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DENSITY-DEPENDENT PROCESSES IN THE POPULATION DYNAMICS OF A BIRD ECTOPARASITE *CERATOPHYLLUS GALLINAE*

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Abstract. The hen flea *Ceratophyllus gallinae* (Siphonaptera: Ceratophyllidae) is a common ectoparasite in Blue Tit *Parus caeruleus* nests, whereby adult fleas draw blood from the incubating female host and from the nestlings. Host–parasite interactions are influenced by density-dependent growth of the parasite population, which may result from competitive exploitation or interference between parasite individuals, or from density-dependent host defenses. Understanding density dependence in host–parasite interactions is important for understanding the evolution of parasite virulence and of host defense. Here we investigate density-dependent processes in the dynamics of flea subpopulations. In a first experiment, intended to establish the importance of density dependence in this host–parasite system, the founder density of parasites was manipulated, and reproductive rates of parasites measured. A second experiment was designed to identify the demographic levels at which density dependence arises in the growth of the hen flea population. The experiments show that parasite reproductive rates significantly decrease with increasing founder density. The patterns of egg production, larval production, and adult flea survival suggest that competition between larvae is the main process behind the density-dependent pattern of subpopulation growth. Host mortality, body condition, and blood hematocrit level of the female host and the nestlings were not significantly affected by parasite load, suggesting that adult fleas are not food limited at high densities.

Key words: Ceratophyllus gallinae; *competition; flea production and mortality vs. density; host condition vs. flea density; infestation with three flea founder densities; larval competition during bird incubation;* Parus caeruleus; *reproductive rates; survival; virulence.*

INTRODUCTION

Density-dependent growth or survivorship in parasites has been demonstrated for various host–parasite systems involving macroparasites, such as helminths (Moss 1971, Croll et al. 1982); ectoparasites; and microparasites (Ni and Kemp 1992, Novella et al. 1995). Density-dependent patterns of parasite population dynamics may be the outcome of competition, that is the negative effects that parasites have on conspecifics by consuming or controlling a limited resource (Keddy 1989, Izraylevich and Gerson 1995). Such patterns can also arise due to density-dependent behavioral (Edman et al. 1972, Murray 1987) or immune responses of the host (Schofield 1982, Randolph 1994), and, in practice, it is not always easy to differentiate between these two mechanisms, which are not mutually exclusive (Begon et al. 1990).

It has been suggested that the aggregated distribution of many parasites may act to enhance density-dependent regulation of parasite abundance (Anderson 1981). Competition among parasites at various developmental stages on or within single hosts should reduce the impact of the parasite on the host population (Hudson and Dobson 1997). Thus, competition is included in various models of parasite population dynamics as an important component in the evolution of virulence (Bull 1994, Read 1994, Ebert and Herre 1996). Within-host competition should select for genotypes with high growth rates, which is often associated with high virulence. This could lead to overexploitation of the host, and, at the population level, selection could then favor a lower level of virulence (May and Nowak 1994, Van Baalen and Sabelis 1995, Frank 1996).

This study experimentally investigates density-dependent processes in Blue Tit nests that are multiply infected with the avian ectoparasite *Ceratophyllus gallinae* (Schrank). Blue Tits, *Parus caeruleus,* and other hole nesters of the *Parus* guild are the main hosts of this flea species (Tripet and Richner 1997*a*). Infestations by bird fleas are atypical, compared to ''classical infections,'' in that the parasite lives in the host's dwelling, rather than on the host itself. The parasites breed during the birds' nesting period when the host is available for regular blood meals. The larvae feed on organic debris found within the nest and on undigested blood excreted by adults (Rothschild and Clay 1952, Lehane 1991). Two overlapping flea generations are produced between the host's egg-laying period and fledging of its young. First generation adult fleas appear around hatching time of the nestlings and join the sur-

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viving founder fleas for larval production. At the end of the nestling period, the first generation and second generation larvae spin cocoons and remain dormant for several months (Tripet and Richner, 1999). These offspring will eventually infest a new host at the beginning of its breeding attempt (see Plate 1). *Ceratophyllus gallinae* has been shown in Great Tits, *Parus major*, to decrease host fitness by reducing both offspring quality and survival (Richner et al. 1993). Furthermore, it has been shown that Great Tits avoid nest sites with high flea loads (Oppliger et al. 1994), desert heavily infested nests (Oppliger et al. 1994, Merilä and Allander 1995), and alter other behaviors when raising young in infested nests (Christe et al. 1996*a*, *b*).

In a first experiment (Experiment 1) we examine the effect of founder density on the reproductive rate of the ectoparasite in order to establish whether, and to what extent, density-dependent processes occur in this host–parasite system. A second experiment (Experiment 2) is designed to identify the demographic levels at which density dependence arises in the growth of the parasite population. The role of competition in shaping flea demography, and its importance as a driving force in the evolution of flea virulence, is discussed.

PLATE. 1. A female *Ceratophyllus gallinae* freshly emerged from the cocoon where she remained dormant during the winter months. If she finds a host, she will feed regularly, develop a voluminous abdomen, and continuously lay eggs unless grooming kills her. Old eggshells laid on a feather of the nest cup lining during the last breeding season can be seen in the background. Photograph by F. Tripet and C. Gindro.

MATERIAL AND METHODS

Study area

This study was conducted in spring 1994 and 1995, in a 60 ha forest, 8 km southwest of Basel, Switzerland (47°32′ N, 7°32′ E). The forest harbors a dense population of Blue Tits, *Parus caeruleus*, breeding in nest boxes and natural cavities. Previous researchers working on this population on other topics reported high natural infestation rates by *Ceratophyllus gallinae* (Zhandt, *personal communication*). Between 1990 and 1994, before the present experiments, both the bird and the flea population were left unmanipulated.

Experiment 1

This experiment examines the effect of founder density on the reproductive rate of the parasite. Following an experimental infestation with three different founder densities, the size of the larval cohorts was measured at different breeding stages of each pair of birds.

In January 1994, we replaced the old nest boxes with new ones (model ''Varia,'' built by the Swiss Ornithological Institute). They consist of an outer plastic cover and an inner wooden box in which the birds build their nest. In 1994, at the beginning of the Blue Tit breeding period, we visited the nest boxes every other day and recorded onset of laying, number of eggs laid, start of incubation, and hatching date of nestlings (referred to as day 0 of the nestling stage).

Since variation in nest mass could influence flea development (Eeva et al. 1994, discussed in Heeb et al. 1996), we standardized nest size to an average height of 8 ± 1 cm, the mean nest size in 1994, by adding or removing material from the column of moss under the nest cup. This manipulation at the end of egg laying never led to nest desertion. When the birds laid their second egg, we heat treated the nest material, using a microwave oven, to kill all existing parasites. During the heat treatment, the nests were placed in plastic bags to prevent excessive desiccation, and they were sprayed with 4 mL of water after the heat treatment. The nests were randomly infested with 6, 20, and 50 adult fleas on the second day of incubation. Rather than systematically controlling the sex ratio at infestation, which was too time consuming, we opted for the following randomization procedure, which ensured that the mean sex ratio did not differ among treatment groups. Nests were collected in January within the study area and stored in plastic bags. Before picking fleas for infestation, three to five randomly chosen, heavily infested nests were put in a large glass container, and the nest material was thoroughly mixed. As a consequence of the mechanical disturbance, and the elevated temperatures at this time of year, all fleas were hatched at the time when the fleas were collected from the nest material (see Humphries [1968] for the effect of mechanical disturbance and temperature as triggers for the hatching of fleas). A large number of fleas were then collected in a small pile, and then either 6, 20, or 50 fleas picked in random order. Host nests were randomly assigned to the treatment groups. Thus, the design ensured equal mean sex ratios at the three density levels. However, heterogeneity of variance between the three experimental groups in the measures of parasite population growth is expected to arise, since random picking of a few individuals for infestation with six fleas

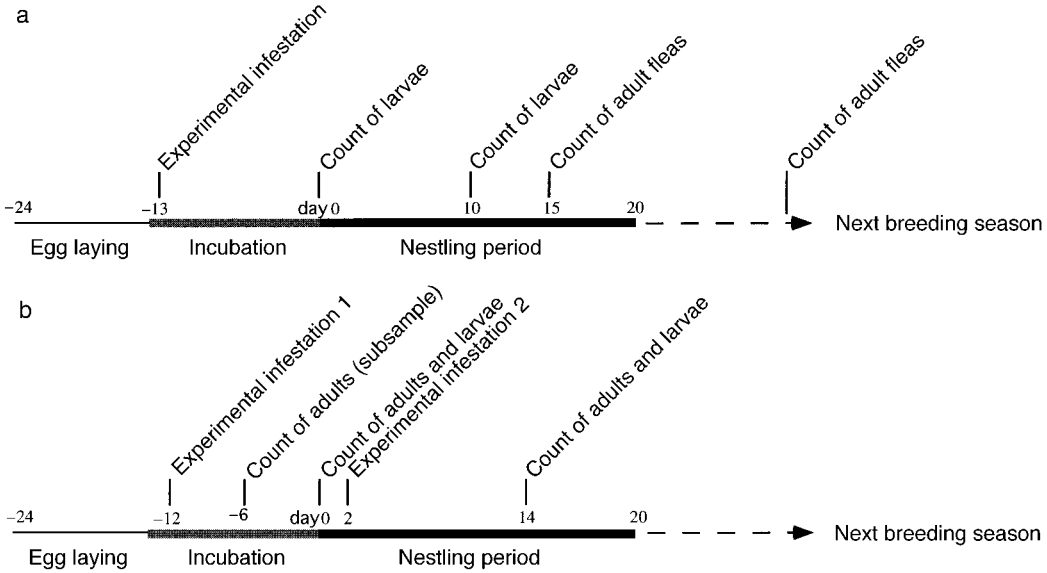

FIG. 1. (a) Experimental protocol in Experiment 1, in relation to sequence and duration of the major events in breeding Blue Tits nests. (b) Experimental protocol in Experiment 2.

leads to greater variance in the founder sex ratio than random picking of many individuals with 20 or 50 fleas. We used nonparametric analyses when heterogeneity of variance was observed.

Measures of parasite population growth.—Four measurements of flea population size were made during each bird's breeding attempt (Fig. 1a): (1) we counted the number of flea larvae produced by founder adults on day 0 of the nestling period (i.e., at hatching). The following procedure was adopted when counting day-0 larvae: We first divided the nest under study in two parts. The top part of the nest, with its nest cup, was kept intact and gently shaken above a plastic dish until no more larvae fell from it. It was then placed back in the nest box, so that the adult birds could feed and incubate their young during the rest of the manipulations. The bottom part of the nest was thoroughly mixed above a metal mesh. The fine fraction of nest material that contained the larvae, and the larvae from the nest cup were then mixed, spread on the plastic dish and divided into eight sectors. We counted the live larvae from two randomly chosen sectors. After counting, we rebuilt the bottom part of the nest and reintroduced the larvae under the nest cup. (2) The same method was used on day 10 of the nestling period (i.e., the mid-nestling period). At that stage, the larvae are produced by founder and first generation adult fleas. By that time, the nest cup is damaged considerably by the female bird's cleaning activities and trampling by the nestlings, thus allowing us to inspect all of the nest material. (3) Adult fleas (founder and first generation combined) were counted on day 15 of the nestling period (Fig. 1a). We spread the coarser fraction of the nest material in a plastic dish and left it undisturbed for 30 min, while counting the fleas from the finer material. The fleas from the coarser fraction were counted as they aggregated on top of the material. After counting, we rebuilt the nest and reintroduced the fleas into the nest material. (4) At the end of the bird's breeding period, after the young fledged, we sealed all cracks in the nest boxes with tape and set a flea trap on the front hole of the nest box. The flea traps were modified from Bates (1962). They consist of a transparent Plexiglas cylinder (length, 6 cm; diameter, 5 cm), which is closed at one end by a nylon mesh and at the other end by a transparent lid, with a hole in the center (hole diameter 2.5 cm). The connection between the nest box entrance hole and the hole in the lid of the trap was made by a 6 cm long rigid plastic tube. The plastic tube was fitted halfway into the transparent cage and acted as a funnel. First and second generation offspring, which hatched from cocoons and dispersed, accumulated in the traps and were collected on the 15th of every month during summer, autumn, and winter. The offspring remaining in the nests after the winter were sampled in mid-March 1995 using the method used for larval counts. The final number of offspring produced (i.e., the reproductive success) of founder fleas during each bird's breeding attempt was calculated as the sum of dispersing fleas and the fleas counted from the nest material (Fig. 1a). In order not to include adult fleas present in the nest during the nestling period in these final counts, we excluded from our calculation adults dispersing within 2 wk after fledging.

Calculation of reproductive rates.—Reproductive rates were calculated as number of larvae or imagos per flea originally introduced. Because of heterogeneity of variances, no parametric analysis could be performed to test for changes over time in number of offspring produced per flea originally introduced. We

therefore calculated the regression coefficients of the lines between the measure at hatching and midnestling period and from midnestling period to the end of nesting. These slopes, equivalent to larval production per 10 d, were analyzed nonparametrically.

Host body condition and hematocrit level.—Adult birds were captured on nestling day 15, by means of a spring-loaded trap that shuts the entrance hole as the bird enters the nest. We sexed the birds according to the presence or absence of a brood patch, weighed them to the nearest 0.1 g using a Sartorius portable 1200 electronic balance, and measured their tarsi to the nearest 0.1 mm using a calliper. We took the same measurements on the 15-d-old nestlings, but we did not sex the young. One 50-μl capillary tube of blood was taken from the brachial vein of each adult and from four randomly picked nestlings per brood. It was centrifuged in order to measure the hematocrit level (proportion of red blood cells per total blood volume). The variable 'body condition' was calculated, for both adults and nestlings, as the residual of the regression of body mass on tarsus length. It is a measure that expresses how much a bird deviates from the mean relationship between body mass and body size.

The original sample size was 49 nests. The birds deserted three nests before the end of incubation, and two broods failed just after hatching. The number of larvae at the end of incubation and midnestling periods were therefore counted in 46 and 44 nests, respectively. Adult fleas were counted from 43 nests. One bird pair started a second brood before we set the flea trap, two nest boxes were stolen, and one flea trap was destroyed. The final number of fleas produced was recorded from 41 nests. In four cases, one of the adult birds died and the surviving partner took care of the young. They were excluded, and the analysis involving the birds' breeding parameters were therefore conducted on the data from 40 nests. Hematocrit levels were measured from 35 out of 40 females.

Experiment 2

The second experiment, performed in 1995, focuses on density-dependent larval production and adult flea survival, in order to differentiate between various processes that could lead to density dependence.

Measure of fecundity and survival during the birds' incubation period.—On the first day of incubation, Blue Tit nests were temporarily collected, put into a plastic bag to prevent desiccation, and heat treated using a microwave oven. The nests were then sprayed with 4 mL of water and put back in the nest box. The following day, the nests were randomly infested with 6, 20, or 50 adult fleas, following the same procedure as in Experiment 1. A total of 72 nests were infested using this method. Birds deserted three nests during incubation. Twenty-one randomly chosen nests were analyzed for adult survival on day 6 of incubation, and 48 nests were analyzed on day 12. The following procedure was used on day 6 or 12 in order to analyze the nest contents. We temporarily swapped the bottom wooden part of the nest box, containing the nest to be analyzed, for one containing a dummy nest made of cotton wool and heat-treated moss. This did not seem to affect the birds in their routines and allowed us to inspect the original nests for parasite development and adult survival during incubation (Fig. 1b). The bottom part of the nest and the nest cup material were dismantled separately, and adult fleas and larvae from both layers were collected with tweezers. The nests were then rebuilt and heat treated using a microwave oven in order to kill any remaining fleas. When this was done (after 4 h, on average), we replaced the parasite-free nest in the nest box so that every bird pair received its original nest. The adult fleas collected on day 6 and the flea larvae collected at day 12 were immediately stored in 70% ethanol, for later estimation of parasite survival rates and reproductive rates. The larvae collected at day 6 were discarded. Adult fleas that were recaptured from the 48 nests analyzed on day 12 of incubation were stored in one 10 mL glass tube per nest and left undisturbed for 24 h. During this time, the females laid the eggs contained in their abdomen directly on the inner surface of the tubes. We counted the eggs directly using a binocular microscope and calculated the mean egg production per female for each nest.

Measure of parasite fecundity and survival during the bird nestling period.—The nests were randomly reinfested, on the second day after hatching of the young birds, with either 45 or 90 adult fleas. These loads were within the range of the number of fleas counted in the nests during the 1994 nestling period in Experiment 1. Twelve days later, we collected the nest material from all nests and immediately heat treated it with a microwave oven for later estimation of parasite numbers and adult survival (Fig. 1b). Birds were provided with artificial parasite-free nests for the remaining 5 d before fledging of the young. A total of 43 nests were manipulated this way. Five bird pairs deserted during the nestling period; two were of the 45-flea group and three were of the 90-flea group. For the analysis, we also excluded two bird pairs with known polygamous males. The resulting sample sizes were, therefore, 18 nests infested with 45 fleas and 16 nests infested with 90 fleas. The nests collected on day 14 of the nestling period were stored in plastic bags. They were later entirely dismantled and searched for larvae and intact adult flea bodies.

Calculation of reproductive rates.—Reproductive rates were calculated as number of larvae per surviving flea. Heeb et al. (1996) found a mean immigration of 5.8 fleas per nest between nest building and fledging of young Great Tits, *P. major*. Consequently, adult survival during incubation and the nestling period was not calculated as the number of survivors per flea originally introduced, because random immigration may have in-

TABLE 1. Number of parasites (mean ± 1 SD) in breeding Blue Tit nests that were experimentally infested with 6, 20, or 50 fleas. Numbers in parentheses indicate number of nests, *n*.

Nestling period	Experimental flea load		
	6	20	50
Day 0 larvae	252 ± 221 (15)	420 ± 282 (17)	723 ± 382 (14)
Day 10 larvae	679 ± 325 (14)	1143 ± 699 (16)	1424 ± 331 (14)
Day 15 adults	29 ± 14 (15)	55 ± 20 (15)	58 ± 31 (13)
Final adults†	1612 ± 818 (12)	2637 ± 1073 (15)	2518 ± 1059 (14)

† End of nestling period.

flated the measure of survival at low founder densities. Density effects on adult survival were investigated by fitting to our day-6 and -12 counts of surviving fleas the following model, which assumes a constant mortality rate *m* (over time) and random immigration *i* of wild fleas:

$$\frac{dN}{dt} = -mN + i. \quad (1)$$

Eq. 1 can be solved analytically to express $N(t)$, the number of surviving fleas at time t (expressed in d), as a function of m, i, and the number of founder fleas N_0:

$$N(t) = \frac{-e^{-tm+\log(i-mN_0)} + i}{m}. \quad (2)$$

Estimates of the terms *m* and *i* were obtained by fitting Eq. 2 to our data. The residuals of the model were analyzed for the effects of density and time of survival.

Statistical analyses were performed using the SYSTAT Statistical Package (Wilkinson 1992) and JMP software (Sall and Lehman 1996). Data were checked for normality and heterogeneity of variances and were transformed where needed. Significance levels are two tailed.

RESULTS

Experiment 1

Parasite population growth.—The number of larval fleas at nestling day 0 and nestling day 10 significantly increased with increasing founder flea density (at day 0, $F_{2,43} = 9.15$, $P < 0.001$; at day 10, $F_{2,41} = 8.04$, $P = 0.001$) (Table 1). The same was true for the number of adult fleas at day 15 ($F_{2,40} = 7.56$, $P = 0.002$) and the final number of adults ($F_{2,38} = 4.02$, $P = 0.026$) (Table 1).

Founder density influenced the number of offspring produced per founder flea, as shown by the decrease of the slopes of parasite population growth (Table 2, Fig. 2) with increasing founder density. This effect was significant both between day 0 and 10 of the nestling period (Kruskal-Wallis, [KW] = 20.67, $n = 44$, $P < 0.001$) and between day 10 and 20 (KW = 20.74, $n = 40$, $P < 0.001$). The slopes themselves significantly increased from the first to the second half of the nestling period (Wilcoxon, $Z = 3.54$, $P < 0.001$). There was also a strong negative effect of founder flea density on the growth rates of the adult cohort from infestation to the nestling period (Fig. 3a; KW = 25.89, $n = 43$, $P < 0.001$). Rates of increase of the adult cohort from day 15 to the end of the nestling period were calculated after correcting for earlier density effects. This was done by subtracting the number of larvae found at mid nestling period from the final offspring number. The resulting rates did not differ significantly between the three experimental groups (Fig. 3b; $F_{2,33} = 1.96$, $P = 0.156$).

TABLE 2. Regression coefficients describing parasite population growth (mean ± 1 SD). Numbers in parentheses indicate number of nests, *n*.

Nestling period	Experimental flea load		
	6	20	50
Days 0–10	69.5 ± 34.9 (14)	35.1 ± 27.4 (16)	14.0 ± 8.9 (14)
Days 10–20	167.5 ± 125.0 (11)	80.3 ± 44.8 (15)	21.9 ± 19.1 (14)

Note: Slopes are based on number of offspring produced per founder flea during days 0–10 and days 10–20 of the nestling period in Blue Tit nests.

Host survival, body condition and hematocrit levels.—There was no significant difference in hatching success or nestling survival between nests infested with 6, 20, and 50 founder fleas. This was also true for the body condition and hematocrit levels of adult females and nestling Blue Tits at day 15 of the nestling period (Table 3).

Experiment 2

Parasite fecundity and mortality during incubation.—The number of eggs laid by the surviving female fleas, measured at the end of the bird's incubation, did not decrease with flea density (Fig. 4a). Instead, fleas collected from low-density nests tended to lay fewer eggs (KW = 5.67, $n = 48$, $P = 0.058$), and there was

FIG. 2. Number of offspring per founder flea (mean ± 1 SE) at three stages of the Blue Tits' nestling period.

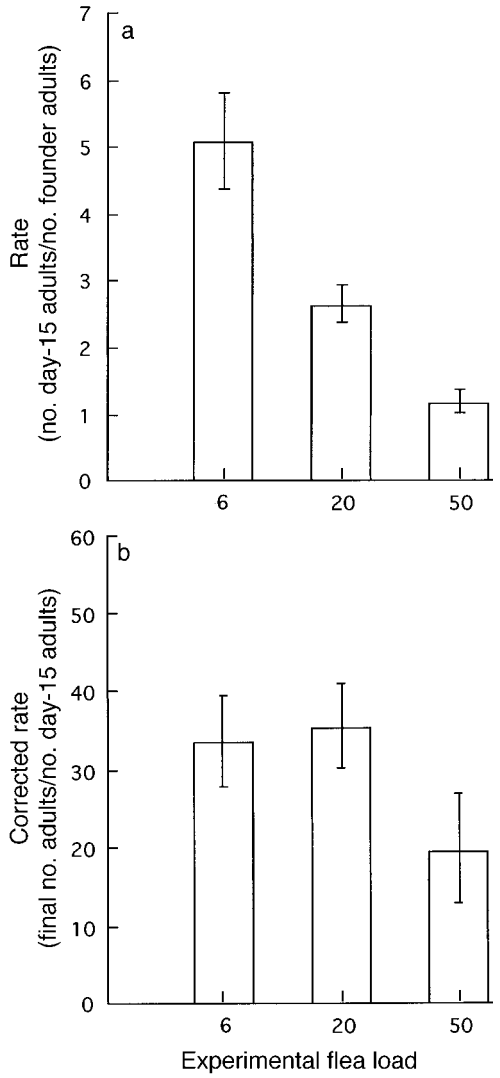

FIG. 3. (a) Rate of increase (mean ± 1 SE) of the adult cohort, from infestation to day 15 of the nestling period, in relation to founder density. (b) Corrected rate of increase (mean ± 1 SE) of the adult cohort, from day 15 to the end of the nestling period, in relation to founder density. The rates were corrected for density effects occurring before the middle of the nestling period by subtracting the density-dependent number of larvae found at mid nestling period from the final offspring number.

also more variance in this group (Bartlett, chi-square = 7.96, $n = 48$, $P = 0.019$) (Fig. 4a).

The number of flea offspring produced during host incubation, calculated per surviving female flea, was negatively affected by density of founders (KW = 19.45, $n = 48$, $P < 0.001$) (Fig. 4b). Flea survival, calculated as the residuals of Eq. 2, did not differ significantly among the three experimental groups (KW = 1.03, $P = 0.597$), nor between day 6 and 12 (Mann-Whitney, $U = 386$, $P = 0.124$). Eq. 2 fitted to our data accounts for 93.9% of the observed variance in survival. Fitted survival curves are shown in Fig. 5a. Mortality m was estimated at 0.053 fleas/d and immigration i at 0.179 fleas/d.

Parasite fecundity and mortality during the nestling period.—The number of offspring per surviving female flea did not differ significantly between the two experimental flea densities (Table 4). There was, however, a significant carry-over effect of the founder density during incubation on the number of offspring per surviving flea (Table 4, Fig. 4c). The time at which adult and larval fleas were removed from the nests, day 6 or 12, did not significantly affect the production of larvae during the nestling period (Table 4). Survival, calculated as the residuals of Eq. 2, did not differ significantly between the two groups, and there was no carry-over effect of the flea density during incubation on survival (ANOVA of flea density during the nestling period, $F_{1,28} = 0.45$, $P = 0.501$; flea density during incubation, $F_{2,28} = 1.43$, $P = 0.256$; interaction $F_{2,28} = 1.14$, $p = 0.334$). Eq. 2 fitted to our data accounts for 65.9% of the observed variance in survival. Survival curves and sample sizes are shown in Fig 5b. Survival was only recorded at time t = day 12. Estimated adult mortality m was 0.054 fleas/d and immigration i was 0.192 flea/d.

DISCUSSION

As shown in Experiment 1, the reproductive success of fleas breeding in Blue Tit nests is significantly affected by the number of conspecifics in the same nest. Our measurements of the larval cohort, day-15 adults, and final adult numbers, all show strong density-dependent population growth (Fig. 2). Except for one nest box that was used by a Blue Tit for a second breeding cycle, the boxes in our study area were all used for a single bird-breeding event. Therefore, most fleas breed once per year, and their offspring overwinter within the nests as first and second-generation cocoons. Thus, the total offspring number per founder flea reflects the mean annual reproductive rate of founders, and reproductive rates at low flea density were about five times higher than those at high density. These patterns need to be interpreted cautiously. In Experiment 1, fleas might have immigrated between the heat treatment at the beginning of egg laying by birds and the experimental infestation at the beginning of incubation. Since reproductive rates are calculated per founder flea (i.e., 6, 20, or 50 individuals), part of the measured density effects might have been confounded by wild immigrant fleas reproducing in the nest together with our founders. In Experiment 2 the nests were heat treated immediately before infestation at the beginning of incubation, and reproductive rates were calculated per surviving female flea. Thus, the potential influence of immigrant fleas was eliminated; nevertheless the same density-dependent patterns were found.

Density-dependent mechanisms

We found no significant effects of flea numbers on adult female hosts, on nestling condition, or on female

TABLE 3. Mean hatching success and survival of nestlings and condition and hematocrit levels of nestlings and adult female Blue Tits that were experimentally infested with 6, 20, or 50 fleas.

Response variable	Experimental flea load			*F*	*P*
	6	20	50		
Hatching success (%)	91.9 ± 3.1 (12)	93.2 ± 2.5 (15)	93.7 ± 2.3 (13)	0.12	0.889
Nestling survival (%)	95.6 ± 2.5 (12)	98.1 ± 1.0 (15)	97.9 ± 1.1 (13)	1.60	0.215
Nestling condition (residual, g/mm)	0.561 ± 0.007 (12)	0.569 ± 0.006 (15)	0.552 ± 0.009 (13)	1.01	0.374
Nestling hematocrit level (%)	50.1 ± 1.7 (12)	47.9 ± 1.3 (15)	48.6 ± 0.9 (13)	0.66	0.54
Female condition (residual, g/mm)	0.549 ± 003 (12)	0.559 ± 0.006 (15)	0.548 ± 008 (13)	1.04	0.363
Female hematocrit level (%)	52.7 ± 0.7 (10)	54.5 ± 1.3 (12)	56.2 ± 1.4 (13)	2.08	0.141

Note: All measures are means ± 1 SE, and numbers in parentheses indicate number of broods.

or nestling hematocrit levels (Experiment 1). Thus, fleas do not seem to be directly resource limited. However, we can not exclude the possibility that food limitation occurs even in the absence of an effect on the hosts. For example, hosts with a high flea load may produce a higher cutaneous immune reaction and, therefore, have less palatable blood for fleas. The number of eggs laid by surviving female fleas, collected at the end of incubation, was not negatively affected by flea density. One would expect flea fertility to decrease as a result of competitive interactions between female fleas for access to suitable feeding sites on the host. Fertility could also decrease if the host's preening intensity increased with parasite density (Hart 1992, 1997), or if a cutaneous immune reaction (McLaren 1990, Wikel 1996) altered the feeding behavior of the parasite in a density-dependent way. The comparatively low egg production in the group with six founder fleas is therefore puzzling. It may be that fleas at low densities exhibit lower fertility because of less mating or reduced sexual selection opportunities (Harvey and Bradbury 1991, Eberhart 1996).

Adult flea mortality was high throughout the bird incubation and nestling period (Experiment 2), but not significantly dependent on density. The mean life expectancy of adult fleas, calculated from Eq. 1, is ~10 d. Adult flea survival is expected to decrease with increasing host defenses. These defenses can be behavioral responses (Hart 1992, 1997), such as preening or nest sanitation (Christe et al. 1996*a*), or immune responses (Baron and Weintraub 1987, McLaren 1990; Wakelin and Apanius 1997). Our results suggest that, within the range of parasite densities created by our experimental infestations, there was no change in the hosts' investment in defenses that significantly affected parasite survival. This may not be surprising, given that the negative effects of fleas might simply be compensated by increased food intake by the host (Tripet and Richner 1997*b*) at the expense of behavioral defenses (Tripet, *unpublished data*). The cutaneous immune responses to regular fleabites are also thought to often culminate in desensitization, rather than in an increase in defenses (Benjamini et al. 1961, Nelson 1987, Jones 1996). New evidence suggests that infested female tits could transmit antibodies to the nestlings via the egg, thus increasing their resistance or tolerance to fleas or associated pathogens (Heeb et al. 1998). Interestingly, these authors did not find that this increase translated into a significant decrease in flea survival or reproduction (Heeb et al. 1998). Thus, although the presence of fleas may correlate with an increase in antibody production, it is not yet clear whether this response is directed against fleas, or against secondary infections due to fleabites.

It seems that flea density affects flea population growth before the appearance of the first generation adults, i.e., during the host's incubation period (Experiments 1 and 2). Larval competition for food during host incubation, resulting in a density-dependent production of first generation adult fleas, is the simplest explanation for the density-dependent pattern of the adult cohort measured at day 15 in Experiment 1 (Fig. 3a). The larvae of a few species of *Ceratophyllus* fleas have been reported to eat eggs and dead larvae of their own species (Marshall 1981), and it may be that the amount of cannibalism in the nest determines the size of the larval cohort. However, we cannot exclude the possibility that competition for pupation sites, i.e., competition for space, also occurs at that stage. Some larvae could be forced to delay their metamorphosis until the end of the nestling period, because the nest cup area heated by the incubating female is limited. Also, once the nestlings are hatched, female hosts may clean deeper into the nest (F. Tripet, *personal observation*) and thus prevent further pupation near the heat source.

In Experiment 1, no significant density effect was found on the rates of increase of the adult cohort from day 15 to the end of the nestling period (Fig. 3b). This suggests that competition between larvae decreases during the nestling period. The results of Experiment 2, which show significant density effects during host

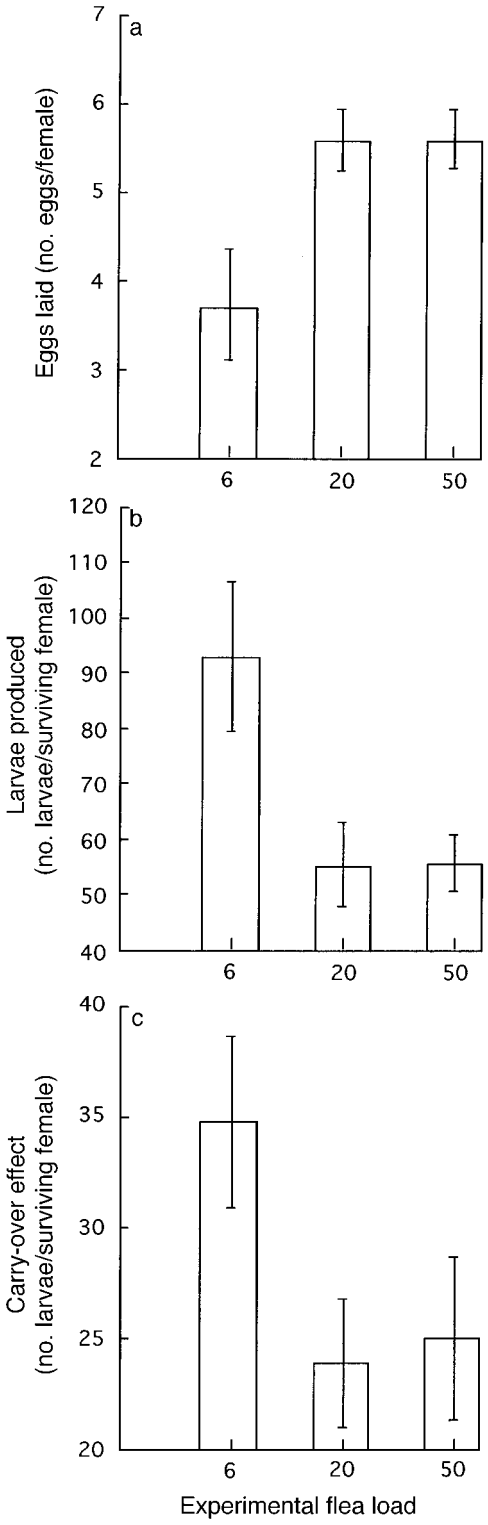

FIG. 4. (a) Number of eggs laid per female flea (mean ±1 SE), collected at the end of incubation in Experiment 2. (b) Number of larvae produced per surviving female flea (mean ±1 SE), as measured at the end of incubation in Experiment 2. (c) Carry-over effect of the founder density during the incubation period on the number of larvae produced per surviving female flea (mean ±1 SE) during the nestling period.

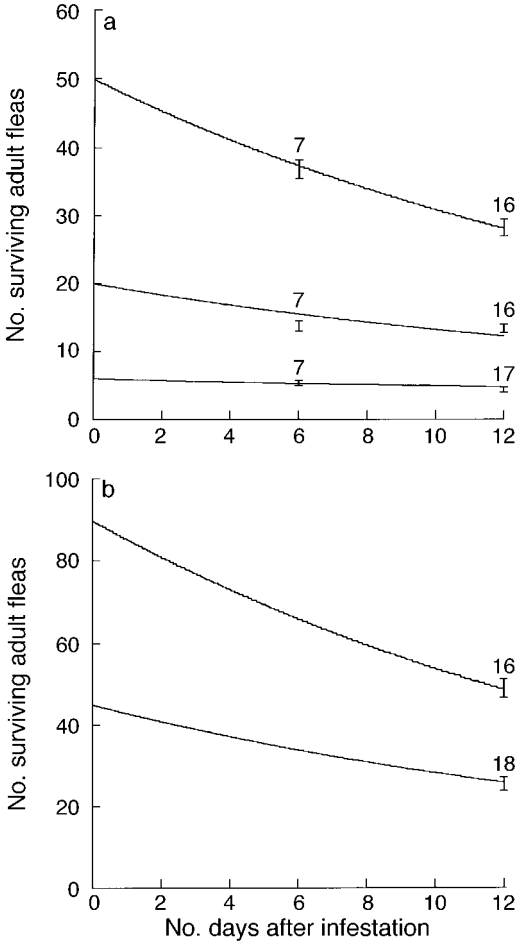

FIG. 5. (a) Survival curves (fitted by Eq. 1) for adult fleas during the birds' incubation period in nests experimentally infested with 50, 20, and 6 individuals (top, middle, and bottom lines, respectively). Shown are sample sizes and ±1 SE for actual measurements. (b) Survival curves (fitted by Eq. 1) for adult fleas during the birds' nestling period in nests experimentally infested with 90 and 45 individuals (top and bottom lines, respectively). Shown are sample sizes and ±1 SE for actual measurements.

incubation (Fig. 4a), but not during the nestling period, lend support to this suggestion. This pattern is paradoxical, because one would expect fiercer competition as the larval cohort increases in size. One possible explanation may lie in the diet of the larvae, which, in addition to the blood feces excreted by adults, feed on organic material such as the hair and feather lining of the nest cup and other debris (Rothschild and Clay 1952). The larvae might first use up this limited supply of organic material from the nest itself before relying mainly on undigested blood produced by the parents. Thus, density effects could be measurable only during the phase of competition for nutrients from the nest cup. Once these resources are used up, the number of offspring produced should be proportional to the number of adults providing food. This would also explain

TABLE 4. Multifactorial analysis testing for the carry-over effects of founder density during incubation and for direct effect of founder density during the nestling stage on the number of larvae produced per surviving female flea during the birds' nestling period.

Factor	Sum of squares	df	Mean square	*F* ratio	*P*
Founder density at incubation (6, 20, 50)	962.94	2	481.47	3.83	0.035
Founder density at nestling period (45, 90)	133.37	1	133.36	1.06	0.312
Time of removal of adults and larvae (day 6 or 12)	8.45	1	8.45	0.07	0.797
Density at incubation × density nestling period	428.07	2	214.03	1.70	0.202
Time of removal × density at incubation	338.94	1	338.94	2.70	0.112
Error	3265.06	26	125.58	···	···

Note: Also included in the model is the time at which the adult fleas and larvae were removed from the nest at the incubation stage.

how our flea density manipulation during incubation, by changing the amount of resources already consumed by larvae, could lead to carry-over effects on flea reproductive rates during the nestling period (Experiment 2).

Density dependence and virulence

As we have found, flea reproductive rates are density-dependent, suggesting that resources are limited. However, the lack of apparent effect on the host suggests that host resources are not limited. Thus, flea virulence, measured as the fitness cost to the host imposed by high density, resource use, or population growth rate of the parasite (for this definition of virulence see Bull 1994, Read 1994, Ebert and Herre 1996), seems rather low.

An analogy can be drawn between pathogens or parasites within host bodies and parasites inside host nests. *C. gallinae* achieves one or two ''vertical'' generations within a host's breeding cycle. The offspring then usually undergo ''horizontal'' transmission, because a new host will often use the nest the following year. Other fleas leave the nest between the two host breeding events and jump on passing birds (Bates 1962, Humphries 1968). These individuals are horizontally transmitted through dispersal. Thus vertical transmission occurs within nests, and horizontal transmission occurs among nests. Therefore, the opportunity exists for selection to act both within and among nests, as is the case for parasites and pathogens within and among bodies of hosts.

Within nests, intraspecific competition among larvae will not affect host fitness, if larvae solely use resources found in the nesting material (e.g., organic debris), and the larval stage will then not be truly parasitic. This decoupling of larval population processes to the host fitness may explain why hosts appear to suffer no costs of parasitism, and fleas may be considered avirulent. However, because adult fleas provide resources (blood feces) to larvae, larval growth rates and density are influenced by the exploitation of hosts by adults. Thus, larval fleas can be considered virulent, in the sense that increases in their population density, growth rate, or efficiency may reduce fitness components of hosts, because larvae require resources that only adults can provide via feeding on host blood.

Selection within nests may be for increased virulence, because flea genotypes that exploit hosts more efficiently and reproduce at higher rates would be at an advantage. But that may be counteracted by two processes. First, as a consequence of the fleas' metapopulation structure, reproductive cycle, and facultative dispersal strategies, in a natural situation the mean relatedness between individuals is expected to be higher within nests than among nests. Thus, subpopulations of parasites can be considered as demes (Wilson 1980). Increased relatedness in flea demes has been proposed as a possible explanation for the evolution of the ''shared'' parental investment in the production of feces for the larval cohort (Hinkle et al. 1991, Silverman and Appel 1994). Kin selection (Hamilton 1972) should also favor reduced population growth rates (Frank 1996), and lower virulence. Second, selection among nests against higher virulence may occur due to avoidance and desertion by birds of heavily infested nests (Du Feu 1992, Oppliger et al. 1994, Merila and Allander 1995). These host behavioral defenses may result in virulence being traded against parasite fitness. In theory (May and Nowak 1994, Van Baalen and Sabelis 1995, Frank 1996), among-nest selection for subpopulations with lower reproductive rates could, therefore, partly counteract within-nest selection for higher reproductive rates. Among-nest (interdemic) selection in our system is made more plausible by the likely high relatedness of fleas within individual nests.

The consideration of host life history evolution under parasitism provides yet another perspective on our observation of an apparent lack of negative effects of fleas on nestlings. Virulence consists of a decrease in some components of host fitness (Read 1994, Ebert 1998), and the effects of parasites may be expressed in the host's present and/or future reproductive success. Indeed, adult birds increase their feeding rates to the young in order to compensate for flea effects, and this might carry costs in terms of future reproduction (Tripet and Richner 1997*b*; Richner and Tripet 1999), as predicted from theoretical models (Perrin et al. 1996). Thus fleas may indeed be virulent, and the effect may

not be detectable on nestlings, but show up on adults later in their life. Ectoparasites might well act on the trade-off between current and future reproduction.

The density dependence seen within nests may represent the mechanism by which flea population growth is regulated and virulence toward hosts reduced. Both interdemic selection and kin selection may have caused fleas to be sensitive to their subpopulation density, thus avoiding deme extinction that would be caused by abandonment of nests. Unfortunately, this argument still leaves unexplained the fact that density dependence acts on larvae and not on adults. At present, we cannot reconcile this issue. Further research should quantify genetic relatedness of fleas within and among nests, and assess the relative importance of within- and among-nest selection on virulence.

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