

Possible fitness consequences of experimentally advanced laying dates in Great Tits: differences between populations in different habitats

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Summary

1. In birds, early breeding individuals generally reproduce more successfully than late breeding individuals. The lack of response to this selection could be explained by resource constraints during the egg production period.

2. *Parus* species can learn from the mismatch experienced between breeding time and nestling food availability and subsequently adjust their breeding time accordingly. In two Great Tit populations, breeding time was manipulated by creating an artificial food peak. This allowed us to study fitness consequences of manipulated breeding time in the following year without the confounding effects of food supplementation.

3. In one population, manipulated females advanced their laying dates in response to the artificial food peak. However, sample sizes were too low to quantify fitness consequences. In the other population, no response to the treatment was found. This difference could be caused by differences in resource availability in early spring between the two habitats. Low resource availability in early spring could also explain the lack of response to selection observed in one population.

Key-words: Climate change, food supplementation, *Parus major*, resource constraints, selection on breeding time

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Introduction

In many bird species reproductive success generally declines during the breeding season (Perrins 1970). Since breeding time is heritable (van Noordwijk, van Balen & Scharloo 1981; van der Jeugd & McCleery 2002; Sheldon, Kruuk & Merilä 2003), this directional selection should lead to an evolutionary change in breeding time. However, no such change has yet been reported and the lack of response to selection on breeding time has become the classical example for evolutionary stasis (Merilä, Sheldon & Kruuk 2001).

This paradox could be explained if resource availability at the time of egg production constrains an advancement of egg-laying dates (cf. Perrins 1970). A single egg can weigh up to 10% of the female's body mass and is costly in terms of energy, nutrients and future fitness (Monaghan, Nager & Houston 1998; Stevenson & Bryant 2000; Visser & Lessells 2001). Temperatures and food supply increase during spring and, consequently, there may be a date before which egg-laying is not possible or is too costly. It may also be possible that essential resources such as certain proteins or calcium are simply not available in early spring

because certain prey species (insects, snails) have not yet appeared.

Resource constraints on egg-laying could explain why laying dates in a Dutch population of Great Tits (*Parus major*) have not shifted despite a change in the phenology of their caterpillar prey (Visser *et al.* 1998). Rising spring temperatures advance the hatching of caterpillars (Visser & Holleman 2001) and the date when their biomass is maximal has advanced by about 2 weeks over the past 15 years (Visser, Holleman & Gienapp 2006). Since caterpillars form the main food supply for Great Tit chicks (Perrins 1991; Naef-Daenzer, Naef-Daenzer & Nager 2000), the birds should track the changes in caterpillar phenology. However, annual mean laying dates have advanced by only about 3 days over the same period. An alternative explanation, suggested by Visser *et al.* (1998), for the mismatch between breeding time of the birds and the phenology of caterpillars is that the birds are currently responding to the wrong cues to time their breeding. The caterpillars hatch at the beginning of May to feed on the fresh leaves of their host trees. The Great Tits however, have to initiate egg-laying about a month earlier to match the nestling period with the period when caterpillars are most abundant. The birds can therefore not react directly to the appearance of the caterpillars but have to use other cues, which used to

indicate caterpillar phenology reliably. Nowadays these cues may no longer be so accurate.

The ideal experiment to test these two hypotheses is to induce earlier laying than is normally found and to measure fitness consequences. Experiments to manipulate laying date have been carried out frequently: in food supplementation experiments an advancement of laying dates was generally found (Nager, R  gger & van Noordwijk 1997 and references therein). These experiments, however, are unsuitable to evaluate fitness consequences because the birds were given additional resources and hence the potential fitness costs of early egg production will be underestimated.

Great Tits use the synchrony between nestling period and food supply that they experience in one year to 'fine-tune' their timing of breeding in the following year (Nager & van Noordwijk 1995). Individuals that breed earlier than optimal delay breeding in the following year, while individuals that breed too late advance their breeding. This ability to learn has also been shown experimentally in Blue Tits (Grieco, van Noordwijk & Visser 2002). Individuals given an artificial food peak late during the nestling period delayed their breeding in the following year. This approach offers an elegant way to manipulate breeding time and to evaluate fitness consequences because the birds are not given any additional resources in the year when their laying dates are manipulated.

Materials and methods

STUDY SPECIES AND AREAS

Our study was carried out in 2001–03 in two Dutch nestbox populations of Great Tits followed since 1955/56. One study area, Oosterhout, is a relatively small woodland of 11 ha, in which 120 nestboxes are placed. The woodland is situated near the river Waal on a river floodplain with rich clay soils. The vegetation consists mainly of large Pedunculate Oaks (*Quercus robur*), Maple (*Acer pseudoplatanus*) and Ash (*Fraxinus excelsior*) with rich undergrowth of Cherry Laurel (*Prunus laurocerasus*), Rowan (*Sorbus aucuparia*) and Common Elderberry (*Sambucus nigra*). The other study area, the Hoge Veluwe, is part of a larger wooded area on poor sandy soils. The vegetation is a mixture of Pedunculate Oak (*Quercus robur*), Red Oak (*Q. rubra*), Birch (*Betula pendula*) and Scots Pine (*Pinus sylvestris*) with sparse undergrowth and interspersed with open heath land. The study area covers 171 ha with about 400 nestboxes.

STANDARD FIELDWORK

Nestboxes were checked weekly to determine clutch size and laying date, i.e. the date when the first egg of a clutch was laid. Since successive eggs are generally laid at 1-day intervals and clutch size usually exceeds six eggs, weekly checks give accurate laying dates. All

nestlings were ringed with aluminium rings at the age of 7 days and weighed at the age of 15 days. Adults were caught during the chick-feeding period and identified by colour and aluminium rings or ringed if unringed.

Caterpillars make up the main food source for Great Tit nestlings in these two study areas and timing relative to caterpillar phenology is the main determinant of reproductive success (van Noordwijk, McCleery & Perrins 1995; Visser *et al.* 2006). To determine caterpillar phenology, caterpillar droppings (frass) were collected under oak trees at six locations in Oosterhout and seven locations on the Hoge Veluwe. Caterpillar biomass was calculated from the frass using the equation of Tinbergen & Dietz (1994). The date when the calculated biomass was highest was defined as 'caterpillar peak' and used as a reference point for caterpillar biomass. As a measure of relative timing (asynchrony), the difference between the day the chicks were 9 days old and the 'caterpillar peak' was calculated. An age of 9 days was chosen as reference because at this age Great Tit chicks grow fastest and their food demands are highest (Gebhardt-Henrich 1990; Keller & van Noordwijk 1994).

FOOD SUPPLEMENTATION

To manipulate the birds' experience, supplementary food that mimicked an artificial food peak was provided (cf. Grieco *et al.* 2002) in 2001 in Oosterhout, 2002 in the Hoge Veluwe. Once egg-laying had started in a nestbox, the nestbox was randomly assigned to one of three groups. In one group of birds (manipulated) the birds should be given the perception that they were breeding too late and hence the artificial food peak was earlier than the chick-feeding period. The treatment started on the day at which the eighth egg was laid or at the onset of incubation if clutch size was smaller than eight (4 out of 21 clutches). The food (mealworms, *Tenebrio molitor*) was offered in small trays (approx. 5 cm × 3 cm × 4 cm) inside the nestbox to ensure that no birds, other than the manipulated pairs, had access to the food. The amount offered increased, in a stepwise manner, from 3 g on day 1 to 20 g on day 7, remained constant until day 9 and then decreased stepwise to 3 g on day 15. The treatment therefore stopped about the time the chicks hatched, since incubation normally lasts 13 days. Nestboxes were visited daily; the remaining food was removed and weighed to the nearest 0.1 g and the 'scheduled' amount of fresh food given. Food-supplemented birds might be in better condition and breed earlier in the second year because of carry-over effects, not because of their manipulated experience. To control for such effects, we provided a second group (control-supplemented) with the same amount of food following the same schedule but starting on the day the chicks hatched. Consequently food supplementation stopped shortly before the chicks fledged. Since this group received the artificial food

peak during the chick-feeding period we did not expect any effect of experienced mistiming on breeding time in the second year. A third group of birds (unmanipulated) did not receive any food to serve as a natural reference group. Owing to the small number of breeding pairs, this third group had to be omitted in the Oosterhout experiment. In the second year of the experiment, a number of first-year breeding females (recruits or immigrants) entered the breeding population and we compared laying dates of the manipulated females to their laying dates. To correct for age effects (Wheelwright & Schultz 1994; Robertson & Rendell 2001), the long-term difference in laying dates between first-year breeders and older females for the Oosterhout study area was calculated (1.46 ± 0.38 (SE), $n = 41$ years). This was compared with the difference between manipulated and first-year breeding females and the difference between control-supplemented and first-year breeding females. Dates (e.g. laying) are reported as April days, with 1 being 1 April and 31 being 1 May.

Results

TREATMENT

In Oosterhout (2001) 28 breeding pairs were food supplemented (14 manipulated and 14 control-supplemented pairs). In the Hoge Veluwe (2002) 42 pairs were food supplemented (21 manipulated and 21 control-supplemented pairs) and 41 pairs were not food supplemented at all (unmanipulated). Neither laying dates nor reproductive success differed between treatment groups

(laying date: $F = 0.85$, $df = 2, 107$; $P = 0.43$; no. of fledged chicks: $F = 1.05$, $df = 2, 104$, $P = 0.35$; mean chick weight: $F = 0.19$, $df = 2, 96$, $P = 0.83$, all corrected for areas). This was even true when only females that survived until the second year were considered (laying date: $F = 0.06$, $df = 2, 57$, $P = 0.95$; no. of fledged chicks: $F = 0.25$, $df = 2, 56$; $P = 0.78$; mean chick weight: $F = 0.07$, $df = 2, 48$, $P = 0.94$, all corrected for areas). When mealworms were offered during the chick feeding period, pairs consumed significantly more than when offered during incubation ($F = 39.2$, $df = 2, 66$, $P < 0.001$).

RESPONSE TO TREATMENT

Survival from the first to the second year of the experiment did not differ between treatment groups or areas (treatment: $\chi^2 = 0.53$, $df = 2, 107$, $P = 0.77$; area: $\chi^2 = 2.21$, $df = 1, 107$, $P = 0.14$). Changes in laying dates from the first to second year of the experiment (corrected for environmental effects) depended on the extent of asynchrony with the food peak (Hoge Veluwe: $F = 47.4$, $df = 1, 44$, $P < 0.001$; Oosterhout: $F = 10.9$, $df = 1, 9$, $P = 0.009$). Females that bred earlier than the natural food peak delayed laying in the second year and females that bred later than the food peak advanced in the second year (Figs 1 and 2). This response to the mistiming experienced was stronger in Oosterhout than in the Hoge Veluwe (area \times asynchrony: $F = 4.39$, $df = 1, 51$, $P = 0.04$). After correcting for the asynchrony experienced, manipulated females bred on average 5.6 days later than control-supplemented in Oosterhout ($F = 5.139$, $df = 1, 9$, $P = 0.0496$) (Fig. 1)

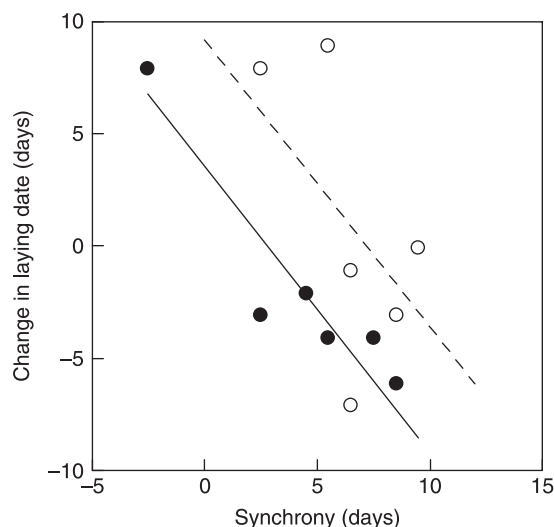


Fig. 1. Oosterhout experiment: change in laying date from first to second year vs experienced asynchrony with the caterpillar food peak in the first year (closed dots and solid line: manipulated females; open dots and dashed line: control-supplemented females). The change in laying date was significantly affected by experienced asynchrony ($F = 11.0$, $df = 1, 9$, $P = 0.009$) and experimental treatment ($F = 5.2$, $df = 1, 9$, $P = 0.049$). Manipulated females bred on average 5.6 days earlier than control-supplemented females.

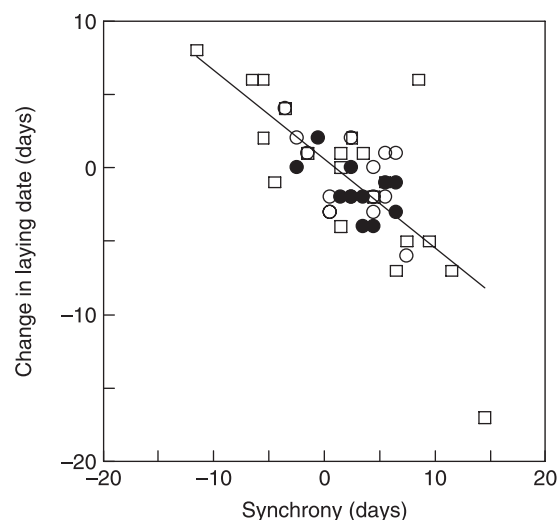


Fig. 2. Hoge Veluwe experiment: change in laying date from first to second year vs experienced asynchrony with the caterpillar food peak in the first year (closed dots: manipulated females, open dots: control-supplemented females, open squares: unmanipulated females). The change in laying date was significantly affected by experienced asynchrony ($F = 44.9$, $df = 1, 43$, $P < 0.001$, solid line) but not experimental treatment ($F = 0.12$, $df = 2, 43$, $P = 0.89$).

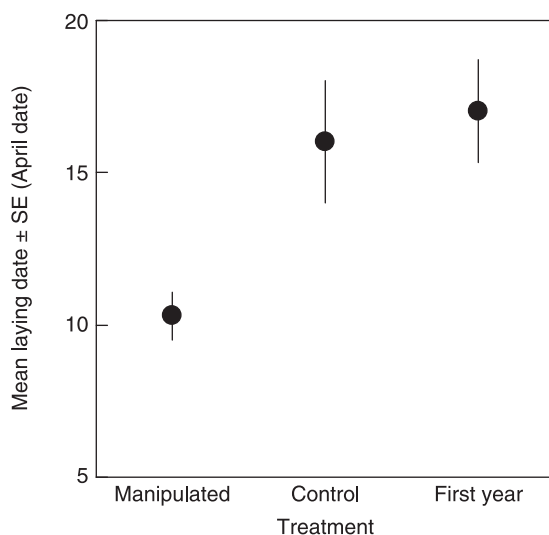


Fig. 3. Oosterhout experiment: laying dates of manipulated, control-supplemented and first-year breeding females in 2002 (second year). The difference between control-supplemented females and first-year breeders is not significantly different from the expected difference between older and first-year breeders, while manipulated females bred significantly earlier than expected for older females (see text for details).

while there was no difference between treatment groups in the Hoge Veluwe (treatment: $F = 0.12$, $df = 2,43$, $P = 0.89$) (Fig. 2). The difference in the response of the treatment groups between areas was significant (treatment \times area: $F = 4.65$, $df = 1,51$, $P = 0.036$).

However, we cannot conclude from this that manipulated females advanced their laying date because the same pattern would arise if control-supplemented females delayed laying. Owing to the small breeding population in Oosterhout it was not possible to have a third group of unmanipulated females, but first-year breeders can serve as an unmanipulated 'outgroup'. Ten first-year females, either immigrants or recruits, bred in the study area in the second year. Their mean laying date was 17.0 ± 1.68 (SE), compared with 10.3 ± 0.76 (SE) for manipulated and 16.0 ± 2.00 (SE) for control-supplemented females (Fig. 3). While the difference between first-year breeding and control-supplemented females (1.0 ± 2.03 (SE)) was not significantly different from the long-term difference between first-year breeding and older females (see Materials and methods) ($t = 0.976$, $df = 56$, $P = 0.83$), the difference between first-year breeding and manipulated females (6.7 ± 0.85 (SE)) differed significantly from the long-term difference ($t = -5.736$, $df = 56$, $P < 0.001$). This indicates that manipulated females do indeed advance their laying date.

FITNESS CONSEQUENCES

Although in Oosterhout laying dates between manipulated and control-supplemented females differed by about 5 days, reproductive success did not

differ between groups (mean chick fledging weight: $F = 1.66$, $df = 1,10$, $P = 0.22$; no. fledged chicks: $F = 0.54$, $df = 1,10$, $P = 0.48$). Survival from the second year of the experiment to the following year did also not differ ($\chi^2 = 0.028$, $df = 1,10$, $P = 0.87$).

In the Hoge Veluwe reproductive success did not differ between groups (mean chick fledging weight: $F = 1.87$, $df = 2,32$, $P = 0.17$; no. fledged chicks: $F = 0.71$, $df = 2,44$, $P = 0.50$). Survival from the second year of the experiment to the following year also did not differ between groups ($\chi^2 = 1.36$, $df = 2,44$, $P = 0.51$). However, overall survival probability was unusually low ($= 0.10$) and differed significantly from survival probability from first to second year ($= 0.59$) ($\chi^2 = 14.4$, $df = 1,83$, $P < 0.001$).

Discussion

Parus species can learn from previously experienced synchrony with their nestling food supply to 'fine-tune' laying dates in the following year (Nager & van Noordwijk 1995; Grieco *et al.* 2002). This learning ability was used to manipulate breeding time in two populations of Great Tits and to quantify fitness consequences of advanced laying dates. In one study area (Oosterhout), manipulated females bred significantly earlier in the second year of the experiment than control-supplemented females (Fig. 2). This cannot be an effect of improved female condition due to food supplementation because control-supplemented females received the same amount of food and actually consumed more. Since the manipulated birds bred earlier than normal (and this was achieved without any manipulation to the females in that year), this allowed us to look at possible negative fitness consequences of advancing laying dates. Unfortunately, overall recruitment was too low to allow statistical testing; in total only seven fledglings from 2002 recruited in 2003. Neither adult survival, number of fledged chicks nor fledging weight differed between manipulated and control-supplemented females. However, sample sizes were small and consequently test power was low. We thus cannot conclude that there is no cost of advancing laying dates.

Interestingly, we found different results when repeating the experiment in another population (Hoge Veluwe). In this population experimental treatment did not affect change in laying dates from the first to the second year (Fig. 2). Although sample sizes were initially twice as large as in the Oosterhout experiment, it would have been impossible to detect any differences in adult survival because general survival probability from the second to the following year was unusually low: only 5 out of 49 females bred in 2004, probably caused by the low beech crop (P. Gienapp & M. E. Visser, unpublished data; cf. Perdeck, Visser & Van Balen 2000). This is also the reason why no results from two other experiments carried out in 2003 are reported. In that year, 40 breeding pairs in the Hoge

Veluwe and 30 breeding pairs in Warnsborn (another nestbox study area) were food-supplemented. From these 70 food-supplemented females, only 7 survived to the second year, too few to test for a treatment effect on laying date.

However, the intriguing result remains that females in the Hoge Veluwe did not advance their laying date while females in Oosterhout were able to. There was no difference in the amount of mealworms consumed between the areas (treatment \times area: $F = 0.06$, $df = 1, 56$, $P = 0.81$), which might explain this difference. Hence, we should look for other ecological factors as a possible explanation. The two areas differ substantially in their vegetation type, Oosterhout is dominated by large, old trees with abundant undergrowth, while the Hoge Veluwe is a mixed oak–pine forest with much less undergrowth on poor sandy soils. This difference in vegetation may translate into a difference in insect abundance or phenology. In rich, mature woods such as Oosterhout, shrubs and herbs flower and unfold their leaves early because they have to make use of the sunlight before it is blocked by the closing canopy. In such woods vegetation growth of the understorey starts earlier than in poor woods with a less closed canopy. The phenology of many insect species is linked to vegetation phenology and this could lead to an earlier availability of insects, an important protein source for egg production, in Oosterhout than in the Hoge Veluwe.

Obviously, calcium is another important resource that is needed during egg production. Small birds cannot rely on stored calcium resources in the bones (Perrins 1996). Snails form the main supply of this resource and their abundance is probably higher in Oosterhout (but see Graveland & Drent 1997 for the absence of a relationship between calcium availability and breeding time). Unfortunately, no data on insect or snail abundance in early spring are available and it is also not entirely clear which food sources are used by Great Tits during egg production. Results from a radiotracking study of Great Tits in the Hoge Veluwe indicated that females mainly foraged in coniferous trees during the prelaying period (own data) but clearly more data on prey choice and its abundance in early spring are needed.

The availability of resources that are needed for egg production probably differs between the two areas. Consequently, only the Oosterhout birds were able to advance laying dates in response to our experiment. The manipulated females in the Hoge Veluwe may have refrained from advancing their laying dates because either the necessary resources were not available, or the cost of acquiring them would have outweighed the fitness benefits of earlier laying.

Because of warmer springs caterpillar phenology has advanced in the Hoge Veluwe by about 2 weeks over the past 15 years, leading to selection for earlier breeding (Visser *et al.* 1998). However, the birds' laying dates have not changed over the same period and two hypotheses have been proposed to explain this

(Visser *et al.* 1998). First, the birds are currently responding to the wrong cues to time their breeding. Second, an advancement of laying dates is hampered by low resource availability in early spring and a related high cost of laying early. Our results strongly hint at the latter hypothesis being true. This means that we should not expect an evolutionary response to the observed selection on breeding time because the benefit of laying earlier is outweighed by the cost of doing so. This also means that the currently observed mistiming of the Great Tits may increase which may eventually lead to a population decline.

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