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Costs of avian incubation

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Selection on clutch size and laying date in a great tit population: natural and experimental variation compared.

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ABSTRACT

The direction and magnitude of natural selection on reproductive traits are both of importance to understand life history variation. Here we report on an analysis of fitness variation in relation to natural variation in laying date and clutch size from a 10 year study on a great tit population (*Parus major* L.) and compare the results to experimental evidence for selection on clutch size from earlier work in the same population. The fecundity (local recruits per clutch) and viability (local survival of the parents from the clutch) selection on standardized laying date and clutch size of the first clutch was estimated using linear regression techniques. Non-linear effects of laying date and clutch size possibly leading to stabilizing or disruptive selection were estimated, as was the effect of the interaction between laying date and clutch size on selection. Overall, the direction of fecundity selection on laying date was negative (standardized selection gradient = -0.085), and on clutch size positive (0.011), but these effects were not significant. There was, however, an interaction between clutch size and laying date on fecundity selection. Selection on clutch size was positive for early birds and negative for the later birds. Such a pattern selects for a steeper clutch size- laying date reaction norm than observed. Annual variation in the direction of fecundity selection with respect to laying date corresponded to nestling growth patterns. When young grew better earlier in the season selection on laying date favoured early clutches. This suggests a causal role for seasonal patterns in food availability or food provisioning to the nestlings in explaining patterns in fecundity selection. For the females, viability selection on clutch size and laying date was not distinguishable from zero. For the males viability selection on *laying date* differed significantly between years but overall there was no effect. With respect to *clutch size* for the males there was a negative viability selection when calculated over the whole material (standardized selection gradient -0.129). The integration of the results of the earlier clutch and brood size experiments predicted no net selection on clutch size, a conclusion that is consistent with the patterns in selection described here on the basis of natural variation. However, the lack of stabilizing selection on clutch size contrasts with the earlier results on the basis of the experimental variation and remains unexplained.

INTRODUCTION

Measuring natural selection in action is one of the great challenges in ecology. Ideally we should know how fitness co-varies with variation in a large number of traits and how this co-variation is affected by the environment. Together with knowledge on the genetic basis of variation in these traits this should enable us to indicate how micro-evolution would proceed. Different approaches have been taken.

Experimental changes of brood size (Perrins & Moss 1975, Nur 1986, Pettifor *et al.* 1988, Gustafsson & Sutherland 1988, Tinbergen & Daan 1990, Orell *et al.* 1996, Both *et al.* 2000), clutch size (Sanz 1997, Visser & Lessells 2001, Hanssen *et al.* 2005), and timing of reproduction (Verhulst & Tinbergen 1991, Nilsson & Svensson 1993a, Brinkhof *et al.* 1993, Norris 1993a, Verhulst *et al.* 1995, Svensson 1997) have been performed to estimate fitness consequences of variation in these traits. With such experiments small genetic changes in the trait are simulated, as it were. The fitness consequences of these “mutations” can be interpreted as an estimate of a segment of the fitness landscape over the trait values. The strength of such an approach is that, especially in the case of clutch or brood size manipulations it is possible to randomize parental and environmental quality over the treatments, allowing within-individual estimates of the fitness landscape. A drawback is that some manipulations are difficult to perform, like the manipulation of the number of eggs laid (Monaghan & Nager 1997, Visser & Lessells 2001), or imply methods that are hard to control for in the design, like the manipulation of hatching date.

An alternative approach to estimate selection pressures acting on clutch size or laying date is to use the phenotypic variation in the trait and quantify the fitness effects related to the variation in these traits (Lande & Arnold 1983). This method relies on the natural variation in the trait and consequently estimates selection in relation to the between-individual phenotypic variation in the trait. This approach has led to a large body of estimates on selection pressures (Kingsolver *et al.* 2001) and on analyses of fitness variation in relation to clutch size and laying date simultaneously (Sheldon *et al.* 2003). The approach differs from the experimental approach because it estimates a fitness surface over all sources of variation in the trait (individual, both environmental and genetic), while the experimental approach strives to measure effects of individual variation in a particular trait in isolation independent of environmental variation. The phenotypic approach on the other hand has the advantage that it can include multiple traits relatively easily.

Neither of these approaches in itself is ideal to estimate selection in a population. The experimental approach gives an estimate of the within-individual fitness differences in relation to the manipulated trait (Grafen 1988), but does not account for phenotypic differences between individuals. With respect to clutch size this can lead to the picture that each individual's own clutch sizes maximize

fitness, but that individuals with larger clutches have higher fitness than individuals with smaller clutches (Daan *et al.* 1990, Tinbergen & Daan 1990). In other words within individuals there is stabilizing selection for clutch size, but between individuals the selection pressure on clutch size can be directional and strong (Fig. 4.1). The conclusion is that manipulation experiments may adequately estimate selection pressure at the individual level but are not adequate to estimate selection at the population level, while the study of phenotypic variation is also not adequate to estimate selection because it is based on phenotypic variation that includes environmental and genetic variation. In principle the approaches can be combined, estimating both the effects of experimental and natural variation simultaneously but this needs big data sets. Recently, also for extensive datasets animal models have been used to estimate selection using phenotypic trait variation (Merila *et al.* 2001, Kruuk *et al.* 2002, Sheldon *et al.* 2003, Postma 2005, Brommer *et al.* 2005). In these models pedigree information is used to minimize the effect of common environments within the pedigree assuming that deeper pedigrees randomize genes better over environments. This method can generate an estimate of the response on selection on the genotypes. In addition some types of variation can be accounted for by statistical correction (like differences between years).

We advocate using the different approaches in the same population to learn more about selection in action. In this paper we concentrate on describing fitness

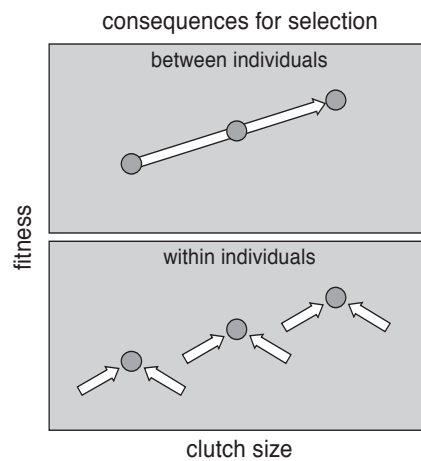


Figure 4.1. Hypothetical patterns in fitness clutch size relationships. The dots indicate clutches of three individual females that are adapted to their local circumstances. Upper panel: Between-individuals selection is directed towards higher clutch sizes as indicated by the long arrow. Lower panel: Within-individuals clutch size is locally adapted, selection works towards each of the natural clutch sizes as indicated by the arrows. In this example selection within individuals is stabilizing while selection between individuals directional.

variation in relation to trait variation by calculating the standardized selection using regression approach (Lande & Arnold 1983) and compare these results to the experimental approach. We have studied fitness consequences of clutch size variation in a great tit population using the experiments. We found strong indications for positive selection on clutch size on the basis of brood size manipulation experiments (Tinbergen & Sanz 2004), but also evidence for negative selection on clutch size based on experiments manipulating clutch size during the incubation phase (de Heij *et al.* 2006). Taken together, on the individual level selection for clutch size appeared to be stabilizing.

In this paper we analyze 10 years of population data to describe the patterns in fitness variation in dependence of phenotypic variation of clutch size and laying date simultaneously. The aim was to (1) describe the patterns of covariation between fitness and both laying date and clutch size simultaneously, (2) see whether we can explain variation in these patterns in terms of biological phenomena like nestling growth and condition and (3) compare the observed fitness landscape over the natural variation in trait values with the fitness landscape based on experimental variation in the trait values.

METHODS

General

The study area comprises a young mixed forest in the Lauwersmeer area in the northern Netherlands (planted in 1974–1975, 53°20' N, 06°12' E) and was described in Tinbergen (2005). Since 1980 the area had a small number of nest-boxes that were used by great tits (*Parus major*). From 1994 onwards, around 200 nest-boxes were available in eight plots. Nest-boxes were checked for occupation by great tits from mid April onwards at least once a week and the number of eggs, occurrence of incubation, and the number of young were recorded. Laying date of the first egg was estimated from the assumption that one egg per day was laid (Kluijver 1951). Clutch size was defined as the number of eggs incubated. We made more frequent nest visits around the expected day of hatching. Hatch date was taken as the date on which we recorded the first egg in the clutch to hatch. In a number of cases hatch date was deduced from the estimated age of the offspring. Adults were captured with spring traps when the young were 7–10 days old (day of hatching = day 0) and were ringed with individually numbered rings. At the same occasion the young were ringed as well. Nestling mass was measured in a sub-sample of nests when the young were 14 days old. Weekly nest checks were continued till mid July to detect later clutches and register their success as in the first clutches. Also the parents of these late clutches were identified. Late clutches were assigned to the female that had been identified from a first clutch in the same season.

Data used

We used data from 10 years (1994 to 2003) including recapture data in the subsequent year. Over this period we used a constant effort in ringing and recapture. First clutches were defined as clutches with a laying date not more than 30 days after the first laying date in that year. Clutch sizes below 5 and above 15 were not included in the analysis ($n = 19$) and neither were clutches with unknown laying date or clutch size ($n = 35$). The large clutches were excluded because they were in a number of cases produced by more than one female, the small ones because it was not always clear that they were complete clutches. To describe the variation in clutch size and laying date we used all remaining first clutches ($n = 1140$, Table 4.1).

Table 4.1. Basic data for 1994 – 2003 on laying date (day 1 = april 1st) and clutch size of all first clutches in the Lauwersmeerpopulation of great tits. For data selection see methods.

year	laying date	SD	clutch size	SD	N
1994	26.75	3.72	10.37	1.58	93
1995	26.76	4.51	9.90	1.42	99
1996	33.48	6.03	9.17	2.02	101
1997	30.88	5.74	9.68	1.79	112
1998	18.38	6.29	9.83	1.54	103
1999	22.42	5.94	8.94	1.93	133
2000	19.25	4.66	9.29	1.67	123
2001	31.52	4.47	8.90	1.65	118
2002	17.99	5.15	9.22	1.24	119
2003	26.53	5.15	8.22	1.69	139
overall	25.23	7.45	9.29	1.76	1140

Sample for selection estimates

For the estimates of fecundity (in terms of recruits) and viability (in terms of parental survival) selection gradients related to clutch size and laying date we used a sub-sample of the first clutches of which the young were ringed or were known to have died before fledging and where the identities of the parents were known. We excluded pairs that had their broods enlarged or reduced (1995, 1997, 1998, Sanz & Tinbergen 1999, Tinbergen 2005) or that had their clutches manipulated during incubation (2000, 2002 and 2003); de Heij *et al.* (2006). In one year (1996) we handicapped parents (Sanz *et al.* 2000). These nests were also excluded from the analysis. This subset without the manipulated pairs consisted of 682 first clutches from 512 females. We treated these clutches as independent observations. Of this subset we measured nestling mass on day 14 for 453 nests spread over all the 10 years (day 0 is hatching date of first chick).

The frequency distributions of laying date and clutch size were very similar between the experimental ($n = 458$) and the non-experimental ($n = 682$) samples as judged from the comparison between the annual means and standard deviations for both laying date and clutch size (t-test for dependent samples, all $p > 0.2$, $df = 9$).

Fitness estimates

We estimated fitness in relation to the traits laying date and clutch size for offspring and parents separately. For each pair we summed the number of local recruits (fledglings recaptured as first year breeding bird in the study area) produced from first and later clutches of the female involved. In addition we registered female and male local survival (adults recaptured as breeding bird in the study area next year). Recapture rate of adults in this population as based on simultaneous estimates of survival and recapture rate was high (0.90, $SE = 0.06$, Tinbergen & Sanz 2004). Because our aim in this paper was to estimate selection gradients we did not use mark-recapture techniques. Because we analyze parental survival over one year and because parents tend not to disperse (Tinbergen 2005) the estimates of their local survival can be upgraded to estimates of real survival by correcting for the recapture rate.

Estimating selection

We estimated standardized selection gradients following the general approach of Arnold and Wade (Arnold & Wade 1984a, Arnold & Wade 1984b). In detail the procedure was as follows (Fairbairn & Preziosi 1996): Trait values of the first clutch were standardized per year by subtracting the annual mean trait value from the individual trait value and by dividing this difference by the annual standard deviation of the trait. In this way the variance as well as the mean of the traits was equal between years. To estimate fecundity selection, we used the number of recruits produced by a parent in a particular year (from early and late clutches) divided by the mean recruitment rate of that year (relative recruitment). For viability selection we used parental local survival till next year (male or female, dead = 0 and recaptured = 1) divided by the mean survival of that year (relative survival). These fitness transformations result in a mean annual fitness component of 1 for each year and facilitate comparisons of relative strength of selection between studies.

We calculated a number of aspects of selection (Brodie *et al.* 1995). To describe *univariate* selection on the trait values laying date and clutch size, we regressed the relative fitness estimates on each of the standardized trait values separately. This selection measure refers to a directional effect of selection on the trait value. To detect stabilizing or disruptive selection we added a squared standardized trait value term as explanatory variable to the regression.

We also calculated *bivariate* selection gradients (Brodie *et al.* 1995), partly

because standardized clutch size and laying date were correlated ($r = -0.16$, $p < 0.001$, $n = 1140$). This approach explains variation in relative fitness from one trait value independently of the other (both standardized trait values entered simultaneously as explanatory values in a multiple regression). We also included a squared standardized trait value term to detect stabilizing or disruptive selection. In addition, we tested for effects of the interaction between the two standardized trait values on relative fitness, a measure for the dependence of the gradient in selection of one trait on the value of the other trait.

Statistics

We tested the significance of the different aspects of selection found by the linear least square method using Poisson (fecundity selection) or logistic (viability selection) regression techniques with a similar model structure. In these analyses we did not use relative fitness, but analysed the raw material of number of recruits and parental survival. Full models that estimate year specific effects of standardized traits, quadratic trait effects and interactions between trait effects are very complicated. The most complicated model had both standardized trait values, their squares and the interaction between the standardized trait values as dependent variables. As all these variables might affect fitness differentially between years, the interaction of the factor year with each of these explanatory variables ($df = 9$) was tested. For Poisson and logistic regressions we adjusted the scale parameter in the case of overdispersion using the Pearson's χ^2 correction (Statistica 7). Tests were based on likelihood ratios.

Power

We calculated the power for the standardized univariate selection gradients as calculated for Tables 4.2, 4.4 and 4.5. The mean number of nests that we used was 68 per year, resulting in a power of around 0.2 (standard deviation of the coefficient = 0.7) to detect a standardized fecundity selection gradient on both laying date and clutch size of 0.1 at $p < 0.05$. This is not very high. However, the power for the selection gradient for the combined years ($n = 682$) was substantial: around 0.95 to detect a selection gradient in 0.1 at $p < 0.05$. For the viability selection for both sexes the power was comparable. We conclude that if selection between years was in a consistent direction, we should be able to detect it.

Estimating the fitness landscape

In order to judge the direction of selection with respect to clutch size and laying date, we have to integrate the fitness effects on the parents with the effect on the offspring. In earlier work we have approached this by simplifying the fitness estimate assuming age independent survival and reproduction (Tinbergen & Sanz 2004):

$$\lambda = F + P. \tag{1}$$

where λ is the rate of increase of the population, F is the annual reproduction and P is the annual survival (see also Charnov & Krebs 1974).

To estimate F we need a correction for dispersal (Tinbergen & Daan 1990, Tinbergen 2005). Knowing that the population increased on average with 6% per year and that recapture probability was 0.9 (Tinbergen & Sanz 2004), and assuming that dispersal out of the study area has been equal to immigration, we can calculate the fraction of recruits that dispersed. Knowing the mean adult local survival (0.393) and the number of female recruits (0.355) per female and also assuming that adults do not disperse (Tinbergen 2005) we can calculate that a fraction of 0.58 of the recruits has dispersed. Using these corrections we calculated the fitness surface in the following way. We fitted a regression (Poisson for fecundity and binominal for survival of male and female separately) on the raw data with the explanatory variables standardized laying date and standardized clutch size and their interaction. We calculated the fit of these regressions on the basis of the significant effects. From these fits we calculated the lambda from (1) using:

$$P = (\text{fit female survival} + \text{fit male survival}) / (2 * \text{recapture probability}) \text{ and} \\ F = 1.58 * (\text{fit fecundity} / (2 * \text{recapture probability})).$$

RESULTS

Variation in clutch size and laying date

First brood mean clutch size in this population was 9.3 (SD = 1.8, $n = 1140$) and laying date 25.2 (April date, SD = 7.5, $n = 1140$). The mean clutch size declined significantly over the ten year study period ($\chi^2 = 11.43$, $p < 0.001$, Fig. 4.2A, Table 4.1). Laying date revealed no clear trend over the years ($\chi^2 = 1.20$, $p > 0.2$, Fig. 4.2B, Table 4.1).

The slope of the clutch size laying date relationship differed between years (Fig. 4.3, ANCOVA, interaction laying date times year: $\chi^2 = 29.6$, $df = 9$, $p < 0.001$, controlled for year, $n = 1140$). It was negative in all but one year (2001) with coefficients ranging from -0.21 to +0.05. The mean coefficient calculated over all years was -0.060 and differed from 0 (one sample t-test, $t = -2.83$, $df = 9$, $p < 0.05$). It was of similar magnitude as the slope relating mean clutch size and laying date between the years ($r = -0.08$, NS, $n = 10$).

Fecundity selection on laying date and clutch size

In the univariate analysis, the linear selection gradient on standardized laying date was negative in seven out of ten years, and significantly so in one year (one sample t-test, $df = 9$, $p > 0.10$, Table 4.2). The differences in slopes between the years were not significant (Poisson regression: $\chi^2 = 12.44$, $df = 9$, $p > 0.18$). Overall, using all breeding attempts independently of the years, the gradient in

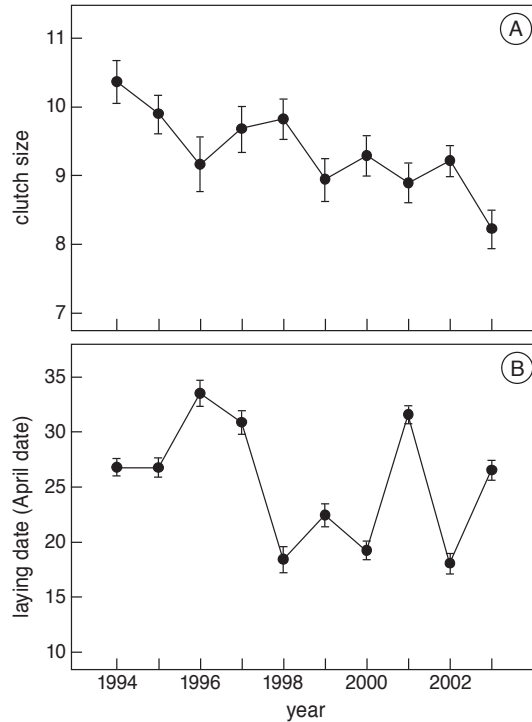


Figure 4.2. The mean clutch size (A) and lay date (B) in our study population over the years (bars indicate SE).

fecundity selection approached significance: early breeding attempts tended to have more recruits ($\chi^2 = 3.39$, $df = 1$, $p < 0.07$, $n = 682$). We interpret this as an indication, albeit a weak one, for negative directional fecundity selection on laying date in this population.

The univariate selection gradient on clutch size was negative in four out of ten years, but in no single year differed significantly from zero. Overall the gradient in selection on standardized clutch size was weak and not significant (Table 4.2). The non significance of the quadratic effects (Table 4.2) suggests no stabilizing or disruptive selection on laying date or clutch size. In the bivariate analysis of the fecundity selection we estimated the effects of laying date and clutch size simultaneously. These estimates were very similar to the univariate linear estimates given above (Table 4.2). Again there were no years in which we detected a significant quadratic effect of laying date or clutch size.

There was an interaction between standardized laying date and standardized clutch size on fecundity. Whether we included or excluded the quadratic effects, in seven out of ten years the coefficient of this interaction was negative. When we

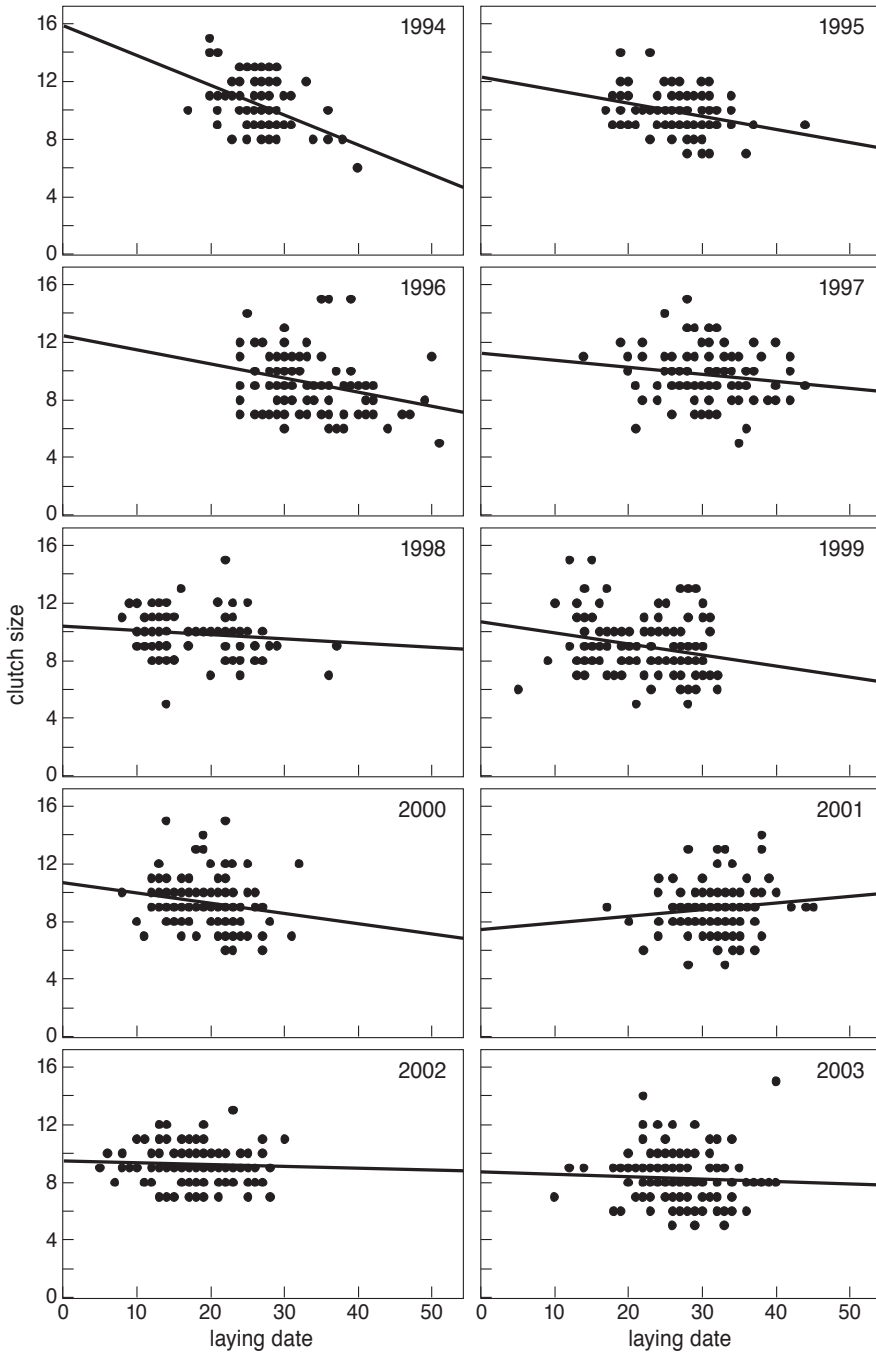


Figure 4.3. The relation between clutch size and laying date for 10 years with linear least square regression lines indicated.

Table 4.2. Annual variation in fecundity selection on laying date and clutch size. The slope estimates the linear univariate standardized selection gradient (one trait value), the bivariate slope the bivariate standardized selection gradient (fitness estimate regressed on lay date and clutch size simultaneously), the effect of the squares of the standardized trait values on the selection gradients are estimated both in the univariate and the bivariate regression. Also the interaction between the standardised trait values in the bivariate regression with (5) and without (6) the squares in the analysis is given.

(1) Values calculated in regression (normal distribution, identity link) with explaining variables laying date (LD) or clutch size (CS), (2) including either LD and LD² or CS and CS², (3) including LD and CS, (4) including LD, CS, LD², CS², (5) including LD, CS, LD², CS² and LD*CS, (6) including LD, CS and LD*CS. Selection gradients in bold were significant (p<0.05) judged on the base of Poisson regressions corrected for overdispersion.

laying date										
year	Univariate				Bivariate				Inteaction	SE
	Slope (1)	SE	Square (2)	SE	Slope (3)	SE	Square (4)	SE		
1994	-0.010	0.164	-0.006	0.086	-0.018	0.179	-0.026	0.088	-0.281	0.206
1995	-0.080	0.192	-0.173	0.154	-0.055	0.204	-0.183	0.154	-0.016	0.266
1996	-0.147	0.246	-0.309	0.232	-0.139	0.250	-0.338	0.231	0.044	0.282
1997	0.018	0.184	-0.112	0.151	0.002	0.184	-0.144	0.150	0.221	0.206
1998	-0.407	0.192	0.006	0.147	-0.399	0.195	0.009	0.145	-0.193	0.244
1999	-0.184	0.132	0.210	0.128	-0.179	0.135	0.262	0.131	-0.472	0.122
2000	-0.348	0.179	-0.205	0.174	-0.351	0.182	-0.266	0.177	0.070	0.196
2001	0.001	0.170	-0.081	0.109	-0.017	0.173	-0.108	0.108	-0.288	0.201
2002	-0.084	0.171	-0.087	0.126	-0.084	0.171	-0.091	0.126	-0.109	0.194
2003	0.256	0.153	0.083	0.092	0.238	0.154	0.069	0.092	-0.284	0.190
all	-0.085	0.055	-0.022	0.039	-0.088	0.056	-0.024	0.039	-0.117	0.056

clutch size										
year	Univariate				Bivariate				Inteaction	SE
	Slope (1)	SE	Square (2)	SE	Slope (3)	SE	Square (4)	SE		
1994	-0.014	0.165	0.104	0.116	-0.021	0.179	0.112	0.117	-0.190	0.134
1995	0.095	0.194	0.055	0.130	0.076	0.204	0.079	0.129	0.013	0.181
1996	0.068	0.248	-0.115	0.184	0.042	0.250	-0.143	0.187	0.060	0.289
1997	-0.235	0.185	-0.163	0.132	-0.235	0.184	-0.177	0.131	0.269	0.205
1998	0.118	0.194	0.001	0.116	0.044	0.195	0.014	0.114	-0.145	0.216
1999	0.064	0.133	-0.099	0.097	0.024	0.135	-0.152	0.098	-0.340	0.116
2000	0.047	0.181	0.170	0.112	-0.020	0.182	0.234	0.112	-0.056	0.184
2001	0.087	0.171	0.215	0.097	0.090	0.173	0.236	0.097	-0.124	0.183
2002	-0.020	0.173	-0.022	0.155	-0.022	0.171	-0.036	0.153	-0.122	0.190
2003	-0.180	0.154	-0.102	0.101	-0.153	0.154	-0.094	0.099	-0.201	0.186
all	-0.002	0.055	0.020	0.037	-0.016	0.056	0.023	0.037	-0.114	0.054

tested the mean estimates of the coefficients for the interactions in the different years, they suggest a trend but did not differ significantly from 0 (one sample t-test with and without quadrates: mean = -0.13, $t = -1.96$, $p = 0.08$, respectively with quadrates mean = -0.08, $t = -1.58$, $df = 9$, $p = 0.14$). The interaction was, however, significant when we excluded the year effect (Poisson regression: interaction term $\chi^2 = 5.63$, $df = 1$, $p < 0.02$, Table 4.3). This implies that fecundity selection on clutch size differed between early laying and late laying birds (Fig. 4.4). In birds with a relatively early first clutch there was a positive selection on clutch size, while in later birds there was negative selection for clutch size.

Table 4.3. Details on the Poisson regression showing how the number of recruits produced per nest depended on standardized clutch size (st. clutch size) on standardized laying date (st. lay date) and their interaction. Early in the season fecundity selection on clutch size was positive, late in the season negative.

	estimate	SE	Wald Stat.	p	log likelihood
intercept	-0.4880	0.0566	74.382	0.000	-611.472
st. laying date	-0.0434	0.0563	0.595	0.441	-611.091
st. clutch size	0.0119	0.0556	0.046	0.830	-610.923
laying date*clutch size	-0.1346	0.0497	7.325	0.007	-607.439

Viability selection on laying date and clutch size

Viability selection on laying date and clutch size turned out to be similar for the univariate calculation (standardized survival regressed on the trait value of interest) and the bivariate calculation (standardized survival regressed on both trait values of interest, clutch size and laying date, simultaneously). We present the statistics on the bivariate selection coefficients only. The gradient in viability selection for clutch size in females did not differ from zero (one sample t-test bivariate: annual mean = -0.076, $df = 9$, $t = -1.761$, $P > 0.1$, Table 4.4) nor was there a difference for laying date (one sample t-test bivariate: annual mean = 0.0003, $df = 9$, $t = 0.007$, $p > 0.9$, Table 4.4). The results for the univariate viability selection (analysis not shown) were similar. There was one significant positive quadratic effect suggesting disruptive selection on clutch size, but the interaction year times squared clutch size was not significant. This means that we were unable to show differences in the quadratic effects between the years. For females there is thus little evidence for viability selection.

For males, a significant effect on the gradient in viability selection existed with respect to clutch size (Table 4.4). Males that cared for larger clutches were less likely to survive. This was so in eight of ten years (one sample t-test on the bivariate slopes, mean = -0.114, $t = -2.30$, $df = 9$, $p < 0.05$). With respect to laying date there was no overall effect on the gradient in selection (one sample t-test on

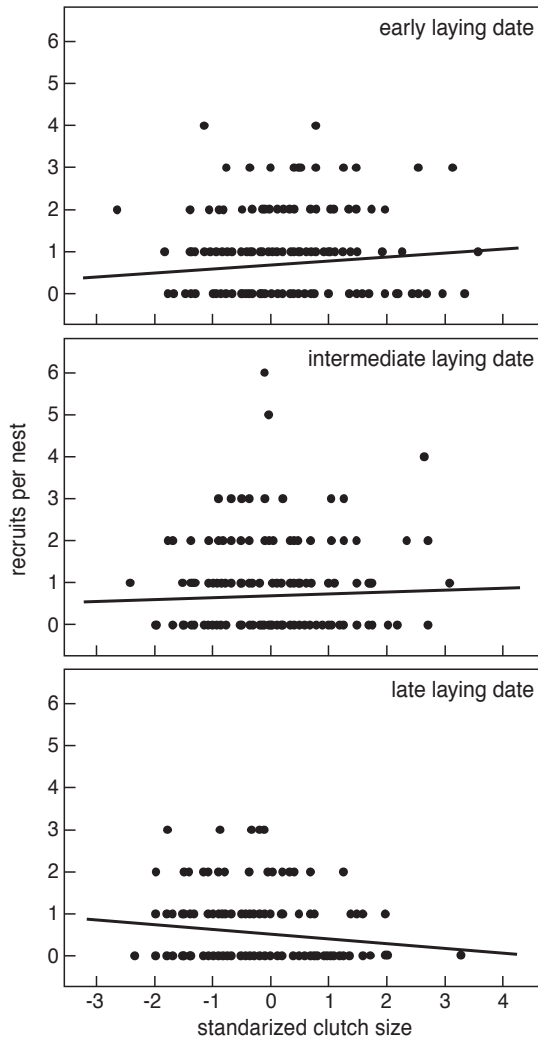


Figure 4.4. Fecundity selection on clutch size changes with laying date. Early in the year there was positive selection on clutch size (top panel), late in the year negative (bottom panel). $n(\text{early}) = 227$, $n(\text{intermediate}) = 228$, $n(\text{late}) = 227$.

the bivariate slopes, mean = -0.005 , $t = -0.059$, $df = 9$, $p > 0.9$). There were two years with a significant deviation from zero, in 1997 there was positive selection on laying date and in 2002 it was negative (Table 4.5). The year times laying date interaction was significant ($\chi^2 = 19.8$, $df = 9$, $p < 0.02$, controlled for year, laying date and clutch size). For the whole sample, and independently of year, there existed a negative directional viability selection on clutch size, but not on laying date, for male parents in this population (Table 4.5).

Table 4.4. Annual variation in female viability selection on lay date and clutch size. The slope estimates the linear univariate standardized selection gradient (one trait value), the bivariate slope the bivariate standardized selection gradient (fitness estimate regressed on lay date and clutch size simultaneously), the effect of the squares of the standardized trait values on the selection gradients are estimated both in the univariate and the bivariate regression. Also the interaction between the standardised trait values in the bivariate regression with (5) and without (6) the squares in the analysis is given. (1) calculated in regression (normal distribution, identity link) with explaining variables laying date (LD) or clutch size (CS), (2) including either LD and LD² or CS and CS², 3) including LD and CS, (4) including LD, CS, LD², CS², (5) including LD, CS, LD², CS² and LD*CS, (6) including LD, CS and LD*CS. Selection gradients in bold were significant ($p < 0.05$) judged on the base of logistic regressions.

laying date											
year	Univariate				Bivariate				Inteaction	SE	
	Slope (1)	SE	Square (2)	SE	Slope (3)	SE	Square (4)	SE			
1994	-0.178	0.144	0.004	0.075	-0.180	0.156	0.025	0.077	0.323	0.182	
1995	-0.058	0.168	0.000	0.135	-0.011	0.178	-0.016	0.135	0.051	0.235	
1996	-0.069	0.215	-0.091	0.203	-0.099	0.218	-0.096	0.203	-0.534	0.250	
1997	0.109	0.161	-0.066	0.132	0.090	0.160	-0.089	0.131	-0.018	0.182	
1998	-0.159	0.168	0.081	0.128	-0.140	0.170	0.085	0.127	-0.233	0.216	
1999	0.190	0.116	0.211	0.112	0.167	0.118	0.211	0.114	0.061	0.108	
2000	-0.136	0.157	-0.076	0.153	-0.154	0.159	-0.070	0.155	0.043	0.173	
2001	0.139	0.149	-0.073	0.096	0.188	0.152	-0.056	0.096	-0.162	0.178	
2002	0.127	0.151	0.111	0.111	0.124	0.150	0.148	0.111	0.092	0.172	
2003	0.013	0.134	0.119	0.081	0.012	0.134	0.120	0.080	0.131	0.168	
all	0.018	0.048	0.025	0.035	0.008	0.049	0.024	0.035	0.009	0.049	
		ns		ns		ns				ns	

clutch size											
year	Univariate				Bivariate				Inteaction	SE	
	Slope (1)	SE	Square (2)	SE	Slope (3)	SE	Square (4)	SE			
1994	0.066	0.144	-0.097	0.101	-0.005	0.156	-0.111	0.103	0.207	0.117	
1995	0.140	0.169	0.156	0.113	0.136	0.178	0.159	0.113	-0.118	0.158	
1996	-0.136	0.216	-0.019	0.160	-0.155	0.218	-0.017	0.164	-0.528	0.253	
1997	-0.295	0.162	-0.037	0.115	-0.289	0.160	-0.033	0.115	-0.009	0.179	
1998	0.129	0.169	-0.011	0.101	0.103	0.170	-0.002	0.100	-0.235	0.189	
1999	-0.140	0.116	0.006	0.084	-0.102	0.118	-0.024	0.086	0.065	0.101	
2000	-0.061	0.158	-0.146	0.097	-0.091	0.159	-0.128	0.098	0.128	0.161	
2001	-0.201	0.149	-0.040	0.085	-0.239	0.152	-0.061	0.086	-0.199	0.161	
2002	-0.119	0.150	0.282	0.135	-0.116	0.150	0.306	0.135	0.055	0.167	
2003	-0.005	0.135	-0.006	0.088	-0.003	0.134	0.009	0.087	0.120	0.162	
all	-0.066	0.048	-0.010	0.032	-0.065	0.049	-0.011	0.032	0.009	0.047	

Table 4.5. Annual variation in male viability selection on laydate and clutch size. The slope estimates the linear univariate standardized selection gradient (one trait value), the bivariate slope the bivariate standardized selection gradient (fitness estimate regressed on lay date and clutch size simultaneously), the effect of the squares of the standardized trait values on the selection gradients are estimated both in the univariate and the bivariate regression. Also the interaction between the standardised trait values in the bivariate regression with (5) and without (6) the squares in the analysis is given.

(1) calculated in regression (normal distribution, identity link) with explaining variables laydate (LD) or clutch size (CS), (2) including either LD and LD² or CS and CS², (3) including LD and CS, (4) including LD, CS, LD², CS², (5) including LD, CS, LD², CS² and LD*CS, (6) including LD, CS and LD*CS. Selection gradients in bold were significant (p<0.05) judged on the base of logistic regressions.

laying date											
year	Univariate				Bivariate				Inteaction	SE	
	Slope (1)	SE	Square (2)	SE	Slope (3)	SE	Square (4)	SE			
1994	-0.150	0.150	-0.116	0.079	-0.206	0.162	-0.122	0.080	0.088	0.190	
1995	0.279	0.176	-0.061	0.141	0.336	0.185	-0.038	0.140	0.299	0.245	
1996	-0.091	0.225	-0.039	0.212	-0.139	0.227	-0.068	0.211	-0.253	0.260	
1997	0.455	0.168	-0.109	0.138	0.454	0.167	-0.125	0.136	0.019	0.190	
1998	0.015	0.176	-0.109	0.134	-0.033	0.177	-0.122	0.132	0.032	0.225	
1999	0.231	0.121	0.121	0.117	0.161	0.123	0.046	0.119	0.060	0.112	
2000	-0.148	0.164	0.082	0.160	-0.191	0.165	0.039	0.161	-0.051	0.181	
2001	-0.094	0.155	0.032	0.100	-0.110	0.157	0.014	0.099	-0.089	0.185	
2002	-0.439	0.157	0.109	0.116	-0.442	0.155	0.146	0.114	0.092	0.179	
2003	0.129	0.140	0.014	0.084	0.119	0.139	0.000	0.083	-0.222	0.175	
all	0.030	0.051	-0.022 ns	0.036	0.011 ns	0.051	-0.025	0.036	0.026	0.051	

Clutch size											
year	Univariate				Bivariate				Inteaction	SE	
	Slope (1)	SE	Square (2)	SE	Slope (3)	SE	Square (4)	SE			
1994	-0.059	0.151	0.024	0.106	-0.141	0.162	0.058	0.107	0.069	0.122	
1995	0.053	0.177	-0.054	0.119	0.168	0.185	-0.056	0.118	0.203	0.165	
1996	-0.227	0.226	-0.138	0.168	-0.254	0.227	-0.131	0.170	-0.251	0.264	
1997	-0.047	0.169	-0.209	0.120	-0.017	0.167	-0.168	0.120	0.077	0.187	
1998	-0.248	0.177	-0.025	0.106	-0.254	0.177	-0.032	0.104	0.100	0.197	
1999	-0.349	0.122	0.147	0.088	-0.313	0.123	0.145	0.089	-0.010	0.106	
2000	-0.187	0.165	-0.004	0.102	-0.224	0.165	0.013	0.102	-0.056	0.169	
2001	0.059	0.157	0.135	0.088	0.081	0.157	0.154	0.089	0.041	0.167	
2002	-0.093	0.158	0.282	0.141	-0.103	0.155	0.305	0.139	0.053	0.174	
2003	-0.095	0.141	-0.115	0.092	-0.082	0.139	-0.116	0.091	-0.136	0.170	
all	-0.129	0.050	0.007	0.034	-0.128	0.051	0.008	0.034	0.024	0.049	

Correlations between selection effects

Fecundity selection on laying date and clutch size were negatively correlated (univariate $r = -0.68$, $p < 0.05$, $n = 10$). This implies that in years with selection for relatively early laying relatively large clutches were also selected. For the female's gradient in viability selection this correlation (between selection on laying date and clutch size) was -0.71 ($p < 0.05$) and for males 0.14 (NS). Males and females did not experience similar variation in viability selection pressures between years (laying date $r = 0.11$ (univariate) and clutch size $r = -0.03$ (univariate, all NS). There were no significant correlations between fecundity selection and viability selection within males or females (all $p > 0.1$).

Implications of nestling growth

To find a potential mechanism causing annual differences in the direction and strength of the gradient in fecundity selection, we analyzed the available measures of nestling mass at the age of 14 days in relation to laying date. We know that a strong correlation exists between the probability of recruitment and nestling mass in this (J.M.Tinbergen, unpublished data) and other populations (Garnett 1981, Perrins 1988, Tinbergen & Boerlijst 1990, Verboven & Visser 1998, Both *et al.* 1999). Therefore, a relation between the nestling mass and laying date could potentially explain the patterns in selection found. Indeed, the coefficients relating mean body mass of the nestlings at the age of 14 days to standardized laying date differed significantly between years (ranging from -0.62 to $+0.51$, controlled for year, interaction $\chi^2 = 25.86$, $df = 9$, $p < 0.01$). In years when nestlings from early clutches were relatively heavy, bivariate fecundity selection also favoured early clutches more than late clutches and vice versa ($p < 0.05$, Fig. 4.5). This suggests that parameters associated with nestling growth provide a

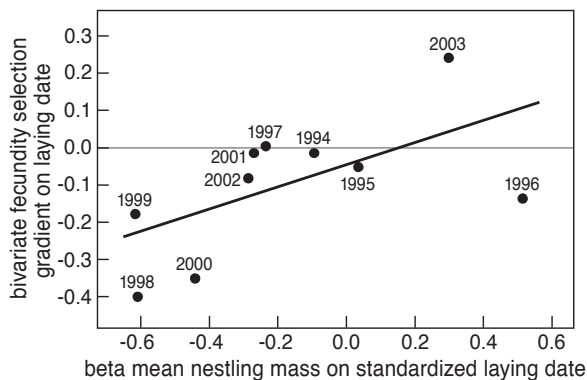


Figure 4.5. The fecundity selection gradient on laying date was related to the slope of the regression of nestling mass on standardized lay date. In years that early young grew better selection favoured earlier breeding and *vice versa*.

potential mechanism to explain patterns in the fecundity selection of laying date. In addition to this date effect nestlings were lighter when born in larger clutches ($\chi^2 = 13.9$, $p < 0.01$, slope = -0.21 g per SD in clutch size). A similar analysis relating aspects of parental mass or condition during the early clutches to laying date or clutch size (not shown) failed to detect any pattern in mass or condition with date or clutch size that could explain variations in the gradient in viability selection.

DISCUSSION

We found no significant fecundity selection (local recruits per clutch) on laying date (bivariate standardized selection gradient -0.088) or clutch size (bivariate standardized selection gradient 0.011) over the whole ten year period. The estimates indicate an average to weak selection when compared to the overview of Kingsolver *et al.* (2001) who showed that mean standardized selection on life history traits was around 0.08 .

Viability selection (parental survival) for laying date in both females (0.008) and males (0.011) was weak and non-significant over the whole period. Opposite viability selection for laying date in males was detected in two out of 10 years: in 1997 late birds survived better while in 2002 early birds survived better. Both effects were quite strong (Table 4.5). No such pattern was found for females. We do not have a biological explanation for these date related survival effects.

For clutch size there was negative viability selection. The gradient in viability selection for females did not differ from zero (-0.065), but for the male it was significant and negative (-0.128). Male viability selection was appreciable: Kingsolver *et al.* (2001) reported that generally *viability* selection on both life history and morphological traits tends to be weak (< 0.1).

Clutch size

The finding that there was an indication for negative rather than positive selection on clutch size is in contrast to the positive selection on clutch size that we detected in our brood size manipulations in the same population (Tinbergen & Sanz 2004). At that time the fact that enlarged broods did better led us to conclude that an overall positive fecundity selection existed in this population. Was this difference caused by annual variation in the direction or strength of selection? From Table 4.2 it can be seen that the fecundity selection with respect to clutch size during the years of the brood size experiments (1995, 1997, 1998) were not very different from the other years. This means that the effect of fecundity selection on clutch size we estimated during the experimental years was not caused by a coincidental year effect but rather that the estimates actually differ between the experimental and the non-experimental approach.

Or was there a difference between the experimental and the non-experimental approach because the brood size manipulations underestimate aspects of the cost associated to clutch size? This may have been so. The birds that had naturally large clutches could have experienced extra costs of egg laying and/or incubation compared to the brood size-manipulated birds that have been presented with their extra chicks “for free”. Experimental work of Visser & Lessells (2001) indicates that egg laying may indeed be costly. The work of de Heij *et al.* (2006) shows that in our study population the incubation phase can generate clutch size related fitness costs. Survival of both males and females was affected by clutch size enlargement during incubation in two out of three years and we concluded that overall selection on clutch size in this population may very well be stabilising because of the survival costs of incubation detected for the parents.

The negative effect of clutch size on male survival in the current analysis (Table 4.5) is consistent with this interpretation. Viability selection in females tended also to be negatively affected by clutch size, but not significantly so. Yet we believe that the pattern is consistent with the hypothesis that there is a cost of incubation for them as well, also because variation in the strength of viability selection between years corresponded to the experimental results of de Heij *et al.* (2006). Female viability selection was, as in the male, weakest in the year 2003. In that season de Heij *et al.* (2006) did not find viability cost to incubation in their experiment (2003 versus 2000 and 2002, Table 4.4). We therefore conclude that there was no strong positive selection on clutch size in this population because the positive selection on brood size acting during the nestling phase (Tinbergen and Sanz 2004) was counterbalanced by the negative viability selection during the incubation phase de Heij *et al.* (2006).

There remains however a discrepancy between the findings with regard to selection on clutch size on the basis of natural clutch size variation (this study) and the conclusions of the experimental work. On the basis of natural variation there was no indication of positive fecundity selection on clutch size while on the basis of the experimental variation there was a clear positive effect of brood size on fecundity. Possibly, laying a large clutch, or incubating it, generates a cost during chick rearing. Because such a carry over effect was not found by de Heij *et al.* (2006) with regard to effects of clutch size during incubation on the chick rearing phase, the most likely candidate is a negative effect of egg laying on the parent’s ability to care for the young during the nestling phase. This calls for further study.

Laying date

Both nestling growth as a function of laying date and fecundity selection on laying date did differ between years; but consistent with the low statistical power (see methods) selection only differed significantly from zero in one year. Yet, because the estimated annual selection gradient correlated significantly with the estimated slope of the mean nestling mass on laying date (Fig. 4.5), we judge the

variation in selection as biologically meaningful. We know that in the study population, as well as in many other populations nestling body mass is a good predictor for local survival (Garnett 1981, Perrins 1988, Tinbergen & Boerlijst 1990, Verboven & Visser 1998, Both *et al.* 1999). Since growth is related to the amount of food ingested, variation in the seasonality in food availability or food provisioning of the parents may well cause the variation in fecundity selection on laying date. In this population the nestlings are, in addition to caterpillars, fed with flying insects (Bibionidae and Tipulidae) that can be extremely abundant over relatively short periods in some years. Apart from differences in the timing of the caterpillar peaks, these flies may have played a role in the variation in the seasonality in the food abundance. Perhaps such food peaks are not very predictable to the parents, resulting in mistiming that impacted nestling growth and subsequent fecundity selection on laying date. Variation of seasonal food availability relative to the laying date of the tits may also explain the negative correlation between fecundity selection on clutch size and laying date. Because relatively early birds lay relatively larger clutches, in years with selection for early breeding, the large early clutches are favoured, while in years with selection for later breeding the late smaller clutches are favoured.

As mentioned in the previous paragraph within years laying date and clutch size of first clutches were negatively correlated (Fig. 4.3). Selection may act via variation in laying date or variation in clutch size or via both and for this reason we presented both univariate and bivariate standardized selection gradients. Yet, we failed to find evidence for consistent directional fecundity selection on laying date or clutch size. This result is in contrast to the findings for the collared flycatcher *Ficedula albicollis* (Sheldon *et al.* 2003) where clutch size and laying date were more strongly correlated. Bivariate analysis of selection in the flycatcher suggested that selection acted primarily via laying date only and not via clutch size, a different pattern from what we observe in the great tit. In our population there was, again in contrast to the collared flycatcher, an interaction between clutch size and laying date on fecundity selection. This means that the clutch size effect on fecundity selection depended on laying date (Fig. 4.4). In early birds there was selection for larger clutches, while in later birds there was selection for smaller clutches. Such a pattern would select for a steeper clutch size laying date relationship if not counterbalanced by viability selection.

Fitness in relation to laying date and clutch size

To discuss the fitness consequences of parental 'choice' of laying date and clutch size we have to integrate the fitness effects for the parents (viability selection) with the effects on the offspring (fecundity selection) in dependence of the standardized laying date and clutch size variation. This is essential when there is an association of fitness components with the trait values on both fecundity and survival. To judge the fitness effects, we had to correct for dispersal, especially

because dispersal tends to be appreciable for great tit *fledglings* but low for parents (Tinbergen & Daan 1990, Tinbergen 2005). Such corrections preclude adequate statistical testing of the whole fitness landscape. We therefore estimated effects of the trait values (standardized laying date and standardized clutch size) for the fitness components separately and calculated their fit. We subsequently corrected the different fits for dispersal and local recapture rate and summed them up to a fitness estimate (λ = the rate increase of the population, see methods for the details of the calculations). The results (Fig. 4.6) show that the fitness surface over the parental laying date clutch size ‘choice’ was also affected by the

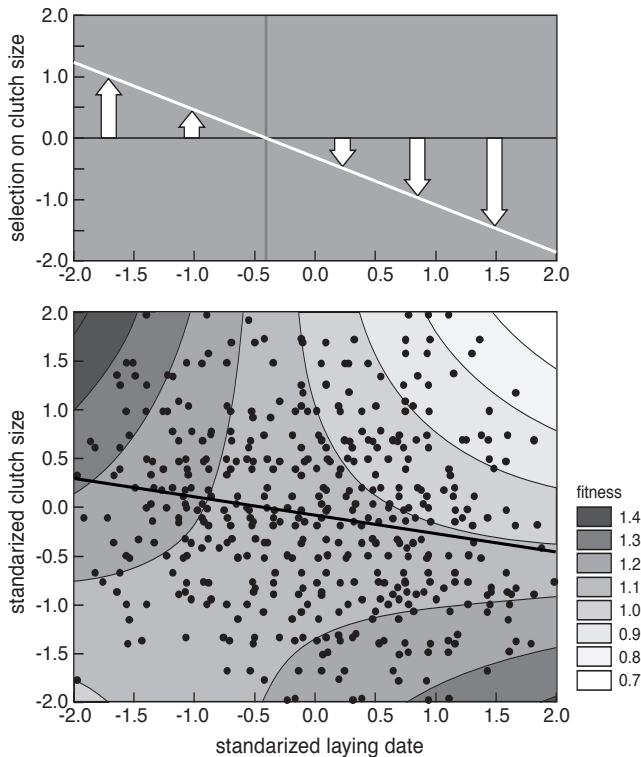


Figure 4.6. Fitness surface over standardized laying date and clutch size (lower panel) and resulting selection on standardized clutch size in relation to standardized laying date (upper panel).

Lower panel: Fitness was defined as the rate of population increase λ . The curved lines are fitness isoclines; dark grey surface colour indicates higher fitness white lower fitness. The fat straight line is the regression of standardized clutch size on standardized laying date based on the data points (closed dots). For relatively early breeders there was selection for larger clutch size and for relative late breeders selection for smaller clutch sizes.

Upper panel: Date dependent selection on clutch size. The arrows indicate the direction and relative strength of selection on clutch size in relation to standardized laying date as deduced from the fitness surface. These patterns indicate selection for a steeper clutch size laying date relationship as actually found (fat line lower panel).

interaction between the two trait values. The general pattern is similar to that on fecundity selection alone. There was a selection for larger clutch size in the early birds and for smaller clutches in the late birds. Selection for a steeper clutch size laying date relationship was thus confirmed when viability selection is included.

This interaction effect between laying date and clutch size on fitness is fascinating. Could nestling growth also underlie this pattern? Indeed in a simple analysis without year effects the relation between nestling growth and clutch size differed for early and late clutches (interaction standardized laying date with standardized clutch size of the first clutches: $\chi^2 = 6.42$, $df = 1$, $p < 0.05$, Fig. 4.7). The nega-

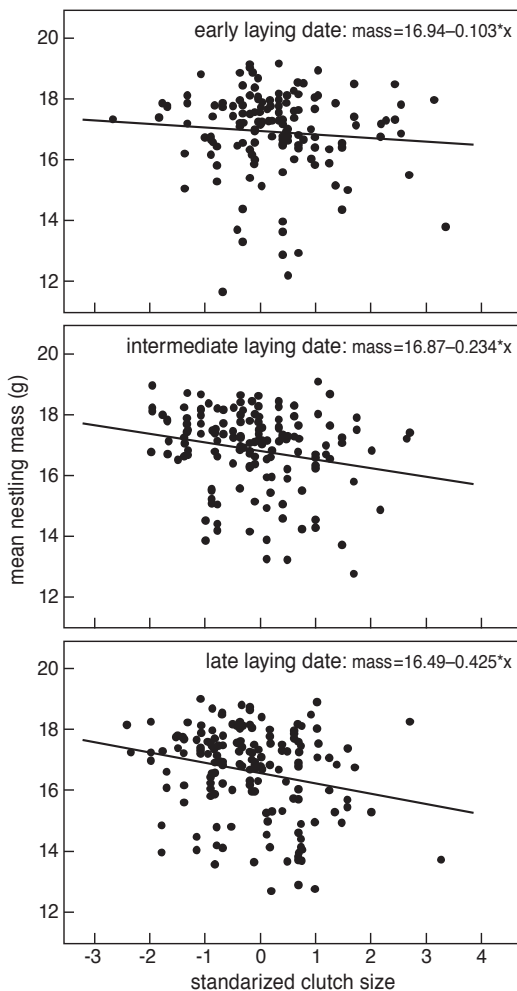


Figure 4.7. The decline of mean nestling mass with standardized clutch size was stronger later in the season. Groups were of around equal size (from early to late $n = 151, 150, 152$).

tive relation between clutch size and nestling growth was less pronounced for the early than for the late birds. Because fecundity comprises nestling number multiplied by the probability of a nestling to recruit this could generate an opposite fecundity selection for early and late clutches. This is the case if nestling mass related recruitment declines with clutch size in the late clutches so strongly that it counterbalances the positive effect of clutch size. We find these patterns suggestive, and we believe that they may explain the fecundity variation with laying date and clutch size.

Micro evolution?

Did the detected selection lead to a micro-evolutionary change? If we assume that there is a genetic basis for variation in clutch size and laying date (van Noordwijk *et al.* 1981a, van Noordwijk *et al.* 1981b, Postma & van Noordwijk 2005b), we would expect a response to selection. To verify this, we compared the direction of selection with the trends over time in our population (Fig. 4.2). We found both a weak indication for negative selection on clutch size due to selection on the male viability fitness component and a decline of clutch sizes over the period of observation. The expected response to selection ($R = h^2 \cdot s$), assuming the heritability of clutch size to be 0.4 (van Noordwijk *et al.* 1981a) and calculating the selection differential on basis of the regression of λ (for calculation of λ see methods) on standardized clutch size (coefficient = -0.021 units per SD in clutch size) amounts to -0.004 eggs per generation, very weak and not strong enough to explain the observed trend (around -2 eggs in 10 year). Possibly an increase in population size caused this decline in clutch size (unpublished data). Consistent with our finding that there was no detectable selection on laying date, we did not detect a trend in the mean annual laying dates of the population either. This contrasts to the pattern that great tits in another area in the Netherlands did breed earlier which has been attributed to global warming (Visser *et al.* 2004, Visser *et al.* 2006), but our ten year study offers limited power in this respect.

Furthermore we detected selection on the steepness of the reaction norm of clutch size on laying date. For this reaction norm there is evidence for a genetic component (Nussey *et al.* 2005, Brommer *et al.* 2005). Thus we might expect a trend towards steeper slopes in the standardized clutch size laying date relationship over the years. The opposite was the case (regression of annual clutch size laying date slopes on year: slope = -0.045, $\chi^2 = 10.81$, $p = 0.001$). There is clearly scope for other ecological factors to explain these patterns.

Whether or not an evolutionary response is expected depends on how the genetic make up of individuals is related to their actual clutch size laying date decisions. In this population the direction of selection on clutch size differed for relatively early and late breeding attempts and also seasonal differences existed in whether it was favoured or panellized to be relatively early. Individual variation in laying date may therefore generate opposite selection on clutch size in different

breeding seasons. This, in combination with influx of birds from elsewhere, may slow down the process of adaptation and prevent local adaptation. In addition, the direction of selection may vary on a small spatial scale (Garant *et al.* 2005, Postma & van Noordwijk 2005a) making it even harder to detect a response.

It is surprising that we did not find more indications for stabilizing selection. We expect the current patterns in clutch size and laying date to be held in place through stabilizing selection. We find no net selection on overall laying date and hardly on clutch size, but a selection pressure for the combination of these two traits: early in the season selection for large and late in the season selection for small clutches. The calculations give no clue in how the current pattern in clutch size and laying date is maintained. Perhaps this is because individuals after all do differ in their “personal” ecological circumstances. We need more combined information about fitness estimates, genetic background and ecological circumstances the birds actually experience to solve this problem of the apparent absence of stabilizing selection.

Concluding we can say that this study revealed intriguing patterns in selection gradients with respect to clutch size and laying date. We found that selection on clutch size and laying date was not independent. Early in the season breeding attempts with larger clutches were selected for while later in the season the opposite was the case. The results of earlier experiments manipulating brood size during the nestling phase and later clutch size during the incubation phase and registering fitness consequences are consistent with the view that there is stabilising selection on clutch size in this population. That we did not detect stabilizing selection on clutch size and laying date on the basis of natural variation in these traits remains a puzzling but fascinating discrepancy with the findings of the brood and clutch size manipulations.

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Energetic and behavioral costs

