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Local adaptation or dispersal? How pied flycatchers cope with climate change

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Chapter 2

The ecological causes of selection for early breeding: Do mistimed pied flycatchers suffer from food limitation?

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Abstract

1. In seasonal environments, timing of reproduction is important for maximizing reproductive output and individual fitness. Climate change alters the phenology of organisms, but to a different extent, so that trophic levels can get out of synchrony and predators could suffer from food limitation.
2. In the migratory pied flycatcher, climate warming has led to increased selection for early breeding. Populations declined in habitats with a large mismatch between brood-rearing and caterpillar peak abundance, suggesting that reduction of food availability causes the increased selection for early breeding. In this paper, we experimentally test whether the ecological cause of increased selection for early breeding is indeed the reduction in food availability for birds that breed late relative to the caterpillar peak.
3. During three years, we delayed hatching of half of the broods by seven days and supplementary fed half of the control and delayed broods during the nestling period. If a mismatch with food is the ecological cause of the increased selection for early breeding, we expected that delayed broods would perform less, unless we food-supplemented them.
4. Interestingly, despite our large sample size (314 nests), we found little support for food limitation to be the main cause of a decline in breeding success. While brood parameters declined with date, recruitment rate was not much higher for early-born offspring, indicating little selection for early breeding. Adult survival declined with offspring hatch date for females, while it increased for males, suggesting a possible conflict over parental care that becomes more severe in late broods.
5. The relatively weak selection for early breeding in this population during the last years is in contrast to what has been reported in earlier studies, while the birds' synchrony with the caterpillar peak did not improve. Patterns of reproductive success were not explained well by caterpillar abundance and phenology, and there was no indication of food limitation in late broods. This suggests that food is not the limiting factor, and that pied flycatchers might be able to use other food sources when caterpillars are scarce.

Introduction

In seasonal environments, timing of reproduction is an important aspect in maximizing fitness of an individual (Daan & Tinbergen 1997). In many bird species, reproductive success and chance of survival to the next year commonly declines during the season (Lack 1968; Perrins 1970), either as a result of changing ecological conditions throughout the season, or because of other date effects influencing relative fitness. Many bird species also lay smaller clutches later in the season (Klomp 1970), which suggests that they adjust to deteriorating conditions.

A number of potential explanations exist why selection for early breeding is such a common phenomenon in many organisms. An individuals' fitness can be affected by environmental factors that vary with date, like deteriorating food conditions (Visser, Holleman & Gienapp 2006; Nagy & Holmes 2005; Newton 1998), changes in parasite prevalence over the season (Altizer et al. 2006) or abiotic factors like day length. Date effects could also arise from scheduling different life-cycle stages into a year (Newton 2008), with moult and possibly migration needing to be performed before a certain date. Whereas all these causes are directly related to date, another possibility is that high quality individuals breed earliest and in the best territories, and that the observed correlations with date are rather the result of this between-individual variation in quality (Verhulst & Nilsson 2008; Wardrop & Ydenberg 2003). For example, in collared flycatchers (*Ficedula albicollis*), healthier females started egg-laying earlier (Gustafsson et al. 1994), and in barn swallows (*Hirundo rustica*), early-arriving males were in better body condition, were able to occupy high-quality territories and attracted high-quality mates (Møller et al. 2008), resulting in high reproductive success. A full understanding of the fitness consequences of breeding date also requires knowledge on the costs of early breeding, due to unfavourable weather and food conditions at the start of the season. Severe survival costs have mostly been found in cases of exceptional weather events (e.g. Brown & Brown 2000). In pied flycatchers (*Ficedula hypoleuca*), survival of females that laid early in the season was found to be lower than for average-timed breeders when spring temperatures were rather low, whereas this cost of early laying disappeared in more recent years when springs were warmer (Drent et al. 2003).

In recent years, climate change has altered the phenology of interacting organisms of different trophic levels at a different pace, and predators and their prey got out of synchrony (Thackeray et al. 2010). Studies have shown that, especially for migratory, hole-nesting passerines, the mismatch between the peak of caterpillar abundance, a main prey type, and timing of breeding has increased, because birds did not advance enough to track the food peak (Saino et al. 2011; Both et al. 2006). In pied flycatchers, climate warming has led to stronger selection for early breeding, and populations declined in habitats with a large mismatch between brood-rearing and caterpillar abundance (Both et al. 2006). This descriptive work suggests that the ecological cause of the recent change in selection for early breeding is a decline in food availability that

hits later breeding individuals more than early breeders (Visser, Holleman & Gienapp 2006; Wiley et al. 2007).

The ecological causes of this change in selection pressures have not been experimentally investigated. There is only a single study, showing that the increased strength of fecundity selection on laying date was correlated with the degree of mismatch between caterpillar and bird phenology (Charmantier et al. 2008). Alternatively, selection on laying date may partly be due to individual variation in quality, and over the course of time, variation in quality among individuals has increased, resulting in an increasing selection. Investigating the cause of a seasonal decline in fitness parameters requires manipulation of the timing of breeding (Verhulst & Nilsson 2008). Results of such experimental studies are ambiguous: most find proof for substantial date effects, but also additional effects of individual or territory quality are found (Verhulst & Nilsson 2008). Some studies also found differing contributions of date and quality in different parts of the season (e.g. Wardrop & Ydenberg 2003; Garcia-Navas & Sanz 2011), for different fitness parameters (Grüebler & Naef-Daenzer 2010) or in different years (Verhulst et al. 1995). Of the studies which found effects of hatch date *per se*, there is only one experimental study showing that food availability was the cause: Delayed broods were performing better when food supplemented relative to controls (Siikamäki 1998). The importance of breeding time, relative to a peak in food abundance, may however differ strongly between species and habitats (see e.g. Dunn et al. 2011). As a result, the consequences of climate change on trophic mismatches and selection for breeding time likely differ depending on ecological conditions (Both et al. 2010). This dependence on habitat features was shown for seasonal variation in flycatcher nestling diets, with caterpillars declining strongly over the season in oak forests in regions with spring warming, but not in coniferous forests (Burger et al. 2012). A full understanding of how climate change-induced trophic mismatches result in changes in selection pressures therefore should take an experimental approach, which also captures natural variation in habitat seasonality.

So far, most experimental manipulations of hatch dates have been conducted in a single or two subsequent years (Magrath et al. 2009; Grüebler & Naef-Daenzer. 2010; Siikamäki 1998; Wiggins et al. 1994), resulting in rather low sample sizes and failure to capture annual variation in experimental effects (if done, year differences were strong, see Verhulst et al. 1995; Török et al. 2004). Fitness parameters were often limited to measures of reproductive success like nestling weight, which only captures part of the variation in recruitment rate in flycatchers and other birds (Sanz et al. 2003; Lobato et al. 2005; Verhulst 1994; but see also Kruuk, Merliä & Sheldon 2001; and in great tits: Tinbergen & Boerlijst 1990).

The aim of our study is to experimentally test the idea, that the increased selection for early breeding in temperate woodland birds is caused by late birds being mistimed with their food peak. For this we carried out an experiment during three subsequent breeding seasons, during which hatching dates of part of a population of pied flycatchers was delayed. This was done in order to investigate date effects while

controlling for the effects caused by quality differences between early and late birds. In addition, we food-supplemented part of the broods in order to study, if food limitation is indeed the cause of a seasonal decline in fitness parameters. We hypothesise that, if timing relative to a narrow food peak causes the decline in fitness over the season, delayed nests will perform less well than controls, unless these delayed nests are food supplemented. We investigated the effects of our manipulation on various fitness parameters and assessed the importance of habitat differences on those relationships.

Methods

Study species

The pied flycatcher is a long-distance migratory passerine wintering in Sub-Saharan Western Africa. It breeds in temperate forests across Europe and is often found in oak or oak-mixed habitat types but also breeds in coniferous (mainly *pinus* ssp) woodlands. It is single-brooded, shows bi-parental brood care and females lay between 4 and 8 eggs (Lundberg & Alatalo 1992), usually one egg per day. Nestlings fledge at an age of around 15 days.

Experimental procedure

The experiment was conducted during three consecutive breeding seasons (2009–2011), in our study site in Drenthe, The Netherlands (NL, 52°49' N, 6°22' E). 1050 nest boxes were subdivided over 12 study areas, and the annual number of pied flycatcher breeding pairs rose from 187 at the start in 2007 to around 350 in 2011–12. All nest boxes were of equal size (inner dimensions: 90*120*230 mm, entrance hole 32 mm). Nest boxes were visited on intervals of 5 days or less from the beginning of April.

We used five treatment categories: delayed nests, delayed nests that received food-supplementation, control nests, control nests with food-supplementation and unmanipulated nests. The control group had the same treatment as the delay group, except the duration of the storage of the eggs before they were returned to the nest and incubated by the female (see below). This is important, because apart from changing hatching date, we also affected the synchrony of hatching of the clutch with our manipulation.

Spring arrival of males and females was monitored and newly built nests were checked daily or every second day for egg-laying. In every season, manipulation was started when enough nests with eggs were available to form groups of all five categories with the same first egg laying date (± 1 –2 days). When nests had 2 eggs, they were randomly assigned to one of the five treatment groups. This was done by assigning nests as they were found during nest-checking, always starting with treatment 1 (control-no food). Eggs were then removed from the nest, replaced with dummy eggs (plastic models of canary eggs) and stored in a plastic cup which was

buried in the ground and covered with a layer (~ 5 cm) of moss or soil at the base of the nest-box tree (a rather cool site, with minimal handling of the eggs). Every subsequent day, nests were checked for egg-laying and newly laid eggs were collected and stored at the day of laying. From the day when clutch size reached six eggs, and no new egg was found the subsequent day, we assumed the clutch to be completed. For smaller clutches, we waited two days before we assumed a complete clutch, to circumvent the possibility of laying gaps and return of the eggs before clutch completion. For control nests, all original eggs were returned to the nest at that point, which meant that for control clutches the delay in hatching was around one day relative to the untreated group. For delayed nests, original eggs remained in the ground for 7 more days in 2009 and 6 more days in 2010 and 2011 and were then returned to the nest, resulting in hatching dates being delayed by 6 (2010 and 2011) or 7 days (2009), relative to control nests. Un-manipulated nests were only assigned (randomly) and checked daily (not checked daily in 2011) but without manipulating the eggs. Some hatching failures occurred in 2009, probably as a result of our manipulation (storing the eggs), therefore protocols were changed towards a shorter delay time (6 days) and cups were kept as dry as possible (containing cotton-wool).

Because cold storage of eggs could affect hatching success of eggs and subsequent development of the young (Wiggins, Pärt & Gustafsson 1998), we compared un-manipulated nests with control, non-supplemented nests to test for an effect of storage. Hatching success was not different between the control nests, where eggs were stored in the ground, and un-manipulated nests (5.66 hatchlings in control vs. 5.77 in un-manipulated nests, $n = 311$, linear mixed model with year as random effect and clutch size as covariate, estimate: 0.11 ± 0.17 SE, $t = 0.66$, $p > 0.1$). We also could not find significant negative long-term consequences of egg storage on fledgling weights (using a linear mixed model, with function `lmer`, the random effects were 'nestbox' and 'year', estimate for un-manipulated nests: -0.01 ± 0.8 SE, $t = -0.17$, $p > 0.01$, $n = 1542$ fledglings, nests with low hatching success were excluded, see below) between control and un-manipulated nests. From this we concluded that our manipulation had at most marginal negative effects on nestling parameters and results should still be meaningful in an ecological context. By excluding nests with low hatching success (occurred more often for delayed nests, see results) in the main analysis, we also avoided biases due to the manipulation.

Food supplementation of the respective treatment groups started at an age of 3 days of the young. Every day, 30 g of mealworms (sprinkled with a vitamin supplement to enhance food quality) were provided in a cup attached on the outside of the nest box. If not all mealworms were eaten when visiting the nest for a new food supplementation, the mealworms were replenished to 30 g. This amount is thought to account for about one third of the daily energy needs of the brood (Verhulst 1994). Parents could use the food for themselves or for their young, and observations confirmed that they used this source intensively for feeding their offspring (unpublished data). Daily food supplementation was continued until the young fledged.

For a number of nests (>10 nests per year) and days during food supplementation we weighed the left-over food when refilling the cups. Data on this is incomplete, but in all three years we found that birds used more of the food later in the season and when they had older chicks. In 2009 (mean intake: 17.9 g/day) and 2011 (mean: 20.7 g/day), the increase in food-use (across all food-supplemented nests, including nestling age effects) with date was similar and very steep (1.37 g/day), while in 2010 (mean: 22.7 g/day) food-use increased less strong (0.5 g/day). Parents of late nests or nests with older nestlings usually used up the complete 30 g of mealworms. Furthermore, our way of food-supplementing broods was comparable to other studies regarding the type and amount of food provided and the interval of provisioning (Siikamäki 1998; Verhulst 1994). We therefore expected our food-supplementation to sufficiently compensate a possible shortage of food in the environment. Although parents were using mealworms to feed their offspring and themselves, food stealing by Eurasian jays (*Garrulus glandarius*) was observed for several nests (6 out of 24 observed nests in 2011, personal observation), often resulting in a complete loss of the daily load and causing additional disturbance to the parents.

At day 7 of nestling age, parents were caught, ringed and measured (weight, tarsus) and nestlings were ringed and weighed. At day 12, nestlings were measured again (fledgling weight and tarsus). In our study area, capture rates of breeding adults are very high (>90%), which is important for estimating return rates. Birds which were not captured were assumed to be dead or to have dispersed outside of the study area.

We assumed that synchrony of the birds' timing of reproduction with the peak of caterpillar abundance is very important for their fitness. To get an estimate of the timing of the caterpillar peak, we sampled frass fall from caterpillars under oak trees throughout the season, for the years 2009 to 2011. Frass was collected using pieces of cheese cloth of 0.25 m² on a wire frame, which were placed under oak trees and emptied approximately every four days (depending on weather conditions, traps can only be emptied when it is dry). The collected frass was dried and weighed, and approximate caterpillar peaks were calculated (Tinbergen & Dietz 1994). Here, we used data from one study plot (from three individual oak trees), which had one of the highest breeding densities of pied flycatchers in our study area, and caterpillar peaks did not differ much between areas (C. Both, personal observation). Furthermore, nestling diet data was available (Burger et al. 2012) for our study site and for the years 2009 to 2011.

Statistics

In the statistical analyses, we incorporated possibly ecological relevant information in addition to the experimental treatment. We choose to analyse the effects of date as observed hatching date, which includes both the natural variation and the experimental delay. Experimental delay was modelled in addition, as a factor with two categories, control and delay (in the model, the effect of delay treatment relative to

control treatment is given). If experimental delay is significant in this analysis on top of the hatching date effect and is positive, this means that we find support for the individual quality hypothesis, i.e. for the same hatching date, birds that originally laid earlier are performing better. If the experimental delay is negative after including hatch date, the experimental treatment negatively affects the quality of the birds through e.g. the prolonged incubation period. Food supplementation was included as a factor, without taking into account that individual birds used different amounts of food. We also included habitat as factor in the analyses, because previous work has shown that effects of phenological mismatches are more severe in oak dominated habitats in warm years (Both et al. 2006; Burger et al. 2012).

We analysed all data using R 2.11.1 (R Development Core Team 2008). For the main analyses, hatch date was centred per year, additional parameters were food supplementation (yes/no), nest treatment (control/delayed) and habitat (per nest-box, 'oak' or 'non-oak' habitat). A list of candidate models was created (see Appendix), and models were compared and ranked by their AIC-values (Burnham & Anderson 2010), and using package *AICcmodavg* in R 2.11.1. For nestling weights as dependent, the AIC-best model contained a three-way interaction between food supplementation, nest treatment and year, indicating year differences in experimental effects. Therefore, data was analysed separately per year, and, for consistency, this was also done for the analysis of fledgling numbers. Return rates of parents and offspring were analysed across all years as no year-interactions were found in the top-ranked models. However, models on adult return rates were analysed separately for males and females, as the combined analysis showed interactions of several parameters with sex, suggesting that males and females reacted differently to our treatment. We also tested models including quadratic effects of date but those were not among the top ranked models (see below).

In each case, the list of candidate models included a global model with biologically meaningful main effects and interactions between parameters of interest, and a minimal model including only parameters to control for: For mixed models (using function *lme*) on individual nestling weights, tarsus length was included in all models (one model per year). In addition, candidate models were used including or excluding number of fledglings as this has sometimes been found to correlate with nestling weights. As random effect we fitted nest-box identity as random intercept. For linear mixed models using number of fledglings as dependent (with function *glm*), clutch size was always included in the models. For adult return rates (using function *lmer* with binomial errors), the minimal models contained only one parameter of interest (fitting the intercept only led to convergence problems). As random effect, year (3 years) was fitted as random intercept. Candidate models were chosen so that all biologically relevant combinations of parameters and interactions were covered. AIC (or AICc for smaller sample sizes, see Burnham & Anderson 2010) values were calculated for each set of candidate models to rank them. AIC weights for the top-ranked models in the sets of candidate models were low (<0.5) which suggests that model

averaging should be used rather than inference from a best model (Burnham & Anderson 2010). We calculated average estimates for the parameters of interest using package *AICcmodavg* in R 2.11.1. For offspring return rates, we used offspring from 2009 or 2010 which could have returned one or two years later (data available until 2012). Because recruitment rates are generally low in pied flycatchers (74 out of 1246 nestlings returned, around 6%), we used a binomial model with clog-log link function (to account for the many zeros in the data, Zuur et al. 2009), and only tested for linear effects of the main parameters of interest, hatch date, delay treatment and food supplementation. Models containing combinations of those parameters were ranked by AICc, and for the top models within a Δ AIC of 2, averaged estimates were calculated. For nestling weights and number of fledglings we also averaged across the top models within a Δ AIC of 2. Those best models had a minimum sum of AIC weights of 0.6 (except in one case: fledglings in 2009, was 0.51). For return rates, AIC weights of models within Δ AIC of 2 were very low (<0.5), and we therefore chose to consider all top models until they reached a sum of weights of 0.6 (different rule but makes it more comparable to analysis of nestling parameters). We used a normal error distribution for number of fledglings, as residuals were roughly normally distributed and poisson errors did not improve distribution, but led to problems with under-dispersion that we could not account for (using quasi-poisson does not give AIC values anymore).

Results

The three study years differed in some potentially important ecological aspects (Figure 2.5). 2009 was a relatively warm spring (mean temperature 16 April–31 May: 12.77°C, mean in same period over the years 1990–2012: 12.13°C), with a very high caterpillar peak occurring around 11 May (oak trees in some cases were defoliated by mid-May; caterpillar abundance was measured using frass nets (Tinbergen & Dietz 1994). In one of our oak-dominated sub-areas, caterpillars defoliated the oaks well before they reached their final size, and most caterpillars died, as did all except one flycatcher nests ($n = 13$). 2010 was cold (mean temperature 10.15°C), and caterpillars were late (peak occurring around May 28) and not very abundant (density less than 30% of the density in 2009, in most areas, but in some areas only 5% of the 2009 density). 2011 was a very warm spring (mean temperature 13.97°C), with caterpillars peaking around 7 May and peak densities being rather similar to 2010. Despite the enormous variation in caterpillar peak dates (20 days), pied flycatcher mean hatching dates of non-manipulated clutches were rather similar between the years (2009: 23.4 May, 2010: 25.3 May, 2011: 20.0 May). Pied flycatcher nestlings thus on average were well timed with a low caterpillar peak in 2010, hatched 12 days after the high caterpillar peak in 2009, and in 2011 hatched 13 days after a low caterpillar peak.

In total, 401 nests were manipulated as control or delay. We excluded nests when they did not fulfill certain criteria: missing parameters, $\leq 50\%$ hatching success

(including predation during the incubation phase), clutch size < 3 eggs. This resulted in 314 nests that could be used in the analyses. In the delayed group, 25 clutches out of 203 failed to hatch, probably a result of our manipulation (moisture in the cups used for storage causing hatching failures and late nests abandoned by females). This was mainly a problem in 2009, when 17 out of 70 clutches (24%) did not hatch (in control group, 6 out of 71). In other years, between 0% and 7% of clutches of the different treatments did not hatch. Clutch sizes were generally larger in 2009 (6.7 ± 0.6 SD) than in 2010 and 2011 (6.3 ± 0.8 SD, similar in both years), but did not differ between treatment groups (females did not lay less eggs as a result of our manipulation, mean of 6.45 eggs for both treatment groups). The treatment resulted in hatching being delayed by about 1 day in the control nests and 8 days (or 7 days) in the delayed nests, compared to un-manipulated nests. The one day extra delay was caused by returning the eggs only one day after the clutch was finished, so that earlier incubation by the female was not possible.

To give an overview of the clean experimental results, we present the data in a graph with mean values (\pm SE) for treatment groups (control or delayed) and food supplementation (Figure 2.1). This is meaningful, because our experimental treatment was balanced, and thus no initial differences in laying date between treatments occurred.

Nestling weight

Candidate models including interactions with year were among the highest-ranking models for nestling weight which suggests strong year-differences in the effects. Therefore we present separate analyses for the different years. Mean nestling weights of all manipulated nests hardly differed between years: 13.97 ± 1.01 g (SD) for 2009, 14.22 ± 0.99 g (SD) for 2010, and 13.96 ± 1.17 g (SD) for 2011.

In 2009, nestling weight did not decline over the season, but was lower in oak habitat compared to other habitats (averaged estimate: -0.17 g ± 0.08 SE, significant in three of the top models, table 2.1). All four top-ranked models contained habitat, once in interaction with food (not significant). There was also a tendency that nestlings of food-supplemented nests were heavier than of non-supplemented nests (estimate 0.29 g). Model (3) contained treatment (Table 2.1) but there was no clear evidence that, with the same hatch date, delayed birds were performing better than early breeders (confidence intervals for delay effect include 0, no quality effect, Table 2.1).

In 2010 and 2011, nestling weight declined over the season (Table 2.1), and the effect was stronger in 2011 than in 2010 weights (averaged estimate 2010: -0.04 ± 0.02 SE (g per day); 2011: -0.1 ± 0.03 SE). This main effect was significant in the best models (Table 2.1). Top-models also contained the delay treatment, which was positive in 2011, but not significant in any of the models, suggesting quality effects in that year (i.e. that naturally early breeders would always perform better than naturally late breeders). In 2011, a weak non-linear effect of date (n.s.) was suggested by

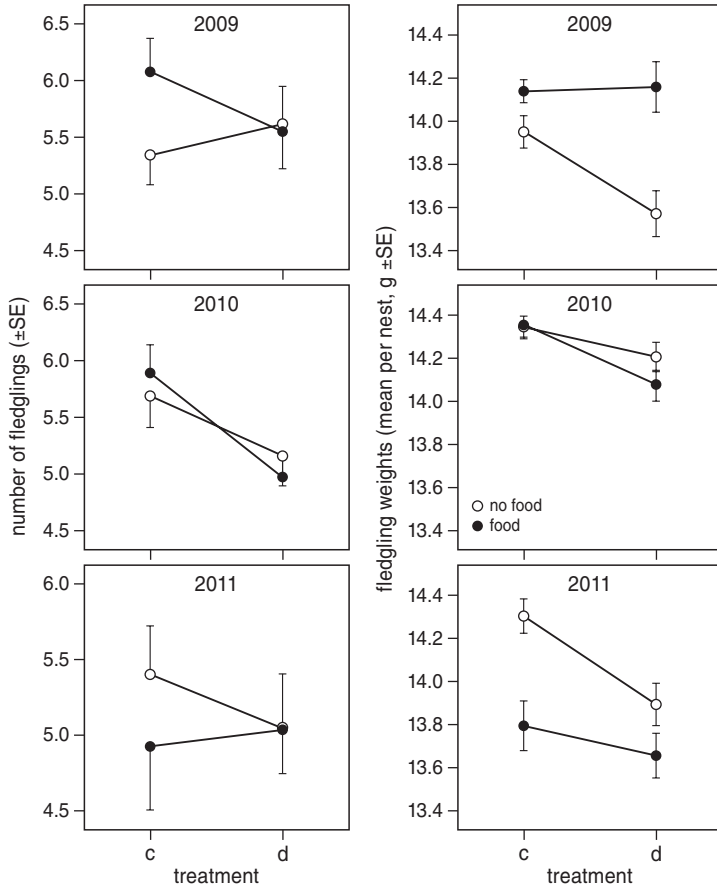


Figure 2.1 Experimental effects on parameters of reproductive success (fledgling weights (right panels) and number of fledglings (left panels), for the experimental groups (control (c), delay(d), and food or no food), separate per year. Averages and SE's were calculated per group.

one of the models, as well as a weak negative effect of food supplementation. In both years, habitat was not an important determinant of nestling weights in the best models (Table 2.1).

Number of fledglings

The number of fledglings was affected in a different way compared to nestling weights (Table 2.2). In 2009, number of fledglings declined with date (averaged estimate: -0.07 fledglings/day ± 0.03 SE, significant in all three models) and there were weak positive effects of the delay treatment, indicating that early birds are of higher quality (accounting for 0.35 fledglings more). Food supplementation only had a weak positive effect on fledgling production (accounting for 0.42 fledglings more in this year, Table 2.2).

In 2010, the number of fledglings declined with hatching date (-0.077 fledglings/day, in this case, the value is derived from a simple regression of fledglings against date because all candidate models contained interactions with date, see also Figure 2.1), and food supplemented nests declined even stronger than non-supplemented nests (Table 2.2). The results indicate a rather strong decline in reproductive output with date, but this was apparently not explained by a shortage of food. Rather, food supplementation shows a negative effect here, possibly because it caused extra disturbance, or because the food was of lower quality than the natural food, but we consider the latter point as unlikely, based on previous studies. The non-significant positive interaction of delay treatment with hatch date may indicate that also individual quality is of some importance, especially when being delayed. In 2011, there was, like in 2009 and 2010, a negative effect of hatch date on the number of fledglings (averaged estimate: -0.07 ± 0.04 SE (fledglings per day), main effect was significant in two models, Table 2.2) which was partly compensated by quality effects (accounted for 0.4 fledglings). In delayed nests, the negative effect of date was less strong compared to controls, again suggesting a weak non-linear date effect (Table 2.2). As

Table 2.1 Best models (within Δ AIC = 2 from all candidate models) using nestling weights (in g) as dependent. Only parameters of interest and present in the best models are shown. Estimates are averaged across models where appropriate. For habitat, the estimate for oak relative to other habitat is shown. Parameters, their significance and AIC weights of best models are presented. Significance: (*) indicates $p < 0.05$ and (.) indicates $0.05 > p < 0.10$.

year	Parameter	Model average				Model rank			
		β	SE	95% CI from	95% CI to	1	2	3	4
2009	delay	0.02	0.16	-0.3	0.33			x	
	food	0.29	0.16	-0.02	0.6	x.	x	x.	
	habitat	-0.17	0.08	-0.33	-0.1	x*	x	x*	x*
	food:habitat	-0.06	0.16	-0.38	0.26		x		
	Δ AIC					0	1.3	1.88	1.99
	AIC w					0.26	0.14	0.1	0.1
2010	birth date	-0.04	0.02	-0.07	-0.01	x*	x*		
	delay	-0.03	0.15	-0.32	0.26	x			
	Δ AIC					0	1.97		
	AIC w					0.48	0.18		
2011	birth date	-0.09	0.02	-0.13	-0.05	x*	x*	x	x*
	delay	0.31	0.21	-0.1	0.73	x		x	
	delay:birth date	0.04	0.04	-0.04	0.13			x	
	food	-0.13	0.17	-0.47	0.2				x
	Δ AIC					0	0.12	1.01	1.51
	AIC w					0.27	0.25	0.16	0.13

we controlled for clutch size in the models, those effects are not the result of the natural decrease in clutch size over the season.

Return rates

Adult males and females showed differing seasonal patterns (Figure 2.2) and were also found to be affected differently by our experimental treatment (top-models contained interactions with sex). We therefore analysed the sexes separately. Interactions with year were not found in the best models, and years are therefore analysed together. Adult females showed lower return rates, the later their young hatched in the previous year, when controlling for treatment, and this was significant in 3 out of the 4 top models (27% versus 34% for an early versus a late group, Table 2.3, Figure 2.2). Females from delayed nests tended to have a higher survival (model

Table 2.2 Best models (within $\Delta AIC = 2$ from all candidate models) using number of fledglings as dependent. Only parameters of interest and present in best models are shown (all models also contained clutch size as covariate). Estimates are averaged across models where appropriate. Parameters, their significance and AIC weights of best models are presented. For 2010, no coefficients are given for the main effects of hatch date, delay and food, because all “best” models contained interactions of those parameters. Significance: (*) indicates $p < 0.05$ and (.) indicates $0.05 > p > 0.10$.

year	Parameter	Model average				Model rank (Best models within $\Delta AIC = 2$)			
		β	SE	95% CI from	95% CI to	1	2	3	4
2009	birth date	-0.07	0.03	-0.13	-0.01	x*	x*	x*	
	delay	0.35	0.38	-0.41	1.1		x	x	
	food	0.42	0.31	-0.19	1.03			x	
	ΔAIC					0	1.4	1.76	
	<i>AIC w</i>					0.27	0.13	0.11	
2010	birth date	0.03				x	x		
	delay	-1.04					x		
	food	-0.02				x	x		
	habitat	-0.16	0.12	-0.4	0.08		x		
	food:birth date	-0.2	0.09	-0.37	-0.02	x*	x*		
	food:delay	1.28	0.66	-0.01	2.57		x.		
	ΔAIC					0	1.7		
<i>AIC w</i>					0.42	0.18			
2011	(intercept)								x
	birth date	-0.07	0.04	-0.15	0	x*	x	x*	
	delay	0.4	0.41	-0.4	1.21		x	x	
	delay:birth date	0.14	0.08	-0.03	0.3		x.		
	ΔAIC					0	0.61	1.21	1.85
	<i>AIC w</i>					0.25	0.19	0.14	0.1

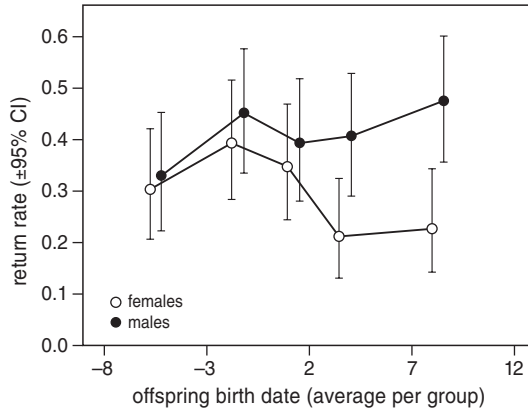


Figure 2.2 Return rates of adult males and females (experimental nests) in relation to offspring hatch dates in the year before. All data (control, delayed, food supplemented nests), regardless of experimental treatment, were used for this graph to show the difference between males and females. Data was grouped into 5 groups with equal sample size ($n = 66$ individuals per group for females, $n = 62$ per group for males), for graphical presentation. Offspring hatch dates were centered per year and averaged per group. 95% Confidence intervals are given per group. For graphical reasons, data points for male return rates are shifted 0.5 along the x-axis to the right.

Table 2.3 Best models (summed AIC weights ≥ 0.60) for adult return rates as dependent (presence-absence data, binomial model), separate for males and females. Mixed models were used with binomial error distribution and log-link-function. Only parameters of interest and present in the best models are shown. Estimates are averaged across models where appropriate. Parameters, their significance and AIC weights of best models are presented.

Return rates females	(Averaged) Model estimates				Model rank (summed $w \geq 0.60$)			
	β	SE	95% CI from	95% CI to	1	2	3	4
hatch date	-0.06	0.03	-0.12	0	x*	x*	x*	x
delay	0.28	0.31	-0.33	0.88		x	x	x
food	0.19	0.24	-0.29	0.67			x	x
hatch date: delay	-0.01	0.07	-0.14	0.12				x
Δ AIC					0	1.24	2.72	3.29
AIC w					0.33	0.18	0.09	0.06
Return rates males								
hatch date	0.05	0.03	-0.01	0.11	x	x*	x	
delay	-0.42	0.29	-0.99	0.15	x	x		
habitat	0.12	0.12	-0.12	0.35				x
hatch date: delay	0.12	0.06	0	0.25	x*			
Δ AIC					0	2.11	2.18	3.27
AIC w					0.34	0.12	0.12	0.07
* $p < 0.05$								

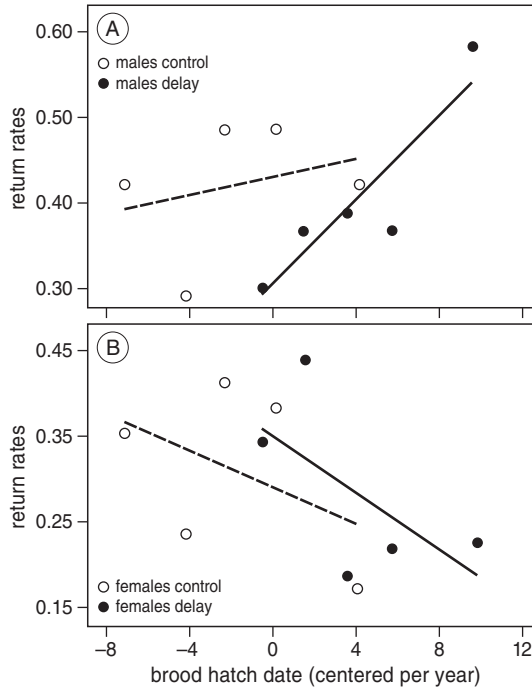


Figure 2.3 Return rates for males (A) and females (B), separate for control and delay treatments. For graphical presentation, return rates are grouped into five groups (= symbols) per treatment with about equal sample size (n between 31 and 35 individuals per group) for hatch date. Mean hatch dates per group and the respective regression lines for the two treatments are shown.

2-4, Figure 2.3), indicating that early birds are of somewhat higher quality and there was a non-significant, positive effect of food supplementation in two of the models. The fourth ranking model also contained an interaction between hatch date and delay treatment indicating a non-linear effect of date (n.s., Table 2.3).

In males, a later hatch date of the young had a positive effect on return rates (estimate: 0.05 ± 0.03 SE, Table 2.3, Figure 2.2). A positive interaction of offspring hatch date with treatment in the best model (estimate: 0.12 ± 0.06 SE) suggests that control and delayed males differed in their return rates over the season (increase was less steep in control males, Figure 2.3). This effect seemed to be driven mainly by the high return rates of delayed males late in the season, and could also be a non-linear overall effect. Food supplementation was unimportant in explaining return rates in males.

Recruitment rate of offspring in the two years after birth was 6% in total, and we found little support for effects of hatch date or the delay treatment (Table 2.4). Early-born offspring therefore did not recruit more often than late-born offspring (Figure 2.4). Analysis showed, that food supplementation of nestlings tended to have a negative effect on subsequent return rates, rather than the expected positive effect, but this was not significant in any of the top models (Table 2.4).

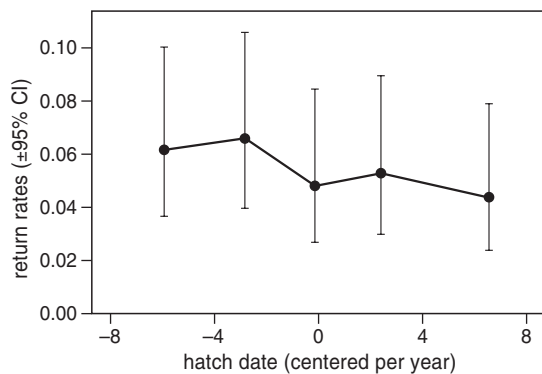


Figure 2.4 Changes in recruitment rates with hatch date (centered per year). For graphical presentation, data was grouped into five groups with equal sample size ($n=229$ individuals per group) and return rates were calculated per group with 95 % confidence intervals; mean hatch dates per group are shown.

Table 2.4 Best models using offspring return rates (presence-absence-data) as dependent. Mixed models were used with binomial error distribution and log-log-function. Only main parameters of interest, present in the best models (within $\Delta AIC = 2$ from all candidate models), are shown. Estimates are averaged across models. Parameters, their significance and AIC weights of best models are presented.

Parameter	Averaged model estimates				Model rank						
	β	SE	95% from	95% to	1	2	3	4 (intercept)	5	6	7
food	-0.43	0.26	-0.94	0.07	x.	x.	x.				x.
delay	-0.33	0.28	-0.89	0.22		x			x		x
hatch date	-0.03	0.03	-0.1	0.03			x			x	x
ΔAIC					0	0.11	0.23	0.67	0.81	1.08	1.74
$AIC w$					0.18	0.17	0.16	0.13	0.12	0.11	0.08

. 0.05 > p < 0.10

Discussion

We could not find convincing experimental evidence, that food limitation is the main ecological cause of declining fitness parameters within breeding seasons. We furthermore found a discrepancy of experimental effects between brood parameters and subsequent survival of offspring: Brood parameters moderately declined over the season in most years and slopes were rather comparable to what was found in previous studies on pied flycatchers (Siikamäki 1998; Veen et al. 2010). Return rates of recruits, in contrast, showed hardly any seasonal decline and seemed not to support the patterns found for brood parameters. The data suggests that, in addition to the observed effects of date *per se*, differences in individual or territory quality partly account for the general decline in fitness over the season.

The lack of strong, negative date effects on fitness parameters, as we expected them, may be the result of little seasonal variation in the environment or of high (unexplained) between-individual variation. Interestingly, caterpillar peak dates varied substantially between years, while laying dates were rather similar (Burger et al., in prep.), so that birds were breeding relatively late in 2009 and 2011, and were well-timed in 2010. This was however not clearly reflected in all of our fitness measurements. When looking at fledging success, like expected, early broods fledged more young in 2009 and 2011, but early broods also profited in 2010, when birds were well-timed with the caterpillar peak and average-timed individuals should not have suffered from a seasonal decline in food abundance. With respect to nestling weights, early breeders produced heavier young than late breeders in 2010 and 2011, but not in 2009. The decline was partly due to date effects, but naturally early-nesting birds were also of higher quality. However, a very important result is that the date effects that we found could not be explained by food limitation, since the positive effects of food supplementation were small and variable between years and this was true for all the different fitness components analysed.

The patterns of moderate seasonal declines in brood parameters were not found back for recruitment rates. For recruits, we found only marginal survival costs: offspring being born late hardly differed from early-born birds in their return rates, and food supplementation did not improve return rates but rather had a negative effect. Although this finding of little survival costs in late birds is surprising at first glance, it is in line with a recent descriptive analysis on selection differentials across years (Visser et al., in prep.). There, selection differentials for laying dates (using number of recruits as fitness measure) for a population of pied flycatchers in the Hoge Veluwe, The Netherlands, show that after strong selection for early laying in the 1990's (with slopes between -0.60 and -0.15) this has diminished again in recent years. Also the selection differentials from our study site from 2007 until 2010 showed only weak selection for early breeding (slopes between -0.28 and 0.04 , from 2007 until 2010, unpublished data). In pied flycatchers, only a fraction of surviving offspring returns within a study site. Differential dispersal propensity might therefore

affect return rates (Doligez & Pärt 2008; Eeva et al. 2008; Schaub & von Hirschheydt 2009) and subsequent calculations of selection differentials. In our study population, however, no differences in dispersal distances between early and late-born birds were found (Burger et al., in prep.).

For female survival, the seasonal decline in return rates is less strong compared to previous studies under conditions of climate warming (Drent et al. 2003). Our manipulation also indicated, that early breeders were of higher quality because delayed females survived somewhat better compared to control females with similar brood hatch dates. Moreover, females survived slightly better when being food-supplemented in the year before. Late-breeding males, against expectations, returned more often than early breeders, and there was no indication of food limitation. Especially for males, we expected early birds to be of higher quality (Møller 2008) and to gain the best territories, but this contrasts with our finding that, for the same hatch dates, delayed males returned less often. Female choice in pied flycatchers appears to be complex (Sirkiä & Laaksonen 2009) and therefore early reproduction in males might be less related to high individual or territory quality. This opposing seasonal pattern of survival between males and females could also indicate a conflict over parental care. Late-breeding males might have a rather rigid annual cycle to ensure own survival and might therefore invest less into parental care and more into self-maintenance: Experimentally delayed males, for example, often started moulting already before their young fledged (Siikamäki 1998; personal observation). The decline in female survival might therefore be caused by females working harder in order to compensate for reduced male care. The lack of strong year differences in survival could arise because adults keep investment in themselves constant between years to ensure survival to the next year while extra energy (e.g. from abundant food) is invested into reproduction (Drent & Daan 1980; Moreno et al. 1999).

One major ecological factor affecting seasonal patterns could be habitat, with certain types of forests having narrower food peaks than others. Regarding seasonal patterns in caterpillars, habitats without oak trees seem generally more stable (less of a food peak), especially with warmer spring temperatures (Burger et al. 2012). Here, only in 2009 habitat type had a significant effect on fledgling weights (resulting in lower weights in oak habitat). In our study area, small-scale habitat parameters which could explain territory quality were measured for a large number of nest boxes, but none of these parameters (e.g. proportion of oak trees, shrub cover, ground layer, all measured around the vicinity of the nest box) had clear effects on reproductive success (unpublished data).

Other mechanisms might cause the lack of strong seasonal patterns in fitness components: As an alternative to caterpillars as nestling food, other prey types might be abundant throughout the season and birds could switch diets later in the season without paying fitness costs. Breeding densities in very seasonal habitats might also have become lower and therefore released competition for the best territories and for nestling food later on. Both et al. (2006) found that numbers declined most in areas of

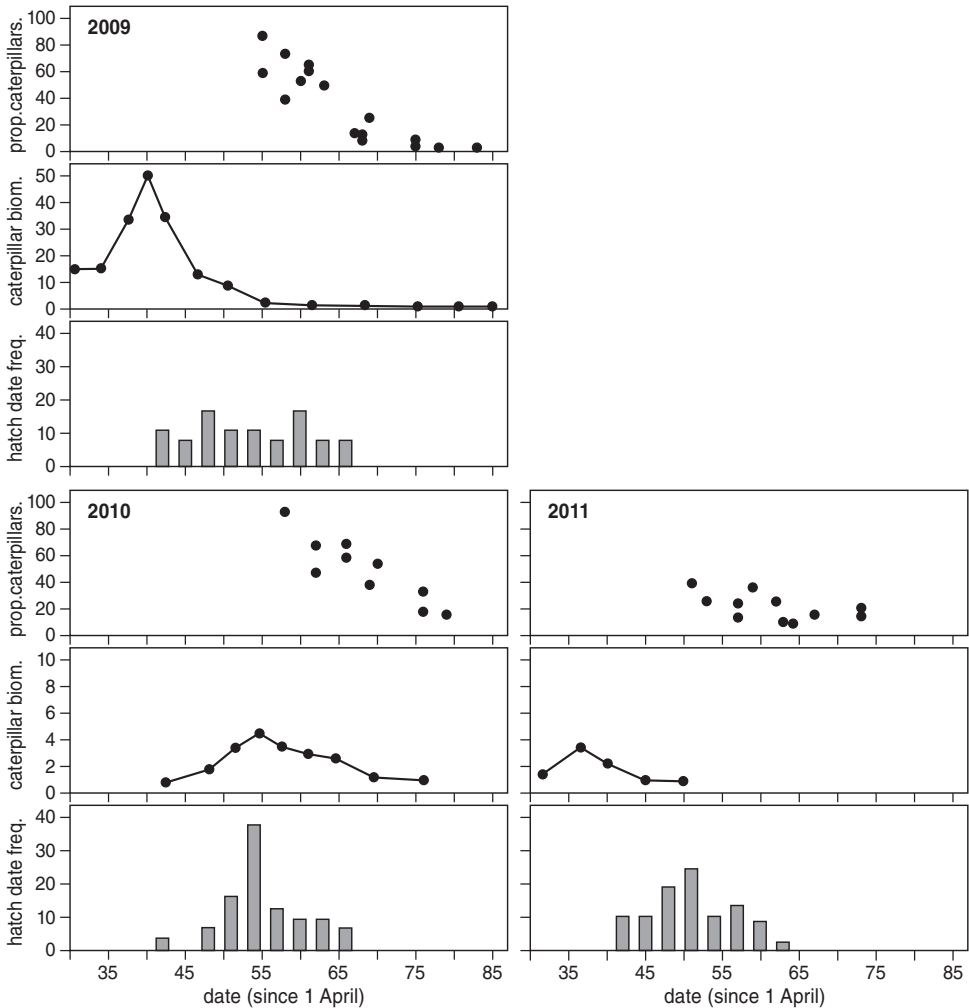


Figure 2.5 Phenological patterns of pied flycatcher hatching date, caterpillar peaks and caterpillar consumption in the three experimental years (2009–2011). For each year the upper graph gives the proportion of caterpillars in the diet of 8–12 d old nestlings, the middle graph the caterpillar biomass (m^2 ; average for the same three trees in each year); the bottom graph is the frequency distribution of hatch date (in bins of three days each). All data are from one of the subareas, which is a mixed area with oak and pine (Dwingeloo). Note that the caterpillar peak in 2009 was about 10 times higher than the other years (scales are adjusted).

greatest mismatches and this could be explained not only by reduced survival but also if birds dispersed to breed in less seasonal habitat patches with, for example, more coniferous trees. In less seasonal habitats, effects due to date *per se* might be rather small. We did not find a consistent difference in the patterns of fitness parameters between habitat types, but this could be because birds chose the habitat that matched their phenotype best (Edelaar, Siepielski & Clobert 2008). If this is so, we will need to

manipulate the settlement of the birds over the type of breeding habitat and compare their performance with birds that were offered a free choice.

In our study, experimental side-effects of delaying clutches were relatively small and, at most, biasing results only slightly towards date effects (Wardrop & Ydenberg 2003; see also e.g. Grueebler & Naef-Daenzer 2010, on incubation costs). Moreover, incubation behaviour was not different for females incubating for one additional week, in terms of duration and frequency of incubation bouts (unpublished data), and male incubation-feeding rate was only related to female incubation behaviour but not to treatment (unpublished data). The lack of a female survival cost of the experimentally induced 50% prolonged incubation period is at variance with the notion that incubation is expected to be a costly process (Monaghan & Nager 1997; Visser & Lessells 2001; de Heij, van den Hout & Tinbergen 2006). If incubation is indeed costly, the real fitness effects of our delay treatment would be higher than what we estimated, and therefore our conclusion that hatching date has little effects on fitness is likely not due to experimental bias.

We can conclude, that food availability was hardly affecting fitness parameters during our experiment, especially with regard to survival. Food is generally considered as one of the main limiting factors for avian reproduction (Martin 1987), and previous experiments on food supplementation with mealworms in pied flycatchers basically supported this view. In a one year experiment in The Netherlands, Verhulst (1994) showed that food-supplemented flycatcher nestlings were not heavier at fledging, but recruited more often. Females attending these broods also had a higher survival rate. His study was done on late breeding pairs only. In an one year experiment in Finland, Siikamäki (1998) carried out a similar experiment as ours, showing that delayed hatching reduced fledgling weight and number, but food supplemented birds did not suffer from this delay. It is difficult to explain, why food supplementation was hardly improving reproductive success in our experiment. With retrospect, for the first two years we had reasons why food was only having a small effect (either very high (2009), or late (2010) caterpillar peaks), but at least in 2011 we have no reason why positive effects of food supplementation were absent (great mismatch, low caterpillar densities, Figure 2.5). In that year, we even found negative effects of food supplementation. We cannot exclude possible limitations of our set-up of food supplementation such as low food quality or the occurrence of food stealing by Eurasian Jays at some nests (personal observation). Also, the contrast from full food supply to no supply might have negative effects for fledglings, especially if there are little alternatives. For all years, we found that, later in the season, birds used more of the provided food: This could arise because birds more often had to feed older nestlings later on, but our data also suggests that late breeders generally used more of the provided food which hints at a lack of preferred alternative prey in the environment. However, we have no proof that late breeders were actually food-limited in any year.

Our experiment can be viewed as mimicking the future consequences of climate change, with predators advancing to a lesser extent than the phenology of their prey

(Both et al. 2010). From previous work it was expected that this increased mismatch between hatching and caterpillar peak would be reflected in reduced survival rates and reproductive success, but this was not consistently found. The observed responses of birds to climate change could still have an adaptive explanation even if birds' responses did not restore the synchrony with a certain food type (Johansson & Jonzen 2012). Qvarnström et al. (2005), for example, suggest that pied flycatchers, compared to collared flycatchers, are better able to cope with declining food availability or food quality. Our results indeed suggest that pied flycatchers possess the flexibility to shift diets or eventually switch habitats, and therefore may depend less on the synchrony with one food type as previously thought.

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Appendix Chapter 2

List of candidate models, using fledgling weights as dependent. All models were mixed models with nest box as random factor using function lme in R. Candidate models for number of fledglings contained the same parameters, but without tarsus length and instead including clutch size into all models (no table shown here).

Parameters

f: food supplementation

t: delay treatment

d: hatch date

h: habitat

l: tarsus length

u: number of fledglings

*: indicated an interaction, lower level interactions or main parameters were always included if parameters were part of the interaction

Candidate models	Parameters
1 (full model)	$f*d*t+f*d*h+d*t*h+t*h*f+l+u$
2	$f*d+d*t+d*h+t*h+f*h+f*t+l+u$
3	$f*t+f*d+t*d+l+u$
4	$f*t+h+d+u+l$
5	$f+t+d+h+u+l$
6	$f+t+d+u+l$
7	$f+t+u+l$
8	$f*t+u+l$
9	$u+l$
10	$f*t+l$
11	$f+l$
12	$f+h+l$
13	$f*h+l$
14	$f+h+t+l$
15	$h+l$
16	$f+t+l$
17	$t+l$
18	$t+h+l$
19	$t+d+l$
20	$d+l$
21	$t*d+l$
22	$f*d+l$
23	$f+h+l$
24 (minimal model)	l

