LONG-TERM LIFE-HISTORY CONSEQUENCES OF ECTOPARASITE-MODULATED GROWTH AND DEVELOPMENT

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Abstract. Many parasites affect the development and survival of offspring. Because passerines exhibit determinate growth, parasites may have lasting effects on phenotypes. The life-history consequences of parasite-induced developmental modifications have rarely been analyzed, and require long-term experimental studies. Here we present the results of a four-year experimental study on the effects of a hematophagous ectoparasite, the hen flea (*Ceratophyllus gallinae*), on growth, survival, and lifetime reproductive success of nestlings of the Great Tit (*Parus major*).

In a design A, half of the nests were heat-treated several times per year to kill parasites, while in the other half, fleas were allowed to immigrate naturally over several years. To allow for the estimation and statistical control of effects due to potentially nonrandom phenotype distribution within this design, a second design, design B, was applied. In design B, all nests were heat treated to kill parasites after nest box occupation, and the subsequent infestation of half of the nests was then randomized in space and time.

In both designs, the fleas significantly reduced nestling body size but did not significantly affect the probability of nestling recruitment as local breeders. Parasitism reduced the clutch size of the nestlings' first recorded clutch, and of the subsequent clutches, and reduced the total number of recruits produced per nestling over the entire study period. Because body size of the recruited nestlings, both at the end of growth and as recruits, was not significantly different between treatments, the reduced fitness was not an indirect consequence of parasite-modified body size.

This study provides experimental evidence for parasite-induced effects during growth on survival and development of offspring and shows the consequences of this phenotypic modification on lifetime reproductive success. It shows that parasite-induced effects during growth are important for understanding optimal resource allocation and life-history evolution under parasitism.

Key words: Ceratophyllus gallinae; *developmental consequences of ectoparasite infestation; future reproductive success; Great Tit; hen flea; lifetime reproductive success;* Parus major.

INTRODUCTION

Parasites, by definition, live in or on hosts from which they derive food, and thereby impair host fitness (Price 1980, Clayton and Moore 1997). Effects of parasitism during growth on survival, future reproduction, and lifetime reproductive success of nestlings are of central interest for the evolution of avian life histories, yet knowledge is still scant despite the ubiquitous occurrence of parasites.

Parasites may impose selection on juveniles by increasing host mortality (Møller 1990, Oppliger et al. 1994), reducing growth (e.g., Richner et al. 1993), and altering host body size. Depending on the trait affected, these short-term costs may have direct effects on future reproductive success. Parasitism may be especially important for species with determinate growth, such as passerines, in which growth ceases halfway through the nestling period. Effects of nest-based ectoparasites on passerine growth can lead to permanent phenotype modifications that affect mortality, fecundity, and other life-history parameters later in life (Gebhardt-Henrich and Richner 1998). Short-term costs of parasites, in contrast, do not necessarily lead to reduced future reproductive success. For example, poor nestling condition due to parasitism (e.g., Møller 1993, Heeb et al. 1999) may be temporary; and future effects on survival, recruitment, and reproductive success will thus depend on the nestlings' potential for compensation, which may have consequences for the trade-off between current and future investment (e.g., Tripet and Richner 1997). Therefore, long-term studies analyzing the effects of parasite presence at a given life stage and the consequences on lifetime reproductive success are essential for quantifying the impact of parasites on host life history.

In a four-year field experiment, we manipulated the load of the hematophagous hen flea (*Ceratophyllus gal-*

linae) in the nests of its natural host, the Great Tit (*Parus major*) (Gosler 1993). We measured both the short-term effects on growth and the long-term effects of parasite-induced developmental modifications on the major life-history traits such as mortality and lifetime reproductive success.

MATERIAL AND METHODS

General methods

To investigate the effects of ectoparasites on survival, local recruitment, and lifetime reproductive success of nestling Great Tits (*Parus major*), we performed a four-year experiment in the Forst, a deciduous forest near Bern, Switzerland (46°54′ to 46°57′ N, 7°17′ to 7°21′ E). In December 1996, 12 study plots were established. A plot consisted of 32 nest boxes arranged geometrically (8 × 4) on a 350 × 150 m grid. To reduce variation in nestling traits, we established the plots in homogeneous forests dominated by beech trees (*Fagus silvaticus*). In addition to the nest boxes in these plots, hereafter referred to as "design A," we set up an additional 88 nest boxes, hereafter referred to as "design B." Nest boxes in design B were established in the forest surrounding the nest boxes of the design A (shortest distance between a plot and a nest box of design B: 231.7 ± 52.1 m [mean ± 1 SE], N = 12). They were randomly located in homogenous beech forest. The distance between two adjacent nest boxes of design B was 70 ± 3 m [mean ± 1 SE].

At the start of the breeding season, we regularly visited all nest boxes to determine clutch size and the hatching date of the nestlings. Nestlings were individually ringed with aluminum rings and were weighed to the nearest 0.01 g using an electronic scale 15 d after hatching. The length of the metatarsus was measured to the nearest 0.1 mm, and the length of the third primary was measured to the nearest 0.5 mm (Svensson 1992). Adult birds were captured 13 d after hatching. We calculated natal dispersal distances by first determining the coordinates of each nest box and then calculating the shortest distance between the currently and the previously occupied box. Throughout this paper, the term "nestlings" refers to young of the initial nests. The term "offspring" is used for the young (F1 generation) of the mature "nestlings."

Experimental procedures

To create infested and uninfested study areas, we split each plot in design A, in January 1997, into two "patches" consisting of 16 nest boxes each (in a 4 × 4 arrangement); patches within each plot were randomly assigned to one of the two treatments. To create similar starting conditions and to ascertain that no nest commensals or other parasites were present at the start of the experiment, we cleaned each nest box of design A and afterwards lined it with 30 g of dry, microwave-treated moss. In February of all subsequent years, the

nest material of the uninfested nest boxes was heat treated to ensure that nest boxes were free of hen fleas (*Ceratophyllus galinae*) before birds constructed a new nest. Although adult hen fleas are blood-feeding parasites that live on nestling birds and adults, their larvae are detritivorous and live in nesting material (Cotton 1970). To prevent immigrating fleas from producing a filial generation (Heeb et al. 1996), we additionally heat treated occupied uninfested nest boxes on the day the birds laid their second egg, on the hatching day, and on the day after fledging. Infested nest boxes were not heat treated, but were otherwise handled similarly. All boxes of the infested patches were infested prior to egg laying with 40 (end of January), 60 (start of March), and 30 (mid March) hen fleas in 1997. The fleas used for the infestation were collected in December 1996 from old bird nests of the same forest. By infesting the nests in the first year of the four-year experiment, we created homogeneous starting conditions among the infested patches. During the subsequent three years, the fleas could reproduce, immigrate, and emigrate naturally in the infested patches. At the end of the experiment (2000), we collected all intact nests (N = 322) and extracted all live and all visible dead arthropods. Infested nests contained significantly more adult hen fleas than uninfested nests (infested nests, 957 ± 76 fleas [mean ± 1 SE]; uninfested nests, 415 ± 33 fleas, Wilcoxon singed rank test, $\chi^2 = 78.56$, $P < 0.0001$). Because hen fleas emigrate directly after the fledging of the nestlings, this measure underestimates the number of hen fleas present. More importantly, hen fleas were not able to reproduce in the uninfested nests and thus the numbers of blood-sucking adults was strongly reduced. Infested nests contained fewer *Protocalliphora azurea* larvae (infested nests, 47 ± 8 larvae; uninfested nests, 29 ± 8 larvae; $\chi^2 = 5.88$, $P = 0.015$), and of fewer ticks (infested nests, 2.1 ± 0.3 fleas; uninfested nests, 1.2 ± 0.3 fleas; $\chi^2 = 5.14$, $P = 0.023$). Beside these hematophagous ectoparasites, no other ectoparasites (e.g., feather lice and hematophagous mites) were found in the nests and on the Great Tits. Thus, observed treatment effects cannot be attributed to other nest-based arthropods.

In design A, birds might have avoided parasites by choosing nest boxes containing the fewest parasites (Christe et al. 1994, Oppliger et al. 1994, Merilä and Allander 1995). Therefore, in design B, we first let birds choose their nest box. Then all occupied boxes were heat treated on the day the birds laid their second egg, and each nest was alternately (in time and space) assigned to either the infested or uninfested treatment. We infested nests of the flea-treatment group with 40 adult hen fleas, while nests of the uninfested treatment group were heat treated again on the hatching day to prevent immigrating fleas from producing a filial generation. In both designs, treatments were applied regardless of which bird was breeding in a given nest box. Before nest construction, in February of each year,

we cleaned all nest boxes of design B to ensure that no fleas occupied the boxes at the start of the following breeding season. The uninfested nests of both designs were thus treated the same way and therefore allowed for the estimation of potential effects of nonrandom host phenotype distribution in design A. In contrast to design A, design B was used in 1997 and 1998 only, while in 1999 and 2000 these nest boxes were used for other studies. All broods initiated were part of the experiment, including late, second, and replacement broods (hereafter referred to as second broods).

Statistics

Except for the survival analysis, measurements of nestlings of the same experimental nest were averaged throughout to avoid pseudoreplication. Because nestling mass, tarsus length 15 d after hatching, and feather length 15 d after hatching were significantly intercorrelated (mass $\times$ metatarsus, $t_{1,383} = 14.03$, $P < 0.0001$; mass $\times$ feather length, $t_{1,383} = 18.48$, $P < 0.0001$; feather length $\times$ metatarsus, $t_{1,383} = 16.42$, $P < 0.0001$), we used a principal components analysis of mass, metatarsus length, and primary length. The first principal component (PC1) explained 75.9% of variance (eigenvectors: mass, 0.576; metatarsus, 0.562; primary, 0.593), and is used as a measure of overall body size.

For the analysis of the nestling traits, we used two different approaches. In the first approach, all nests of both designs were treated as statistically independent data points because the treatments were applied separately to the nests, depending on the timing of egg laying of each female. Data were therefore analyzed using mixed-model ANOVA, with year as a random effect. In a second approach, we analyzed the data from design A to control for nonindependence between nest boxes within a patch and within a plot. For this approach, nested ANOVAs were used. According to the structure of the experimental design, the patch was nested within the plot and within the treatment: patch(plot $\times$ treatment). Plot, patch, and year were modeled as random effects. Both the patch and the plot did not explain a significant part of the observed variance (Appendix A) suggesting that nest boxes were responding as independent replicates. We therefore applied the first approach to all following analyses. This approach has the advantage that an interaction between the design factor (A or B) and the treatment factor (infested or uninfested) can be tested, allowing for the evaluation of nonrandom phenotype distribution in design A. A significant interaction would indicate nonrandom phenotype distribution between treatments in design A. The final model consisted of the design, the treatment, the year factor, and the significant interactions. Nonsignificant interactions were deleted by backward elimination.

Survival estimates (ϕ) of the fledged nestlings were determined on the basis of capture–recapture data, using the program MARK (G. C. White, *unpublished pro-*

gram). We applied Cormack-Jolly-Seber models (e.g., Jolly 1965) to account for potential variation in capture probability among birds of different treatment groups. A total of 2530 fledglings originating from 389 nests were included in the analysis. The survival estimates are based on birds captured as breeders within the study area, referred to as the probability of recruiting locally. This probability is conservative: it underestimates the rate of survival because some individuals may emigrate permanently from the study area, and also because resident nonbreeders remained undetected. Before analysis, we used a bootstrap approach to confirm that the starting model adequately fitted the data (see footnote 4). Because our starting model was overdispersed (1000 simulations, $P < 0.008$) we adjusted the variance inflation factor (c-hat) with the quotient of the observed inflation factor and the mean simulated inflation factor from the bootstraps. Survival analysis was started with the full model including year, age (first year and older than one year; Clobert et al. 1988), flea treatment, and experimental design. Model selection was based on the Aikaike Information Criterion (AIC). Experimental factors were additionally tested using LRT tests (see footnote 4) and Δ deviances and P values are given. The absence of significant effects on recapture probability (Appendix B) shows that estimates of ϕ were not biased by differential recapture probability and/or differential dispersal distances. For the analysis of dispersal distances, data were log transformed to achieve normally distributed residuals in the final model.

We analyzed the nestlings' first event of reproduction using mixed-model ANOVAs that included the following factors: the treatment applied to the nestlings' nest of growth, the experimental design (A, B) of the nestlings' nest of growth, whether the nestling grew up in a first or a second brood, the treatment applied to the nestlings' first recorded brood, the design of the nestlings' first recorded brood, whether it was a first or a second brood, and the sex of the nestling. These factors were modeled as fixed effects, while the year of birth was modeled as random factor throughout. All possible interactions were included in the starting model. The final model was then selected by backward elimination. Because we analyzed the reproductive parameters of the first recorded brood, birds (22 out of 187 individuals) captured later in subsequent years could potentially bias the results. However, the number of years between fledging and recapture ($P > 0.2$) and their interaction with treatment ($P > 0.9$) were not significant in any of the analyses, showing that there was no such effect. Due to technical problems with the balance, the body mass of one recaptured nestling could not be determined; therefore sample size for the analysis of body size equals 186. One recruited nestling fledged before the measurements 15 d after hatching were taken, and for four recruited nestlings, the exact hatching day could not be determined. Sample sizes in the anal-

ysis of tarsus length and body size during the nestling period were correspondingly reduced.

To assess the consequences of flea infestation during development on all future breeding events of a phenotype, the treatment effect on the total number of eggs, fledglings, and recruited offspring produced during the four experimental years per recruited nestling, was analyzed. To obtain equal variances between treatment groups the total number of recruits produced was log-transformed. To account for nestlings born in different years, the number of possible breeding attempts during the study period was included in the analyses. Because several individuals bred up to two times per year and because no single Great Tit bred three times, the number of possible broods per year was set to two. The number of recorded broods and the number of broods in infested nests were entered as covariates, and the nestling's sex was entered as a categorical factor.

Significance levels are two tailed, except for the parasite treatment applied to the nestling's nest. Because the parasite's effects are predictably negative (e.g., Clayton and Moore 1997), directed tests (Rice and Gains 1994) were used, except for dispersal distances, for which the effects of parasites on dispersal are less clear (Clobert et al. 2001). Means and standard errors of the uncorrected original values of the traits in focus are given throughout. For all ANOVAs, the dependent variables were tested for equal variances by Bartlett's test prior to analysis, and normality of the model residuals was evaluated. Analyses were performed with JMP IN 4.0 (Sall and Lehman 1996).

RESULTS

Nestling growth and body size

The body size of nestlings (PC1) raised in infested nests was significantly reduced compared to nestlings of uninfested nests (see Appendix A). There was no significant interaction between the treatment and the design (treatment × design, $F_{1, 373} = 0.53$, $P = 0.47$), indicating that fleas affected nestling body size in both designs similarly. Body size further differed significantly between the four experimental years (Appendix A; Fig. 1), and the interaction between treatment and year was also significant (Appendix A; Fig. 1). There were no significant effects of experimental design, and the number of nestlings in a brood did not affect nestling body size significantly (Appendix A). Nestlings of infested nests had a significantly smaller tarsus 15 d post-hatching (treatment: infested, 19.09 ± 0.04 mm [mean $\pm$ 1 SE]; uninfested, 19.32 ± 0.04; for statistics, see Appendix A). Tarsus length further differed between years, but was not significantly influenced by the number of nestlings (Appendix A). There were no differences in tarsus length between experimental designs (Appendix A), and none of the interaction terms were significant ($P > 0.1$). In a nested analysis of the nest boxes in design A, neither patch nor plot explained a
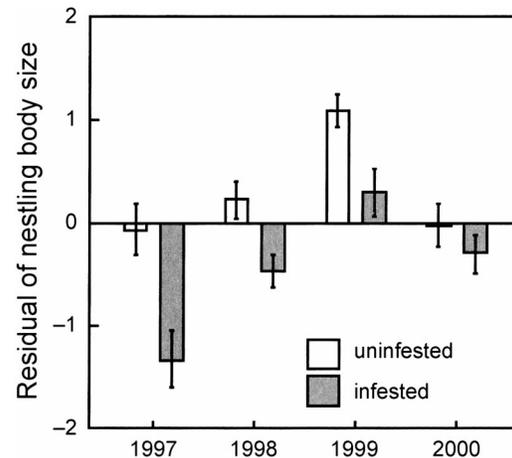

FIG. 1. Nestling body size among years and treatments (infested, uninfested). Values are means $\pm$ 1 SE. For statistics, see Appendix A.

significant component of the variation in tarsus length or body size (Appendix A), but effects of treatment and year were significant. Nestling number explained a significant part of the variation in body size only. Due to smaller sample sizes, the interaction between treatment and year was no longer significant.

Local recruitment of nestlings

Of the 2530 fledged nestlings, 187 (7.4 %) were recaptured as breeders in our study area in one of the subsequent years. The probability of recruiting locally (φ) was not significantly influenced by the flea treatment (Appendix B and C). Age, year, and the interaction between age and year explained a significant proportion of the total variance in φ (Appendix B). Nestling body size significantly influenced the probability of recruiting locally (Appendix B). As Tinbergen and Boerlijst (1990) showed for body mass, birds of an optimal intermediate size survive better than the largest birds. We therefore included body size as a quadratic variable in the model. However, the treatment effect and the interaction between treatment and design were still nonsignificant after inclusion of the quadratic term (Appendix B).

Dispersal distances of locally recruited nestlings

The mean dispersal distance of the 187 locally recruited nestlings was 890 ± 40 m (mean $\pm$ 1 SE). Fledglings of infested nests dispersed slightly but not significantly further (infested, 950 ± 70 m; uninfested, 850 ± 60 m; $F_{1, 181} = 2.33$, $P = 0.12$). Females dispersed significantly longer distances than did males (females, 980 ± 60 m; males, 790 ± 60 m; $F_{1, 181} = 6.67$, $P = 0.01$). Neither design ($F_{1, 181} = 0.69$, $P = 0.41$), year ($F_{2, 181} = 0.24$, $P = 0.78$), nor the interactions between treatment and design ($F_{1, 178} = 0.03$, $P = 0.86$), between treatment and sex ($F_{1, 177} < 0.001$, P
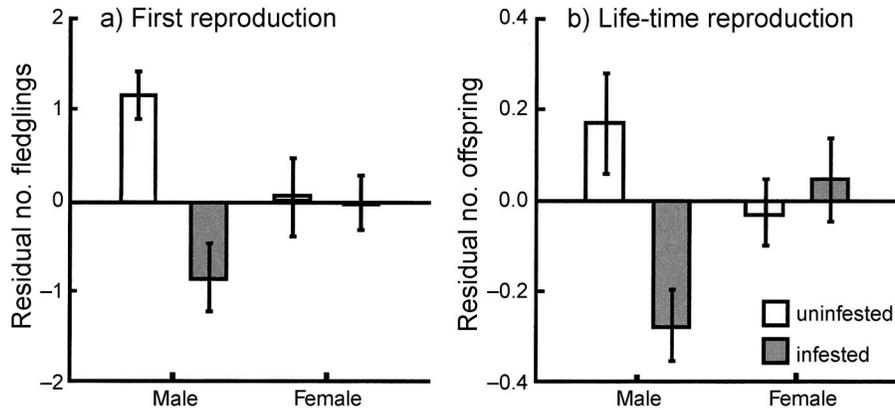
FIG. 2. (a) Sex-specific differential effects of parasite presence/absence during the nestling's first reproduction on the number of fledging offspring produced. Residuals of the model without the interaction are shown. For statistics, see Appendix E and *Results*. (b) Sex-specific differential effects of parasite presence/absence during the nestling's growth on the total number of recruiting offspring produced during a nestling's life. Residuals of the model without the interaction are shown; for statistics, see Appendix G and *Results*. Values are means ± 1 SE.

= 0.98), and between treatment and year ($F_{2, 179} = 1.28$, $P = 0.28$) explained a significant proportion of the variation in dispersal distances.

Body size and first reproduction as a local recruit

Tarsus length and body size of locally recruited nestlings were not significantly different between birds originating from infested and uninfested nests, and there were no effects of experimental design (Appendix D). Males were larger and had greater body size than females (Appendix D). The year of birth affected tarsus length, but not body size (Appendix D). There were no significant interactions between treatment and design, between treatment and sex, or between treatment and year (Appendix D).

The clutch size of the nestlings' first recorded brood was significantly smaller (5.8% smaller) for nestlings raised in infested nests the previous year (Appendix E). However, the treatment applied to the first recorded brood had no significant effect on its clutch size (Appendix E). Second broods consisted of significantly fewer eggs than did first broods, and broods of female nestlings contained slightly but not significantly fewer eggs than broods of male nestlings (Appendix E). There was no interaction between the treatment applied to the nestlings' nest of growth and the nestling's sex ($P > 0.2$), and no interaction between the treatment applied to the nestlings' first recorded brood and the nestlings' sex ($P > 0.2$). There were no significant differences in clutch size between designs of the nestlings' nest of growth, between designs of its first recorded brood, and between years ($P > 0.2$). Both the interaction between the treatment and the design ($P > 0.2$), and treatment and year ($P > 0.8$) were not significant.

The number of offspring that fledged of the nestlings' first recorded brood was not significantly reduced by the parasites applied to the nestlings' nest of growth,

but it was reduced by the parasites applied to its first recorded brood (Appendix E). There were no differences between designs applied to the nest of growth, between nestlings originating from first or second broods, and between years (all $P > 0.1$). However, the number of offspring fledged differed between first and second clutches (Appendix E). The interaction between the treatment and the design was not significant ($F_{1, 173} = 2.01$, $P = 0.16$). Female nestlings did not produce significantly different numbers of fledgling offspring than did male nestlings (Appendix E), and the effect of the treatment was not different between male and female nestlings (treatment × sex, $F_{1, 167} = 1.48$, $P = 0.22$). However, there was a significant interaction between the treatment applied to the nestlings' first recorded brood and its sex (Appendix E; Fig. 2a). Male nestlings breeding in uninfested nests produced more fledging offspring than males breeding in infested nests (individual contrast, $F_{1, 178} = 16.52$, $P < 0.0001$). Female nestlings produced equal numbers of fledging offspring regardless of the parasite treatment (individual contrast, $F_{1, 178} = 0.04$, $P = 0.85$). While male nestlings breeding in uninfested nests produced more fledging offspring than female nestlings breeding in uninfested nests (individual contrast, $F_{1, 178} = 6.47$, $P = 0.01$) there was no difference between sexes in the infested nests (individual contrast, $F_{1, 178} = 1.12$, $P = 0.29$).

Effects of parasite presence during the nestling period on all reproductive events

The total number of eggs produced during the four experimental years by nestlings reared in infested nests was significantly smaller than that of nestlings reared in uninfested nests, and was strongly influenced by the number of clutches produced (Appendix F). Because the number of clutches produced was included in this model, the analysis shows that infested nestlings pro-
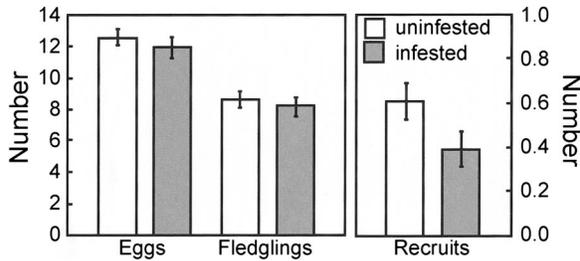
FIG. 3. Effects of parasite presence/absence during nestling growth on the total number of eggs, fledglings, and recruits produced during a nestling's subsequent life. Values are means ± 1 SE.

duced fewer eggs per clutch. There was a slight tendency for the nests of female nestlings to contain fewer eggs per clutch than those of male nestlings (Appendix F). However, there was no significant interaction between treatment and sex ($F_{1, 174} = 0.020$, $P = 0.887$). The number of possible brood events and the number of broods raised in parasite presence did not significantly influence the number of eggs produced per clutch (Appendix F).

The total number of fledging offspring produced was significantly affected by the number of broods a nestling produced (Appendix F). The number of fledging offspring produced per clutch was not significantly different between nestlings of infested and uninfested nests, or between female and male nestlings (Appendix F). There was also no significant interaction between sex and parasite treatment ($F_{1, 175} = 0.240$, $P = 0.625$). The number of broods raised in the presence of parasites also did not explain a significant proportion of the variance (Appendix F).

However, the total number of reproducing offspring produced per breeding event was significantly lower for nestlings reared in infested nests (Appendix F). The total number of reproducing offspring produced depended further on the number of brood events, on the number of possible brood events, and on the number of broods reared in the presence of parasites (Appendix F). Male and female nestlings did not produce significantly different numbers of reproducing offspring per brood event (Appendix F), and there was no significant interaction between parasite treatment and sex ($F_{1, 174} = 0.78$, $P = 0.38$).

Lifetime reproductive success of locally recruited nestlings

To analyze the consequences of parasite presence during growth on lifetime reproductive success, we eliminated the "number of brood events recorded" and the "number of broods raised in the presence of parasites" in the above presented model. This analysis thus measures the effects of parasite infestation during growth on fitness (total offspring produced). Lifetime egg production was not significantly affected by the presence of fleas during growth (Appendix G, Fig. 3),

and was not significantly different between sexes (Appendix G). There was also no significant interaction between sex and parasite treatment ($F_{1, 182} = 0.782$, $P = 0.378$). However, the number of possible breeding events explained a highly significant part of the variance (Appendix G). The same effects were found for the total number of fledging offspring produced (Appendix G; sex × parasite treatment, $F_{1, 182} = 0.984$, $P = 0.323$).

The number of reproducing offspring produced was significantly lower for nestlings reared in infested nests (Appendix G, Fig. 3) and was significantly influenced by the number of possible brood events (Appendix G). Nestlings growing up in uninfested nests produced 0.61 ± 0.08 (mean ± 1 SE) reproducing offspring, while those growing up in infested nests produced 0.39 ± 0.08 reproducing offspring; that is a parasite-induced reduction in lifetime reproductive success of 36.1%. There were no differences between male and female nestlings in the number of reproducing offspring produced (Appendix G), but there was a significant interaction between sex and treatment ($F_{1, 182} = 6.88$, $P = 0.009$), explaining an additional 3.0% of the total variance (Fig. 2b). In addition to the negative effects due to the parasite treatment, males reared in infested nests produced fewer reproducing offspring compared to males reared in uninfested nests (individual contrast, $F_{1, 182} = 16.52$, $P < 0.001$), whereas, in females, the number of offspring produced was similar between treatments (individual contrast, $F_{1, 182} = 0.26$, $P = 0.61$, Fig. 2b). In the infested treatment, the number of reproducing offspring differed significantly between male and female nestlings (individual contrast, $F_{1, 182} = 7.46$, $P = 0.007$), whereas in the uninfested treatment males and females did not differ in the number of reproducing offspring (individual contrast, $F_{1, 182} = 1.46$, $P = 0.23$).

DISCUSSION

Impact of ectoparasites on growth and lifetime reproductive success

This study demonstrates experimentally that ectoparasite presence during juvenile growth reduces the hosts' lifetime reproductive success: the number of eggs in the first and in the following broods was reduced, and the total number of recruits produced was reduced by one third. Because life-history theory assumes that reproduction is costly and competes with all other life-history traits for limited resources (Roff 1992, Stearns 1992), parasite infestation during growth appears to alter the allocation of energy and resources to reproduction, parasite defense, and body maintenance, underlining the importance of parasites for life-history evolution.

Parasites may have impaired the growth of nestlings resulting in smaller adult body size and thus reduced lifetime reproductive success (Gebhardt-Henrich and

Richner 1998). However, body size of the subsample of recruited nestlings measured at the end of the nestling period did not differ between infested and uninfested nestlings ($F_{1, 182} = 0.673$, $P = 0.258$), and there was also no difference in body size between treatments during the first reproduction (see Appendix D). Therefore, parasite effects are not a simple consequence of changes in body size. An alternative hypothesis suggests that parasites may reduce growth rate but that final adult body size will not change (Gebhardt-Henrich and Richner 1998). Our results support this hypothesis: body size of the subsample of recruited nestlings, was smaller in infested nestlings during the nestling period ($F_{1, 177} = 8.889$, $P = 0.002$) but did not differ from uninfested birds after recruitment (see Appendix D). Nestlings reared with parasites apparently allocate their limited resources differently than uninfested nestlings. Thus, the modulated life-history of nestlings reared in parasite presence and the impaired fitness is probably not the result of parasite-modulated body size but of other yet unknown mechanisms.

Nestling body size is a good predictor of the probability of recruiting locally (Appendix B and C). Because the flea treatment reduced nestling body size, it should have explained a significant proportion of the variation in the probability of recruiting locally. Nevertheless, the probability of recruiting locally was not affected by the flea treatment (Appendix B). This discrepancy might be explained by two hypotheses, which are not mutually exclusive. First, nestling body size may not be sufficiently reduced by fleas to cause significant negative effects on the probability of recruiting locally. Second, flea impact on body size might be compensated by a prolonged nestling period, leading to fledglings with similar body size. We found that the duration of the nestling period differed significantly between treatments (treatment: infested, 19.9 ± 0.10 d [mean ± 1 SE]; uninfested, 19.6 ± 0.09 d; $F_{1, 382} = 5.78$, $P = 0.02$), so both hypotheses are supported by this and other studies (Lehmann 1992 [review], Møller 1993, Heeb et al. 1999; but see Richner and Tripet 1999).

The probability of local recruitment tended to be lower ($P = 0.1$) and natal dispersal distances tended to be longer ($P = 0.12$) for infested nestlings (but see Heeb et al. 1999 for another Great Tit population). As flea presence during growth did not significantly affect recapture probability, the probability of local recruitment and thus local survival were not biased by the slightly longer dispersal distances.

Fleas affected clutch size in the nestlings' first recorded clutch, demonstrating that growth in infested nests has long-term costs, as similarly shown in Blue Tits (Richner and Tripet 1999). There was no significant effect of nestling sex and no significant interaction between treatment and sex. Because clutch size was reduced in flea presence in both, male and female nestlings, the observed parasite-induced clutch size reduction is not only the result of female nestlings adjusting clutch size in response to previous infestation level. The females of the infested male nestlings as well had a reduced clutch size, suggesting that other mechanisms, such as assortative mating with respect to previous parasite exposure or with respect to mate quality, may have let to the observed clutch size reduction.

Although clutch size was affected by the parasite presence during growth, no such effects were found on the number of fledging offspring. Moreover, parasites applied to the first clutch significantly reduced the number of fledglings. Both lines of evidence suggest that the number of fledging offspring is controlled by current parasite presence rather than by long-lasting effects of parasites. This hypothesis is further supported by the significant interaction between current parasite infestation and sex, probably reflecting the parasite-dependent reproductive investment of male Great Tits (Christe et al. 1996, Møller 1997).

Interestingly, not only the clutch size during the first reproduction but also the clutch size of all future reproductive events was significantly reduced by the presence of hen fleas during growth. Moreover, the number of recruiting offspring produced per brood was significantly smaller for nestlings reared in infested nests. However lifetime reproductive success (measured as total egg production) did not differ between nestlings reared in infested vs. uninfested nests, suggesting that nestlings grown up in infested nests produced more but smaller clutches than nestlings grown up in uninfested nests. This idea, however, is not supported by our data: there were no differences in the number of clutches produced between nestlings reared in infested (1.43 ± 0.05 [mean ± 1 SE]) and in uninfested nests (1.41 ± 0.07; Wilcoxon signed ranks test, $\chi^2 = 0.08$, $P = 0.78$). Nor were there significant differences in the number of possible brood events (infested, 5.97 ± 0.13 [mean ± 1 SE]; not infested, 5.91 ± 0.16; Wilcoxon signed ranks test, $\chi^2 = 0.06$, $P = 0.81$). Our results may be explained by higher error variance in the lifetime reproductive success analysis.

Although total egg number did not differ, nestlings reared in infested nests produced more than one-third fewer recruiting offspring compared to those reared in uninfested nests. Male, but not female, nestlings produced significantly fewer recruiting offspring when reared in infested nests, suggesting that male fitness is more strongly affected by parasites. Although fleas had a negative effect on the number of recruiting offspring, no such effect was found on the total number of eggs and the total number of fledging offspring produced. This shows the importance of analyzing life-history consequences over several years in terms of reproducing recruits. Our experiment demonstrates that the impact of ectoparasitic hen fleas during growth cannot be compensated for later on, resulting in important lifetime fitness consequences.

Annual differences of the parasite impact

The effects of parasite infestation may differ among years due to variation in host responses that depend on environmental conditions and/or variation in parasite virulence (de Lope et al. 1993, Merino and Potti 1996). Environmental conditions are thus of great relevance for the evolution of resource allocation strategies among reproductive events and for the hosts' life history. We found differences between years in nestling body size and tarsus length 15 d post-hatching, supporting the idea that, environmental variation among years or varying virulence of ectoparasites may result in parasite impacts that differ between years (Allander 1998). However, the interaction between flea infestation and year was significant for nestling body size only, and not for survival and future reproduction, showing that fleas did not affect the fitness-relevant traits differently among years. The consistency of the negative effects of the fleas among years underlines their importance for the evolution of host life histories.

In conclusion, we demonstrated that parasite presence during growth and development of an individual reduced its fitness at all life stages. We show that fleas impact nestling size and reduce nestling body size, which perhaps was compensated for by a longer nestling period. Second, fleas reduced the clutch size of the nestlings' first recorded clutch and of subsequent clutches. More importantly, fleas altered the host's lifetime reproductive success by reducing the number of reproducing offspring. Our experiments revealed long-term fitness consequences and illustrated the inability of nestlings to fully compensate for negative parasite effects during later life. Our results thus show that hen fleas are an important and relatively constant selective force in the evolution of host life history.

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APPENDIX A

A table showing the effect of parasite treatment on body size (PC1) and tarsus length of nestling Great Tits is presented in ESA's Electronic Data Archive: *Ecological Archives* E085-057-A1.

APPENDIX B

A table showing the probability of recruiting locally in relation to age, year, treatment, and experimental design is presented in ESA's Electronic Data Archive: *Ecological Archives* E085-057-A2.

APPENDIX C

A figure showing the probability of recruiting locally in relation to treatment (infested, uninfested), experimental design (A, B), and nestling body size is presented in ESA's Electronic Data Archive: *Ecological Archives* E085-057-A3.

APPENDIX D

A table showing the effect of parasite treatment on body size and tarsus length of locally recruited nestling Great Tits is presented in ESA's Electronic Data Archive: *Ecological Archives* E085-057-A4.

APPENDIX E

A table showing the effect of parasite treatment and treatment of the first recorded brood on the first reproduction of recruited nestling Great Tits is presented in ESA's Electronic Data Archive: *Ecological Archives* E085-057-A5.

APPENDIX F

A table showing the effect of parasite presence during the nestling period on total number of eggs, fledglings, and recruits produced is presented in ESA's Electronic Data Archive: *Ecological Archives* E085-057-A6.

APPENDIX G

A table showing the effect of parasite presence during the nestling period on lifetime reproductive success is presented in ESA's Electronic Data Archive: *Ecological Archives* E085-057-A7.

Appendices

Ecological Archives E000-000-A1

Fitze, P.S., Clobert, J., and H. Richner 2004. Long-term life-history consequences of ectoparasite-modulated growth and development. *Ecology* 00:000-000.

Appendix A. Effect of parasite treatment on body size (PC1) and tarsus length of nestling great tits. Only significant interactions (p<0.05) are shown. A. Model including all data but not testing for heterogeneity between patches and plots. B. Model including only the data of the plot design and accounting for the hierarchical structure of plots, patches, and treatments.

a. Model with nest boxes as independent samples.

	body size				Tarsus length			
	DF	F	p	% variance explained	DF	F	p	% variance explained
treatment	1,374	17.96	<0.0001	4.0 %	1,377	13.32	0.0002	3.3 %
year	3,374	10.32	<0.0001	25.6 %	3,377	2.52	0.06	0.9 %
design	1,374	0.20	0.66	<0.1 %	1,377	<0.01	0.99	<0.01 %
nestling number	1,374	2.75	0.10	0.6 %	1,377	3.56	0.06	0.9 %
treatment x year	3,374	2.73	0.04	1.8 %				

b. Model with nest boxes as dependent within patches and within plots.

	body size			Tarsus length		
	DF	F	p	DF	F	p
treatment	1,295	11.30	0.0006	1,295	8.97	0.003
year	3,295	9.94	<0.0001	3,295	3.10	0.03
plot	11,295	1.60	0.10	11,295	1.29	0.23
patch(parasites, plot)	11,295	1.16	0.31	11,295	0.64	0.80
nestling number	1,295	4.13	0.04	1,295	2.87	0.09
treatment x year	3,295	1.87	0.14	3,295	1.10	0.35

Ecological Archives E000-000-A2

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Appendix B. Probability of recruiting locally in relation to age, year, treatment and design. For each model the Aikaike Information Criterion (AIC), the AIC weight (AICw), the number of parameters (np) and the deviance are given. All models include an intercept, both for survival (ϕ) and recapture probability (p). Linear predictors are age (age) and year (t). Each manipulated factor has two levels: Flea treatment (f): infested, uninfested; Design (d): A, B. Model algebra specification is conforming to MARK (White, 2000). c*t stands for c + t + c.t. Within each set of analyses, models are numbered according to decreasing complexity but ordered according to AIC. The selected model (the model with the lowest AIC) in each set of analyses and the key comparisons between models are presented by bold type..

Model	AIC	AICw	np	deviance	Models compared, hypothesis tested, LRT test
a. Modelling recapture probability (p)					
8. ϕ**(f* d*age*t)p(.)**	**1583.13**	**0.348**	**21**	**23.16**	**7-8, f, p=1**
7. ϕ(f* d*age*t)p(f)	1583.13	0.348	22	23.16	**6-7, d, p=0.65**
6. ϕ(f* d*age*t)p(f+ d)	1587.01	0.050	23	22.95	5-6, age, p=0.41
5. ϕ(f* d*age*t)p(f+ d+age)	1588.37	0.025	24	22.27	4-5, t, p=0.94
4. ϕ(f* d*age*t)p(f+ d+age+t)	1592.35	0.004	26	22.14	**3-4, f.d ,p=0.30**
3. ϕ(f* d*age*t)p(f+d+age+t+f.d)	1593.31	0.002	27	21.06	**2-3, f.t, p=0.77**
2. ϕ(f* d*age*t)p(f+d+age+t+f.d+f.t)	1594.84	0.001	29	20.53	1-2, interactions, p=0.86
1. ϕ(f*d*age*t)p(f*d*age*t)	1611.34	0.000	40	14.30	
b. Modelling probability of recruiting locally (ϕ)					
12. ϕ(f+d+age+t+age.t)p(.)	1562.75	0.231	8	31.20	**10-12, f.d, p=0.35**
16. ϕ**(age+t+age.t)p(.)**	**1563.83**	**0.135**	**6**	**36.30**	**15-16, f, p=0.10**
15. ϕ(f+age+t+age.t)p(.)	1563.88	0.131	7	34.34	**12-15, d, p=0.08**
10. ϕ(f+d+age+t+f.d+age.t)p(.)	1563.89	0.131	9	30.31	**9-10, f.t, p=0.34**
9. ϕ(f+d+age+t+f.d+age.t+f.t)p(.)	1565.79	0.051	11	28.17	8-9, interactions, p=0.89
11. ϕ(f+d+age+t+f.d)p(.)	1573.09	0.001	8	41.54	10-11, age.t, p=0.0008
8. ϕ(f* d*age*t)p(.)	1583.13	<0.001	21	23.16	
14. ϕ(f+d+t+age.t)p)p(.)	1698.57	<0.001	6	169.02	12-14, age, p<0.0001
13. ϕ(f+d+age+age.t)p(.)	1721.23	<0.001	6	193.71	12-13, t, p<0.0001

c. Test of normalizing selection on body size. Modelling probability of recruiting locally (ϕ) using body size (obs) and body size^2 (obs^2) as a covariate.

Model	AIC	AICw	np	deviance	
3. ϕ**(obs obs^2)p(.)**	**1657.35**	**0.88**	**4**	**1649.33**	
2. ϕ(obs)p(.)	1661.25	0.13	3	1655.23	**3-2, obs^2, p=0.02**
1. ϕ(obs^2)p(.)	1686.57	0.00	3	1680.56	**3-1, obs, p<0.001**

d. Modelling probability of recruiting locally (ϕ) including normalizing selection on body size.

Model	AIC	AICw	np	deviance	
5. ϕ**(obs obs^2+age+t+age.t)p(.)**	**1485,89**	**0.16**	**8**	**1469.82**	4-5, f, p=0.59
4. ϕ(f +obs obs^2+age+t+age.t)p(.)	1487.90	0.07	9	1469.81	3-4, obs obs^2 on f, p=0.12
3. ϕ(f, obs obs^2+obs obs^2+age+t+age.t)p(.)	1490.79	0.02	12	1466.64	2-3, **d,obs obs^2, p=0.12**
2. ϕ(f, obs obs^2+d, obs obs^2+obs obs^2+age+t+age.t)p(.)	1492.01	0.01	14	1463.81	**1-2, f.d, obs obs^2, p=0. 56**
1. ϕ(f*d*obs obs^2+age+t+age.t)p(.)	1496.06	0.001	17	1461.77	

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Appendix C: A Figure showing the probability of recruiting locally in relation to treatment (infested, uninfested), experimental design (A, B), and nestling body size. *Ecological Archives* E000-000.
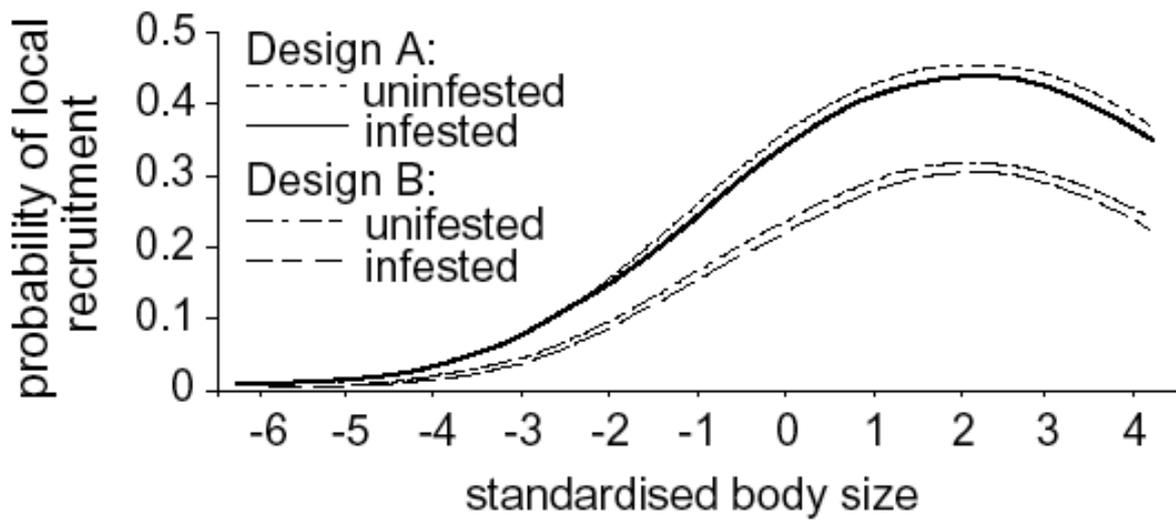
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Appendix D. Effect of parasite treatment on body size and tarsus length of locally recruited nestling great tits. *Ecological Archives* E000-000.

	tarsus length				body size			
	DF	F	p	% variance explained	DF	F	p	% variance explained
treatment	1,181	0.93	0.21	0.4	1,180	0.90	0.22	0.4
year of birth	2,181	2.01	0.14	1.4	2,180	<0.01	1	0.0
design	1,181	0.40	0.53	0.2	1,180	0.60	0.44	0.2
sex	1,180	31.46	<0.001	42.4	1,180	76.38	<0.001	29.5
treatment x design	1,173	0.03	0.86		1,173	0.001	1	
treatment x sex	1,179	1.11	0.29		1,175	0.09	0.76	
treatment x year	2,174	0.51	0.60		2,178	2.21	0.11	

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Appendix E. Effect of parasite treatment and treatment of the first recorded brood (treatment 1st) on the first reproduction of recruited nestling great tits. The final model, after backward elimination is shown.

	clutch size				number fledglings			
	DF	F	p	% variance explained	DF	F	p	% variance explained
treatment	1,179	7.59	0.004	3.6 %	1,178	1.18	0.18	0.6 %
treatment 1st	1,179	1.35	0.15	0.6 %	1,178	9.35	0.002	4.4 %
year of birth	2,179	1.56	0.32	1.2 %	2,178	0.828	0.44	1.9 %
brood 1st	1,179	19.32	<0.001	9.1 %	1,178	7.24	0.008	3.4 %
sex	1,179	2.75	0.10	1.3 %	1,178	2.02	0.16	1.0 %
treatment 1st x sex					1,178	7.15	0.008	3.4 %

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Appendix F. Effect of parasite presence during the nestling period on total number of eggs, fledglings, and recruits produced.

	a. number eggs produced				b. number fledglings produced				c. number recruits produced			
	DF	F	p	% variance explained	DF	F	p	% variance explained	DF	F	p	% variance explained
treatment	1,181	12.92	0.044	0.3%	1,181	0.67	0.26	0.2 %	1,181	4.59	0.02	1.9 %
nr clutches with parasites	1,181	0.42	0.324	<0.1 %	1,181	0.17	0.42	<0.1 %	1,181	7.07	0.01	3.0 %
nr clutches produced	2,181	652.92	<0.001	51.49%	2,181	99.35	<0.001	27.13 %	1,181	13.01	<0.001	5.5 %
nr possible breeding events	1,181	1.95	0.164	0.2 %	1,181	5.00	0.03	1.4 %	1,181	22.92	<0.001	9.7 %
sex	1,181	3.04	0.083	0.2%	1,181	1.15	0.29	0.3 %	1,181	1.07	0.30	0.5 %

Appendix G. Effect of parasite presence during the nestling period on lifetime reproductive success.

	a. number eggs produced				b. number fledglings produced				c. number recruits produced			
	DF	F	p	% variance explained	DF	F	p	% variance explained	DF	F	p	% variance explained
treatment	1,183	0.62	0.27	0.3%	1,183	0.41	0.33	0.2 %	1,182	3.59	0.04	1.6 %
nr possible breeding events	1,183	27.89	<0.001	13.11 %	1,183	26.61	<0.001	12.59 %	1,182	35.15	<0.001	15.3 %
sex	1,183	0.61	0.44	0.3%	1,183	0.78	0.38	0.4 %	1,182	0.51	0.48	0.2 %