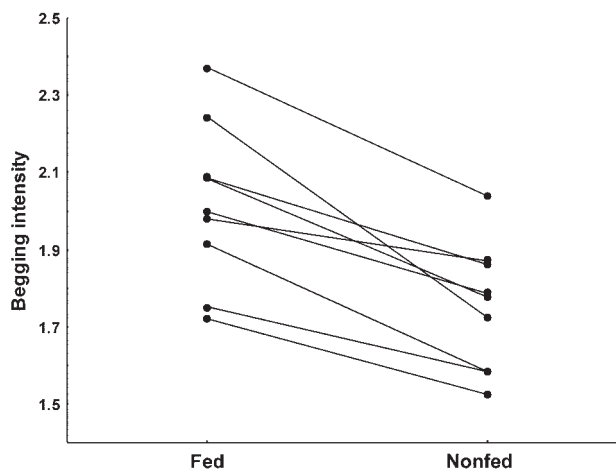


FIG. 1. Mean begging intensity of fed and nonfed Collared Flycatcher nestlings (4 days of age) for each brood.

TABLE 1. Factors affecting food allocation among Collared Flycatcher nestlings. Only those variables that were retained in the model after backward deletion of nonsignificant terms are given. Position (nested within broods) could not be included in the "young" age category (see text).

Age category	Predictor	For two parents			For males only			For females only		
		F	df	P	F	df	P	F	df	P
Young	Begging	26.03	2 and 1,404	<0.001	-	-	-	-	-	-
	Parent	1.74	1 and 3,446	0.187	-	-	-	-	-	-
Old	Begging	43.83	3 and 3,446	<0.001	22.85	3 and 1,650	<0.001	21.29	3 and 1,793	<0.001
	Position	6.92	31 and 3,446	<0.001	3.08	31 and 1,650	<0.001	5.64	31 and 1,793	<0.001
	Parent * position	2.02	31 and 3,446	<0.001	-	-	-	-	-	-

In the mixed model, we were not able to test for the effect of nestling position, probably because of the low feeding frequency of most males and two of the females. This could have caused problems during analysis, because nestling position was nested within broods (i.e. we wanted to test whether position preference exists, not whether a certain position is preferred). Therefore, we performed chi-square tests for each brood and each parent separately, comparing the observed distribution of feedings among positions with the expected distribution based on the number of begging events in those positions. The chi-square and degree-of-freedom values gained in those analyses were summed, and an overall *P* value was calculated (Haccou and Meelis 1994) for male and female parents separately. Although we found that during feedings by both male and female parents, position had an overall significant effect (males:  $\chi^2 = 60.07$ , *df* = 27, *P* < 0.001; females:  $\chi^2 = 134.85$ , *df* = 27, *P* < 0.001), the position effect was significant only in three broods for male parents and two broods for female parents. Thus, that preference is probably not strong. The most preferred positions of male and female parents were different. However, when position effect was tested independently of which parent fed the nestlings, the position effect was still significant ( $\chi^2 = 75.74$ , *df* = 27, *P* < 0.001). The preferred positions of the parents and the overall preferred positions varied among broods, but the nestlings in the middle position generally had a better chance of getting food than the average of each position.

In the manipulation experiment, we received somewhat different results. Distribution of feedings among the four positions deviated significantly from the expected distribution only when female parents fed the nestlings ( $\chi^2 = 70.00$ , *df* = 30, *P* < 0.001). Males had no position preference ( $\chi^2 = 20.56$ , *df* = 21, *P* > 0.1); their food distribution was based on how many times nestlings begged in different positions. When all feeding events of both parents were analyzed together, we found that in some positions, chicks had a better chance of being fed than in other positions ( $\chi^2 = 46.91$ , *df* = 21, *P* < 0.001), probably because of the larger share of female parents in feeding. Because there were no differences in the success of different-sized nestlings, we can conclude that size did not affect food allocation (male parents: paired

$t = -0.40$ ,  $df = 8$ ,  $P = 0.697$ ; female parents: paired  $t = -0.76$ ,  $df = 9$ ,  $P = 0.467$ ; together:  $t = -0.61$ ,  $df = 8$ ,  $P = 0.561$ ).

*Old nestlings.*—Our starting model included nestling position, sex, and begging intensity as factors, nestling condition as covariate, and their interactions with parental sex. After the backward deletion of nonsignificant terms, begging intensity had a significant positive effect on the probability of a nestling gaining food in this age category also (Table 1 and Fig. 2). Nestling position and its interaction with parental sex were also significant (Table 1). When we performed the analysis for male and female parents separately, we found that nestling position affected the food allocation of both parents significantly, so the significant nestling position \* parental sex interaction indicated that the preferred positions of the two sexes are different. The overall position effect, however, shows that, in spite of this difference, chicks still had a better chance of getting food in some positions than in others. That was probably the joint effect of overlapping preferred positions and difference in feeding activity of the two parents. As in the results with young chicks, the preferred positions (i.e. those in which a nestling's chance of obtaining food was greater than the average chance within that brood) of the two parents and the overall preferred positions differed among broods; but again, the middle position was generally successful.

Also as in the "young" age category, paternal attractiveness-dependent sex preference was

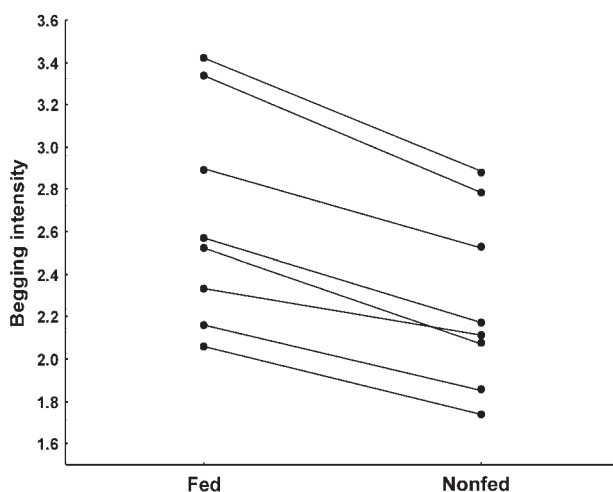


FIG. 2. Mean begging intensity of fed and nonfed Collared Flycatcher nestlings (10 days of age) for each brood.

not found in "old" nestlings (Spearman rank correlation, males:  $R_s = -0.26$ ,  $P = 0.531$ ,  $n = 8$ ; females:  $R_s = 0.57$ ,  $P = 0.139$ ,  $n = 8$ ).

The results of the manipulation experiment supported the above findings. Observed distribution of food among the four positions differed from the expectation, regardless of which parent fed the nestlings (male parents:  $\chi^2 = 101.83$ ,  $df = 33$ ,  $P < 0.001$ ; female parents:  $\chi^2 = 98.64$ ,  $df = 33$ ,  $P < 0.001$ ), and the overall probability of being fed also differed among positions ( $\chi^2 = 89.30$ ,  $df = 33$ ,  $P < 0.001$ ). Success of different-sized nestlings did not differ (male parents: paired  $t = 1.42$ ,  $df = 11$ ,  $P = 0.184$ ; female parents: paired  $t = 0.77$ ,  $df = 11$ ,  $P = 0.460$ ; together: paired  $t = 1.30$ ,  $df = 11$ ,  $P = 0.220$ ).

## DISCUSSION

We aimed to identify the nestling traits that Collared Flycatcher parents use as cues in their food allocation decisions. Our study identified two factors that seem to play such a role. First, several studies have suggested that begging intensity is a reliable signal of offspring need (Hussell 1991, Godfray 1991, Price and Ydenberg 1995, Price et al. 1996, Kilner and Johnstone 1997, Delman 1998, Kölliker et al. 1998, Lotem 1998, Roulin et al. 2000, Saino et al. 2000, Leonard and Horn 2001b), which implies that parents should consider that signal during food distribution among nestlings. Our results show that both parents of this species in both age categories (i.e. 4 and 10 days after hatching) preferred the more intensely begging—and presumably needier—offspring. Here, we might distinguish between short-term need (or hunger) and long-term need (i.e. the energy or food needed until fledging, which is thus dependent on nestling condition), each affecting the begging behavior of nestlings (Price et al. 1996, Lotem 1998). In our analyses, we statistically controlled for the effect of nestling condition on begging intensity, so we can conclude that begging intensity affected food allocation independently of offspring condition.

Position of nestlings also affected food allocation by Collared Flycatcher parents. With young nestlings, results were not concordant. The analysis of broods under natural conditions revealed a position effect for both male and female parents. We also found an overall position effect, when feeding events were

analyzed irrespective of the feeding parent. In the size–position manipulation experiment, however, males did not show preference for any position, whereas the result for females and the overall result were again significant. The reliability of the analysis of position effect was low for the broods under natural conditions, because of the small number of paternal feeding events. Furthermore, position effect could be confounded by parental preferences for certain chicks, which may spend a long time in the same position. In the size–position manipulation experiment, however, position effect could not have been confounded by chick quality, because of the experimental design. Thus, we argue that the results from that experiment are probably a better indicator of the role of position.

The effect of position was much clearer with “old” nestlings. Both female and male parents had preferred positions, and the overall position effect was also significant. Preferred positions of the two parents were significantly different but overlapped, which may be one reason for the overall position effect. Another reason may be the difference in feeding activity of the two parents. Though the preferred positions of male and female parents, and the overall preferred position, differed among broods, the middle position was generally among those in which nestlings’ chance of gaining food was greater than the average chance in the brood. That might also be explained by the overlapping position preference of the parents.

The overall position preference may make nestling competition possible, though its level may be reduced by the difference between the preferred positions of the parents. Similarly to our results, position and begging behavior of nestlings affected food allocation in many species (Gottlander 1987, Leonard and Horn 1996, Kölliker et al. 1998, Whittingham et al. 2003, Porkert and Špinka 2004). However, it is hard to determine the relative importance of those two factors. The relative role of active parental decision versus nestling competition in determining access to food may depend, for example, on food abundance or the ability of parents to meet the needs of their chicks. That is supported by the results of Porkert and Špinka (2004), who found more pronounced position preference in larger broods (i.e. in broods where we might expect stronger competition). In our broods, begging intensity seemed to have a more important role,

given that univariate within-brood analyses (results not shown) more often gave significant results for begging intensity (seven out of nine and eight out of eight cases for young and old nestlings, respectively) than for nestling position (three out of nine and six out of eight cases for young and old nestlings, respectively).

We found three factors—nestling size, condition, and sex—that did not affect food allocation. We expected size and condition preference in the “young” age category, because in species with hatching asynchrony, the last-hatched chick(s) is (are) smaller and possibly in worse condition a few days after hatching (Clotfelter et al. 2000), but size and condition at that age are probably independent of genetic quality. Thus, preference for those chicks can be advantageous, because it can help the chicks to catch up with their nestmates (Stamps et al. 1985) and thus increases the fitness of the parents. However, just before fledging, smaller size and worse condition may indicate not only later hatching, but also slower development attributable to lower genetic quality (Sheldon et al. 1997), infections (Fair et al. 1999), or both. Therefore, preference for chicks in poor condition may not be advantageous, even if food is abundant. The lack of age-dependent size and condition preference may indicate that the disadvantage of the last-hatched chick can be compensated by other parental behaviors, such as preferential maternal investment in late-laid eggs (Rosivall et al. 2005).

One might also expect parental sex-dependent size preference, because female but not male preference for small chicks or chicks in bad condition were found in some species (Stamps et al. 1985, Leonard and Horn 1996). It has been hypothesized that the fitness value of the actual brood can be larger for females, if females have shorter expected lifespans than males (Slagsvold 1997), as was suggested to be the case in passerines (Breitwisch 1989). Thus, females may invest more in chicks in bad condition. The cuckoldry hypothesis (Slagsvold 1997) also predicts a difference in the size preference of male and female parents, if the probability of extrapair mating is changing predictably during the fertile period of females. In Collared Flycatchers, extrapair chicks are expected mainly from the third- and later-laid eggs (Michl et al. 2002), but only the last or last two eggs hatch later (Rosivall et al. 2005); thus, size



preference may not help males avoid feeding of unrelated young. Size and condition differences among nestlings may also be hard to assess, or assessment may be too time-consuming, so that parents may use simpler rules to save time for foraging.

Sex of the offspring did not affect food allocation, either. Sex preference can be expected when the benefits of rearing male and female offspring differ. The lack of general sex preference is not surprising, because in species with sexually size-monomorphic fledglings, offspring sex-dependent survival was found only in case of food shortage (Martins 2004). Furthermore, in a Swedish population of Collared Flycatchers, such a difference was not found even in enlarged broods (Sheldon et al. 1998). However, preference for a certain sex in relation to male quality or attractiveness might still be advantageous for parents, because the reproductive performance of males is strongly affected by their attractiveness (Sheldon et al. 1997, Sheldon and Ellegren 1999). The lack of such a preference may suggest that, in this species, (1) nestlings of the two sexes are not distinguishable or (2) sex-specific differences in their future reproductive values are too small to favor sex-biased food allocation.

In conclusion, food allocation in Collared Flycatcher broods was determined by the begging intensity and position of chicks. Parents showed active and strong preference for the more intensely begging, and presumably needier, offspring. Partial nestling control, however, cannot be ruled out, because parents had preferred positions, for which chicks can compete. The relative importance of parental and nestling control may change, depending on food abundance. To investigate such changes, further studies are needed.

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## 15. Summary

Parental investment has a profound effect on the reproductive success of both parents and offspring. The amount and allocation of parental investment are therefore key questions in behavioural ecology. In iteroparous species, parental investment is distributed over multiple reproductive events, so we have to distinguish two levels of allocation: one within broods and one among subsequent broods. The quality of the young may vary considerably at both levels, so their contribution to parental fitness may also differ. As a consequence, an even allocation of resources is not necessarily the best option for the parents, and preferential allocation is expected to evolve. In my thesis I studied the patterns of various parental investments both between and within avian broods. Two studies focused on sex allocation. We can expect that individuals facultatively manipulate the production of male and female offspring when the costs or the benefits of rearing a chick are sex dependent. Many studies had found adaptive departure from parity in brood sex ratios, still the existence of the phenomenon had been questioned, because of the contradictory results. To help the understanding of the contradictions, I examined brood sex ratio adjustment in the Hungarian population of the Collared Flycatchers (*Ficedula albicollis*) and compared the results to those yielded in a Swedish population of the same species. Contrary to the latter results, we found that male attractiveness was not related to brood sex ratios, but male biased broods were produced late in the season. We discussed the possible explanations for such a difference. One of those, the low detectability of sex ratio adjustment at the brood level, was also examined in detail. In a simulation study I found that this detectability is very low if birds manipulate the sex of the first chick only, which is predicted by some theoretical works. This could explain some of the contradictory results. Investigating the possible reason for biased maternal investment into the eggs of young (one year old) males, we found that their nestlings developed slower and fledged with smaller size except in favourable years. Therefore we argued that the higher testosterone and  $\beta$ -carotene concentration in their eggs is probably a compensation for the poor genetic quality of their chicks. A within brood compensatory maternal investment was also revealed. We found that increasing nutrient investment into later laid eggs successfully reduced the disadvantage of the late hatching chicks in terms of growth and fledging size, even if this investment could not completely eliminate the handicaps. Interestingly, after hatching, small nestlings did not receive further help to catch up. We showed that the food allocation decisions of both male and female parents based on begging intensity and position of the chicks, while nestling size, condition and sex was not related to the probability of being fed. In summary, the results presented in my thesis suggest that many ways of parental investment in Collared Flycatchers are dependent on social / environmental circumstances and this may have fitness consequences.

## Összefoglaló

A szülői befektetés jelentősen befolyásolja a szülők és utódok szaporodási sikerét. Ezért a szülői befektetés mennyisége és utódok közti elosztása a viselkedésökológia kulcskérdése. Többször szaporodó fajoknál a szülői befektetés több szaporodási esemény között oszlik meg, így az elosztásnak két szintjét különíthetjük el: a fészekaljok közötti és fészekaljon belüli elosztást. Mindkét szinten az utódok között jelentős minőségi különbségek lehetnek, ezért nem feltétlenül az egyenletes elosztás a legelőnyösebb a szülők számára. Tézisemben a szülői befektetés különböző formáinak fészekaljakon belüli, és azok közötti mintázatát vizsgáltam madaraknál. Két vizsgálat az ivar manipulációval foglalkozott. Az utódok ivarának manipulációját akkor várhatjuk, ha a hím és tojó utódok felnevelési költsége, illetve hozzájárulása a szülői rátermettséghez eltér. Számos vizsgálatban mutattak ki nem random szegregációra utaló fészekalj ivararányokat, azonban léteznek ellentmondó eredmények is. Az ellentmondások okainak megértéséhez további, összehasonlítható adatokra van szükség. Ezért az ivararány manipulációt egy Svédországban már vizsgált faj, az örvös légykapó (*Ficedula albicollis*) magyarországi populációjában vizsgáltam, és a kapott eredményeket összehasonlítottam a korábbi svéd eredményekkel. Utóbbiakkal ellentétben, nálunk a hímek vonzósága nem korrelált az ivararányval, viszont a költési szezon második felében a hímek aránya a fészekaljakban nagyobb volt. A különbségek lehetséges okai közül, az ivararány manipuláció rossz kimutathatóságát, részletesebben is megvizsgáltam. Egy szimulációs kísérletben kimutattam, hogy ha az ivararány manipuláció az első tojásból kikelő fiókára korlátozódik, mint azt néhány elméleti munka sugallja, akkor a fészekalj szintű kimutathatóság olyan alacsony, hogy ez könnyen ellentmondó eredményekhez vezethet. A tojók hím kor függő tojás befektetésének okait vizsgálva azt találtuk, hogy a fiatal (egy éves) hímek utódai nem kedvező években lassabban fejlődnek és kisebb kirepülési tömeget érnek el, mint az idősebb hímek utódai. Ezért úgy gondoljuk, hogy a fiatal hímek tojásaiban található magasabb tesztoszteron és  $\beta$ -karotin szintek a rosszabb genetikai minőség kompenzációját szolgálják. Fészekaljon belül is találtunk kompenzációs mintázatot a tojás befektetésben. Kimutattuk, hogy a később lerakott tojásokba juttatott többlet tápanyag sikeresen csökkentette az utoljára kikelő fiókák hátrányát mind a növekedés, mind a kirepüléskori méret tekintetében. A kompenzáció azonban nem volt teljes. Ezért is meglepő, hogy kikelés után a kis fiókák nem kaptak további segítséget a felzárkózáshoz. Eredményeink szerint mindkét szülő csak a táplálékkérő viselkedés intenzitását és a fiókák fészekben való elhelyezkedését vette figyelembe a táplálékelosztás során. A fiókák mérete, kondíciója, és ivara nem függött össze a táplálékhoz jutás valószínűségével. Összefoglalva, dolgozatom eredményei arra utalnak, hogy az örvös légykapónál a szülői befektetés számos formáját befolyásolja a szociális / ökológiai környezet, ami rátermettség különbségeket eredményezhet.