Egg laying in the Blue Tit (*Parus caeruleus*): effect of temperature and interaction with food resource

Fabrizio Grieco
ABSTRACT

Egg size and laying interruptions in a Blue Tit population were analysed in relation to changes in temperature within and between three years. A feeding experiment allowed us to study the interaction between the effects of temperature and food consumption on egg-laying. Temperature influenced egg volume in one year of three. The relationship between the two variables was non-linear, i.e. a positive relation was found only at low temperatures. In the other two, relatively warm years, the probability of having laying gaps was negatively influenced by temperature. This was due to the effect of short periods of cold weather.

Egg size variation resulted from the combination of effects of temperature and effects of feeding regimes. Food-supplemented females laid eggs whose volume was less dependent on temperature, even in cold periods. Thus the effect of temperature on egg size changed in different feeding regimes. On the other hand, no evidence was found that good feeding conditions changed the relationship between temperature and the probability of laying interruptions.

Keywords: between-individual variation, egg quality, laying interruptions, within-individuals variation
INTRODUCTION

In birds, egg production and laying are believed to be influenced by ambient temperature. The reason for this is that producing eggs is costly, in terms of energy, nutrients (Perrins 1996, Stevenson and Bryant 2000) and other specific components, such as Calcium (Stlouis & Barlow 1993, Graveland et al. 1994) or amino acids (Houston 1998, Ramsay & Houston 1998). In the Great Tit Parus major, food demand by females increases up to 40% during laying compared to the pre-laying period (Royama 1966a). The daily costs of egg formation in passerines are estimated to be 40% or more of their basal metabolic rate (Ricklefs 1974, Walsberg 1983). However, recent studies suggest that producing large eggs is more costly at low temperature. Stevenson and Bryant (2000) found an association between high energetic expenditure (DEE) and both low temperatures and the production of large eggs. At low temperature, producing large eggs may cause DEE to increase up to four times the basal metabolic rate. According to this study, temperature is viewed as a constraint on egg production early in the breeding season, which may explain the observed changes in breeding phenology of birds in recent, warmer years (McCleery & Perrins 1998, Forchhammer et al. 1998, Crick & Sparks 1999).

The effects of temperature on egg laying may be direct, i.e. low temperature increases the female’s cost of body maintenance at the expense of egg formation. Physiological studies indicate that air temperature has large effects on the energetic needs of birds (Haftorn & Reinertsen 1985). Alternatively, low temperature may reduce the response of gonadal growth to the photoperiodic stimulus (Maney et al. 1999 and references therein). On the other hand, the temperature effects may be indirect, for instance if low temperature decreases the effective availability of prey that contains the energy and/or nutrients needed for egg formation (Perrins & McCleery 1989).

Effects of temperature have been reported on different life-history traits related to egg production and laying, both in descriptive and experimental studies. Tits Parus ssp. laying in colder environments lay at later dates (Kluijver 1952, Perrins 1965, O’Connor 1978, Perrins & McCleery 1989, Nager 1990), while in one study out of three, manipulation of nest box temperature did result in change of laying date in
the expected direction (Nager 1992, Yom-Tov & Wright 1993, H.R. Offereins unpubl. data). Egg size and/or mass have been found to be positively correlated with temperature a few days prior to egg-laying (Ojanen et al. 1981, van Noordwijk 1984, Järvinen & Pryl 1989, Magrath 1992), while in other cases have not (Nager 1990, Järvinen 1991, Yom-Tov & Wright 1993). In these studies, the effect of temperature was tested not always while controlling for other, confounding variables correlated with temperature, e.g. calendar date. However, experimental manipulation of nest box temperature has shown a clear effect of temperature on egg size in the Great Tit (Nager & van Noordwijk 1992), not in the Blue Tit *P. caeruleus* (Yom-Tov & Wright 1993).

Low temperature may also cause delays in clutch initiation (Meijer et al. 1998, Visser & Lambrechts 1999) or interruptions within a laying sequence (Winkel 1970, Winkel & Winkel 1974, O'Connor 1979). Yom-Tov & Wright (1993) demonstrated that an experimental increase in nest temperature caused a drop in the probability of having laying interruptions in Blue Tits.

The different results of studies on temperature and egg quality may be due to the interaction between food resource and temperature. Nager & Zandt (1994) found that Great Tit egg size was smaller when food abundance at the time of egg formation was low. However, the correlation between food density and egg size was evident only when temperature was low. This interaction might be seen the other way round: high food density could reduce the influence of temperature on egg volume or the probability of having laying gaps.

In this study, I have analysed the relationship between ambient temperature, egg volume and laying interruption rate in Blue Tits during three years. A supplemental feeding experiment carried out in a parallel project on Blue Tits provided the opportunity to investigate the interaction between temperature, feeding regimes and egg-laying. By combining temperature patterns and additional feeding experiments in the three years, I have tried to assess the effect of food availability on the relationship between temperature and egg size, and between temperature and laying interruption rate.
METHODS

The study was carried out in the next box population of the Netherlands Institute of Ecology in the National Park “De Hoge Veluwe”, central Netherlands, from 1997 to 1999. The study area comprises four hundred nest boxes in a mixed forest dominated by pine *Pinus* spp. and European oak *Quercus robur* (for details see van Balen (1973)).

**Feeding experiments**

From half March each year, nest boxes were visited regularly at least twice a week for signs of nestbuilding. Because Great Tits were the subject of other experiments, it was not possible to start supplemental feeding in all potential nest sites. Blue Tit (BT) nest sites were identified by means of (1) the form and structure of the nest (Cramp & Perrins 1993) – but species discrimination is easier at later nestbuilding stages – and, more reliably, (2) observation of minute details of behaviour of birds alarming around the nest boxes (Grieco, in press). More information came from nocturnal inspection of nest boxes, since the females roost in the nest box prior to egg-laying. Of the 101 nests provided with pupae, 96 (95%) turned out to be BT nests. Additional ten BT nests were not discovered and provided with food before laying, since they were built very quickly (usually late in the season).

All Blue Tit pairs were offered fly pupae in fixed daily amounts (25 items/day). To prevent other birds from consuming them, the pupae were placed in a small tray inside the nest box, usually attached at the left inner side. Additional feeding started as soon as there were clear indications for BT nesting. This caused supplemental feeding to start at a variable number of days before laying date (average 8.7±5.9 (SD) days). Nineteen nests (25% of all supplemented nests, n=76) were provided with food less than five days before, or even after the date of laying of the first egg. Given that tits collect energy in the three-four days prior to egg laying (Perrins 1979), for those nests the food addition had probably no effect early in the laying sequence.

Personal observations indicate that the females were visiting the nests more frequently than the males (this is not surprising at least in the pre-laying phase, since nestbuilding is performed by the female), and that the females took most of the pupae.
Previous work on Great Tits showed that females supplemented with fly pupae in a similar experiment laid larger eggs than control females (Grieco & Visser 1997), indicating that part of the additional food was actually eaten by the birds.

Routine fieldwork
Focal nests were visited every day and the number of pupae missing was recorded. Food was replenished up to the standard amount. The only difference in the experimental set up between years was that in 1997 food was provided until the date of laying of the first egg (here indicated as laying date), while in 1998 and 1999 until the first day the female was brooding the eggs or the eggs were found warm.

Eggs were numbered and measured the day they were laid. A volume-index of eggs was calculated as $0.5 \times l \times b^2$, where $l$ (length) and $b$ (breadth) of eggs were measured with a calliper to the nearest 0.05 mm. This index, here indicated as egg volume, is a good approximation of the measured volume as well as of the fresh weight of Great Tit eggs (van Noordwijk et al. 1981).

Data on mean daily temperature were obtained from the KNMI weather station at Deelen Airport, c. 5 km from the study area. For each egg laid, I calculated the average temperature during egg formation of individual Blue Tits as the average temperature of the three days preceding the laying of an egg. This was done because, at least in the closely related Great Tit, the phase of rapid follicular growth lasts three to four days (Walsberg 1983). Mean temperature during laying was calculated for each clutch by averaging temperature of all the three-day periods in the laying sequence (including laying gaps). If not otherwise stated, average temperature will indicate average temperature during egg formation. Calendar date was expressed as April date (1= 1 April, 31= 1 May etc.).

Data analysis
Variation in egg volume was analysed with general linear models. To test the effect of temperature, the individual egg was the observation unit while clutch was considered as factor. Eggs laid after an interruption were excluded from the analysis since they were slightly larger than the average in the sequence. To avoid the problem of the huge number of degrees of freedom in such design where variables are highly
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correlated (e.g. date and temperature), the effect of each of those variable was tested last against the residual due to within-day variation in egg volume, where day was considered as factor.

For each nest, I also calculated the coefficient of regression of egg volume on mean temperature in the 3-day period preceding laying of each egg. Only the coefficients based on at least five eggs were included in the analysis.

For each day, the laying gap (LG) rate was defined as the proportion of females which interrupted egg-laying on a certain day within their sequences (i.e., between the first and the last egg laid for each female). To have a better estimate of LG rate, only days with more than four females being expected to lay an egg were included. Laying gap rate was analysed with a generalised linear model with binomial error distribution and logit link function, and the effect of predictor variables on the change in deviance was tested with $\chi^2$ tests. Whenever the residual deviance was high compared to the number of degrees of freedom I applied the William’s correction for overdispersion (Crawley 1993) in combination with F tests.

I analysed a total of 86 clutches where the incubation stage was reached. Of these, seventy-six belong to nests that were provided with additional food. However, in 14 (18%) of those nests the birds did not accept the food, or average consumption was less than one pupa/day (very low food consumption rates could be due to the action of other, intruder birds; pers. obs.). Those nests were pooled together with the nests not provided with food because of identification errors, and were here considered as nests ‘with no food consumption’ (n=24), as opposed to those where food consumption was significant (n=62). The three groups of nests (food not provided, food provided but not accepted, food consumed) did not differ in age or body size of the female, laying date, clutch size or average temperature during egg-laying (ANOVA and Kruskal-Wallis ANOVA, all n.s.). The first two groups might be treated as a 'control-like' under the assumption that birds that did not accept food did so because they either they did not see it or because they were scared by the tray. At least the first option is plausible, as shown by some birds in the third group that started eating up the food after ignoring it totally in the preceding days. However, the first two groups did not differ in average egg volume or in LG rate (general and generalised linear models respectively, all n.s.).
Statistical analysis was performed with SAS v. 6.12 (SAS Institute 1989) and GLIM v. 4.0 (Numerical Algorithms Group 1993).

RESULTS

Temperature in the study period
Although March was slightly warmer in 1997 than in 1998 and 1999, the between-years difference in temperature was reversed in April (Table 1). Average values in April were higher in 1998 and 1999 than in 1997 and the long-term average for that month (7.8 °C), yet temperature in those years showed similar patterns with a marked decrease around mid April, when minimum temperature dropped below 0 °C (see Figure 1). The lowest average daily temperature in the period when eggs were laid (usually comprising the second two decades of April and the first of May) was 2.4 °C in 1998, against 3.8 and 3.2 °C in 1997 and 1999, respectively.

Food consumption
Overall, fly pupae were offered in 1236 nest days (234, 466, 536 in the 3 years respectively).

The additional food offered daily was totally consumed in 39.6% of the nest days, while in 22.3% it was ignored. In the rest of the nest days, food was obviously taken in intermediate amounts. Table 2 shows the between-year differences in food consumption rate, in terms of proportion of nests where the fly pupae were taken in significant amounts (see Methods), and in terms of proportion of items taken daily during the experiment. Food consumption differed among years, both in terms of proportion of pairs accepting food and proportion of pupae eaten (proportion of pairs eating $\chi^2=8.34$, $P<0.05$; amount consumed $F_{2,58}= 14.49$, $P<0.00001$). Post hoc comparisons show that food consumption was similar between 1997 and 1999, and much higher in those two years than in 1998 (Table 2), both in the whole period of food supplementation and in the period prior to laying date. The proportion of pupae taken increased with date in 1998 and more slightly in 1997 (partial correlations:}
Table 1. Temperature data in March and April, for the three study years.

<table>
<thead>
<tr>
<th></th>
<th>T (°C):</th>
<th>March</th>
<th>April</th>
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<tbody>
<tr>
<td></td>
<td>mean</td>
<td>max</td>
<td>min</td>
</tr>
<tr>
<td>1997</td>
<td>7.4</td>
<td>11.5</td>
<td>3.4</td>
</tr>
<tr>
<td>1998</td>
<td>6.9</td>
<td>10.7</td>
<td>2.7</td>
</tr>
<tr>
<td>1999</td>
<td>6.9</td>
<td>10.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Table 2. Food consumption rate during the experiments in the pre-laying period (1997) and pre-laying + laying period (1998, 1999). *) ≥ 1 pupa taken per day. *) includes only nests where ≥ 1 pupa was taken per day. Different letters indicate between-year significant difference after post hoc comparisons (all $P<0.005$).

<table>
<thead>
<tr>
<th></th>
<th>1997</th>
<th>1998</th>
<th>1999</th>
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<tbody>
<tr>
<td>proportion of nests where food was taken* (n):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-laying period</td>
<td>0.52 (23)</td>
<td>0.48 (21)</td>
<td>0.86 (22)</td>
</tr>
<tr>
<td>overall</td>
<td>0.52 (23)</td>
<td>0.96 (27)</td>
<td>0.92 (26)</td>
</tr>
</tbody>
</table>

Median proportion of items taken per day$^a$ [range] (n):

<table>
<thead>
<tr>
<th></th>
<th>1997</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-laying period</td>
<td>0.78 [0.32-1.0] (12)$^a$</td>
<td>0.19 [0.0-0.94] (19)$^b$</td>
<td>0.66 [0.0-0.95] (21)$^a$</td>
</tr>
<tr>
<td>overall</td>
<td>0.78 [0.32-1.0] (12)$^a$</td>
<td>0.44 [0.17-0.81] (25)$^b$</td>
<td>0.68 [0.36-0.95] (24)$^a$</td>
</tr>
</tbody>
</table>

$P<0.005$ and $P=0.08$, respectively), but not with average temperature, in any year (partial correlations, n.s.).

**Egg volume: effect of temperature and date**

Less than 10% of the birds caught each year is known to have bred the preceding season. Consequently, we can treat data from two successive years as independent. There was no significant difference in average egg-volume among years (Table 3; ANOVA, $F_{2,83} = 0.21$, $P>0.80$). Neither was there any significant change in egg-volume (here expressed as deviation from mean egg volume in the population and year) of females breeding in two successive seasons (Wilcoxon matched-pairs test,
Table 3. Average (± SD) egg volume and proportion of laying gaps of Blue Tit clutches at the Hoge Veluwe, in the three study years. Mean egg volume was calculated for each of the \( n \) individual clutches; laying gap rate was calculated for each clutch over the period between the first and the last egg.

<table>
<thead>
<tr>
<th>Year</th>
<th>Egg volume Mean ± SD (mm(^3))</th>
<th>Laying gap rate median [range]</th>
<th>% clutches with at least one gap</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>1020.8 ± 99.4</td>
<td>0.07 [0.0- 0.42]</td>
<td>63.3</td>
<td>30</td>
</tr>
<tr>
<td>1998</td>
<td>1035.7 ± 85.8</td>
<td>0.0  [0.0- 0.58]</td>
<td>32.1</td>
<td>28</td>
</tr>
<tr>
<td>1999</td>
<td>1023.7 ± 87.6</td>
<td>0.0  [0.0- 0.40]</td>
<td>25.0</td>
<td>28</td>
</tr>
</tbody>
</table>

\( T=13, n=10, P>0.10 \). In none of the study years, egg size correlated with tarsus length or body mass of females at the time they had their chicks in the nest (correlation, all \( P>0.05 \)). Females older than second calendar year did not lay larger eggs than yearling females (\( F_{1.70}= 0.11, P>0.7 \); egg volume expressed as deviation from the population mean).

Clutch, or individual female, contributed a large part of the variation in egg volume (effect of clutch: \( F_{83,736} = 20.31, p<0.00001 \)) and explained 65.8% of the total variance. Average temperature in the three-day period preceding laying influenced egg volume, females laying larger eggs in warmer days (\( F_{1,101} = 5.94, P<0.02 \)). I also found a nearly significant interaction between year and temperature (\( F_{2,100} = 2.86, P=0.06 \)), suggesting that the effect of temperature could be different among years (Figure 1). After repeating the analysis for each year separately, ambient temperature predicted significantly egg volume only in 1997 (\( F_{1,35} = 18.26, P<0.0001 \), while in 1998 and 1999, the effect was far from significant (ANOVA, both \( P>0.60 \); Figure 2). Date of laying also influenced the size of the egg laid, but only in 1998 (\( F_{1,33} = 9.12, P<0.005 \); for the other years, \( P>0.20 \)).

The strikingly large volume of the eggs laid on 17 and 18 April 1998 (Figure 2) was due to a few females laying large eggs. Given that many females interrupted laying in that period, the mean egg volume increased above the usual range of values. This suggests that females differed in the ability of laying large eggs and without interruptions. Females that had no laying interruptions laid slightly larger eggs than those females that had at least one interruption, but the difference was not significant.
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Figure 1. Mean volume of Blue Tit eggs laid on a certain date, in relation to average temperature in the preceding 3-day period, in the three study years.

Figure 2. Mean egg volume in Blue Tits (●) in relation to mean temperature in the 3-day period preceding laying (—), in each of the three study years.
However, the interaction between having or not laying interruptions and food consumption was significant ($F_{1,80}=6.80$, $P=0.011$). This indicated that the difference in egg size between females that had laid with and females that laid without interruptions was affected by whether or not food was consumed. In nests where food was not consumed, females laying without interruptions tended to lay larger eggs than the others (Table 4). The difference disappeared in nests with some food consumption, indicating that consuming extra-food compensated for individual differences in the ability to collect enough resource for egg formation.

Summarising, female Blue Tits laid relatively smaller eggs at lower temperatures only in 1997. The absence of clear influence of temperature in the two subsequent years might have been due either (a) to the average higher temperature, or (b) to the effect of supplemental feeding during egg-laying in those years. However, a between-year difference in egg size was still found at low temperatures. Eggs laid at temperatures lower than 8 °C were on average smaller in 1997 than in the other two years (Figure 1; $t_{34}=-2.49$, $P<0.02$; $t$-test, data from 1998 and 1999 pooled), suggesting that food availability, or some unknown factor, led to an increase in egg size. The analysis of egg volume in relation to food consumption (see below) could help establish whether additional feeding had an effect.

Laying gaps: effect of temperature

The proportion of laying interruptions in individual females differed significantly among the three study years, with more laying gaps per day in 1997 than 1998 and 1999 (Table 3; $\chi^2=7.03$, $P<0.05$). However, the fewer laying gaps in 1998 and 1999 could be accounted for by the marked drop in temperature in short periods of the laying season. I analysed LG rate in individual days in relation to temperature. Average daily temperature seemed to affect LG rate, but if it was tested after entering average temperature during the egg formation period in the model, it was no longer significant ($\chi^2=0.08$, $P>0.7$). On the other hand, mean temperature over the threeday period was a good predictor of LG rate after controlling for daily temperature ($\chi^2=8.46$, $P<0.005$). The interaction between year and temperature in the preceding 3-day period was significant ($\chi^2=9.12$, $P<0.01$), therefore I repeated the analysis for
Table 4. Average (+ SD) egg volume (expressed as deviation from the population mean) of Blue Tit clutches laid by females that had laying interruptions and females that did not. Nests are grouped according to whether or not food was taken in significant amounts (see Methods). P values refer to: a) F test in ANOVA; b) a priori contrast analysis.

<table>
<thead>
<tr>
<th>Females</th>
<th>Mean ± SD (mm$^3$)</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All nests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with no gaps</td>
<td>+6.19 ± 76.72</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>with gaps</td>
<td>-5.14 ± 105.95</td>
<td>36</td>
<td>0.11a</td>
</tr>
<tr>
<td>Food not consumed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with no gaps</td>
<td>+46.25 ± 69.27</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>with gaps</td>
<td>-44.27 ± 116.60</td>
<td>11</td>
<td>0.03b</td>
</tr>
<tr>
<td>Food consumed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with no gaps</td>
<td>-8.70 ± 74.84</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>with gaps</td>
<td>+12.07 ± 98.47</td>
<td>25</td>
<td>0.37b</td>
</tr>
</tbody>
</table>

each year separately. Temperature in the egg formation period could not explain variation in LG rate in 1997 ($\chi^2_1 = 0.14$, $P>0.7$), but in 1998 and 1999 its effect was significant (1998: $F_{1,17} = 14.53$, $P<0.001$; 1999: $\chi^2_1 = 4.63$, $P<0.05$; Figure 3). The largest effect occurred in mid April 1998, when average temperature dropped to about 4°C and over 50% of the females interrupted egg-laying (Figure 3). Summarising, the effect of ambient temperature on the probability to interrupt laying occurred in the years when there was no effect on egg size. The higher variation in temperature in short periods of 1998 and 1999 made it easier to detect significant changes in laying gap rate.

**Effects of supplemental feeding and interaction with temperature**

The first question is whether Blue Tits that consumed a significant amount of additional food laid larger eggs than those that either were not provided with or ignored additional food. In 1997 the difference in egg volume between Blue Tits in nests with no food consumption and those exploiting additional food was not significant (average ± SD egg volume relative to population mean: with no food consumption $-5.13±110.59$ mm$^3$ ($n=18$), food consumed $+7.69±83.80$ ($n=12$); $F_{1,28}=$
Table 5. Values (mean ± SD) of regression coefficients $b$ of egg volume on temperature in the 3-day period preceding egg-laying, in nests where Blue Tits did not consume additional food and where they consumed a significant part of it (see Methods) in the three study years. The proportion of positive $b$s is also shown. *Includes also pairs not food-supplemented.

<table>
<thead>
<tr>
<th>year</th>
<th>$b$, Mean ± SD ($n$)</th>
<th>proportion of positive $b$s ($n$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>food not consumed*</td>
<td>with food</td>
</tr>
<tr>
<td>1997</td>
<td>13.92 ± 15.10 (18)</td>
<td>-0.52 ± 15.64 (12)</td>
</tr>
<tr>
<td>1998</td>
<td>10.82 (1)</td>
<td>1.21 ± 13.4 (24)</td>
</tr>
<tr>
<td>1999</td>
<td>-13.08 ± 25.2 (4)</td>
<td>-0.49 ± 30.42 (22)</td>
</tr>
<tr>
<td>all</td>
<td>9.09 ± 19.26 (23)</td>
<td>0.21 ± 21.48 (58)</td>
</tr>
</tbody>
</table>

0.12, $P>0.7$). This result is not surprising since food was offered only until the first egg was laid. However, the first egg, not the second, was slightly larger in females consuming additional food ($t_{27}=-1.68$, $P=0.10$). The difference was significant if only the first eggs laid at temperatures lower than 8 °C were included ($t_{17}=-2.55$, $P<0.02$). Thus low temperature increased the correlation between food availability and egg size.

In 1998 and 1999 the feeding experiment lasted until the onset of incubation, yet the difference in mean egg size between birds exploiting extra-food and the others was not significant (with no food consumption +34.44±74.01 mm$^3$ ($n=6$), food consumed -4.22±86.98 ($n=55$); $F_{1,53}= 1.08$, $P>0.3$; data from the two years pooled). However, this could be due to the small number of birds in the first sample. In none of the three years mean egg volume increased with the average proportion of pupae taken daily (Spearman rank correlation, all $P>0.30$).

Thus, there was no direct evidence that the additional food resources caused a general increase in egg size. Another possibility was that female Blue Tits consuming additional food could lay eggs whose volume was less dependent on ambient temperature than females which were not given additional food or that did not accept it. Given that only in 1997 a sufficient number of nests ‘with no food consumption’ was available, I limited the analysis to this year. The interaction temperature × food consumption was just significant (ANCOVA, $F_{1,350}= 3.84$, $P=0.05$). This could be due to pseudoreplication because eggs within clutches were likely to be laid at similar
Figure 3. Laying gap rate in Blue Tits (●) in relation to mean temperature in the 3-day period preceding laying (—), in each of the three study years. Laying gap rate is here shown for days when at least two females were expected to lay.

temperatures. For each clutch, I calculated a coefficient \( b \) of regression of egg volume on temperature during the three-day period preceding laying. The regression coefficients were, on average, higher for nests with food not consumed than in nests with some food consumption (Table 5; for 1997: \( t_{28} =2.53, P<0.02 \)). Egg size increased with temperature during egg formation, but only in those females that did not consume additional food. In food-consuming females, the regression coefficients
Figure 4. Values of regression coefficients \( b \) of egg volume on temperature plotted against the average temperature over the period of egg formation and laying, in female Blue Tits that did not consume supplemented food (including females that were not food-supplemented) and females that consumed at least part of supplemented food.

were unrelated to the mean proportion of food items consumed (Spearman correlation, \( P > 0.20 \) for all years). Table 5 also shows the values for the other two years, where very few pairs belong to the first group.

Could we explain variation in the \( b \) coefficients within feeding groups? Among nests with no food consumption, \( b \) correlated negatively with the average temperature calculated over the laying period (Figure 4). Thus temperature affected positively egg size within clutches, but only at the lower temperature range. There was not such relationship among nests with some food consumption (Figure 4). The two correlation coefficients differed significantly (\( P < 0.005 \)).

Food supplementation could influence the frequency and duration of laying interruptions. Females that consumed some food made as many interruptions within a laying sequence as females that did not, in all years (proportion of laying interruptions in individual laying sequences: Mann-Whitney U test, all n.s.). Moreover, the proportion of laying gaps was unrelated to the amount of food eaten (Spearman correlation, all n.s.). There was no evidence for an interaction between food
consumption and temperature (for 1997: $F_{2,32}=0.53$, $P=\text{n.s.}$, after correcting for overdispersion; years 1998 and 1999 lack of sufficient number of birds not consuming food). Given that in 1997 food was offered until laying date, the effect could be evident in the very first eggs of the sequence, therefore I focused on the second egg only. Neither the proportion of second eggs that were laid after an interruption, nor the mean duration of such interruptions, differed between females that consumed food and those which did not, at any range of temperature (i.e., below or above 8°C; $\chi^2$ and Mann-Whitney U tests, all n.s.).

Summarising, the interaction between temperature and feeding experiments had an effect on egg size, not on laying gap probability. Blue Tits consuming additional food laid their eggs of size independent of temperature contrary to those that did not consume any, but they did not change the frequency of laying gaps at low temperatures.

**DISCUSSION**

**Egg laying: effect of temperature**

Tits lay their eggs in variable environmental conditions (e.g. Kluijver 1952, Perrins 1965, van Balen 1973, Perrins & McCleery 1989). Low temperature affects a laying female either by increasing its cost of thermoregulation or by reducing the density or the availability of natural food. Although several authors have reported a positive correlation of egg size or mass with temperature, the relationship might be non-linear. Van Noordwijk (1984) found that in a cold year Great Tit egg volume was positively related to temperature, while in another, warmer year this correlation disappeared. Nager & Zandt (1994) conclude that egg size was unrelated to temperature over a broad range of moderate to good conditions. The existence of a non-linear relationship between temperature and egg size or, more generally, quality, where the latter no longer increases once some saturation temperature has been reached, may explain the strong influence of temperature on Blue Tit egg size in 1997 (cold year) and the absence of such influence in 1998 and 1999 (warmer years). I could not find any non-linear pattern within years, between clutches: even in 1997 egg volume increased with temperatures throughout its range (Figure 1). Among the females that
did not consume additional food the increase in egg volume with temperature was 
0.011 cm$^3$/°C, very close to that found by Jones (1973) and Nager & Zandt (1994) in 
Great Tits (approximately 0.01 cm$^3$/°C in both studies).

A non-linear relationship between temperature and egg size resulted, however, 
from the analysis of the regression coefficients $b$ of egg volume on temperature within 
clutches. These represent the response of the individual female to changes in 
temperature. Figure 4 shows that, for those females that did not use any extra food 
resource, this response was dependent on the average temperature the female 
experienced during the whole period of laying. The slopes of the regression 
coefficients $b$ on temperature were negatively correlated with the average temperature 
in that period. This means that females experiencing cold weather laid eggs that were 
positively influenced by temperature, while those laying in warmer days laid the eggs 
whose volume was more independent on temperature.

Once she has started egg laying, a female Blue Tit may respond to poor 
weather conditions either (a) by laying smaller eggs or (b) by interrupting egg laying. 
In both cases, this has consequences on reproductive success. On the one hand, small 
eggs lead to poorer hatching success, lower offspring survival and/or longer nestling 
period (e.g. Jones 1973, Schifferli 1973, Järvinen & Väisänen 1983, Nilsson 1990). On the other hand, laying interruptions may delay hatching and therefore fledging of 
the young, with possible negative effects on their survival and local recruitment 
in egg size allow a female to lay the eggs under a wider range of environmental 
conditions than would be possible according strictly to heritable factors. Laying 
interruptions, on the other hand, probably prevent the female from laying very small 
eggs with poor hatching success. In which conditions, therefore, should a female 
switch from one option to the other?

Blue Tits had more laying interruptions if temperature of the previous three days 
dropped to around 4°C. Similar threshold temperatures have been found in other 
studies (Winkel 1970, Winkel & Winkel 1974, Yom-Tov & Wright 1993). However, 
the time lag between drop in temperature and the effect on laying gap frequency 
varies among studies. For instance, Blue Tits in an English population made more 
interruptions when minimum temperature 4 to 5 days before laying was low (Yom-
Tov and Wright 1993). In my study, mean temperature in that period did not correlate at all with laying gap rate (unpubl. data). These discrepancies might reflect actual differences between populations in the speed of response to changes in temperature (see Ojanen et al. 1981, Järvinen & Pryl 1989 for similar differences in the time period when temperature correlates most with egg size). Alternatively, they may be due to contingent situations, e.g. birds laying in relatively poorer years or areas may be more exhausted during egg formation and therefore be more dependent on the environmental conditions over longer periods. This would translate in variable time lag between drop in temperature and laying interruption.

Individual females also differed in the ability to lay their eggs without interruptions. During the cold spells of April 1998 and 1999 a few females continued to lay their eggs while other stopped. Females laying without interruption laid larger eggs than females that had at least one interruption, indicating that the quality of the individual or the territory is an important component in variation in egg volume and sequence. However, the difference in egg volume between females laying with and without interruptions was apparent only when the additional food was not available or not consumed. Therefore, the use of the extra food produced the additional effect of reducing the inter-individual difference in the ability to collect enough resource for egg formation and laying. The ability of producing large eggs and without interruptions is probably a feature of the individual female, yet the two processes are only partially overlapping, as shown by the absence of effects of food supplementation on laying gap rate.

Egg laying: interaction between temperature and food resource

Some evidence was found that supplementing Blue Tits with food changed the relationship between temperature and egg quality. In 1997, the effect of temperature on egg volume was evident only in those females that did not consume additional food. With low temperature, egg size was a more precise estimate of food availability. This result is similar to that found in a study on Great Tits where egg volume at low, not high temperatures, correlated with estimates of natural food abundance (Nager & Zandt 1994). As expected from the experimental set up in 1997, the effect of
additional feeding was stronger for the first egg in the sequence (see Nilsson & Svensson 1993a for a similar effect).

Despite the fact that food provisioning reduced the influence of temperature on egg size, I did not find a parallel effect on the incidence of laying interruptions. In 1997, birds consuming additional food in the pre-lay phase did not have fewer laying interruptions in a sequence than those that did not, at any temperature range. This is not surprising, since food was offered until laying date (see Nilsson & Svensson (1993b) for a small effect of supplemental feeding during egg-laying). However, the potential effect of food on laying gap rate was not evident even where it could well be expected, i.e. in the second-laid egg. Also, the high laying gap rate during cold periods in 1998 and 1999 despite provision of food during laying seems to indicate that the influence of low temperatures on laying interruptions does not operate purely through energy reserves in the female's body, as suggested by the minimal amount of energy invested in the phase of yolk deposition that is more sensitive to temperature (Yom-Tov & Wright 1993).

In conclusion, the different patterns of egg volume observed in the three study years may be ascribed to the combination of the non-linear effects of temperature (whatever the proximate mechanisms are, i.e., constraints of thermoregulation vs. food availability), with the effects of experimentally increased food availability. On the other hand, the probability to make laying interruptions appears to be primarily a function of temperature changes.

ACKNOWLEDGEMENTS
This paper benefited from comments by Arie van Noordwijk and Rüdi Nager. Thanks to Kate Lessells for valuable suggestions about the statistical analysis of egg volume. Leonard Holleman provided the fly pupae for the experiment, while Christel Mols wrote a Dutch summary. This study is part of my PhD project at the Netherlands Institute of Ecology, Department of Animal Population Biology, funded by the University of Pavia with a Postgraduate Scholarship and the European Commission with a Marie Curie Fellowship (nº ERBFMBICT 971939).