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LARVAL COMPETITION AFFECTS THE LIFE HISTORIES AND DISPERSAL BEHAVIOR OF AN AVIAN ECTOPARASITE

FRÉDÉRIC TRIPET,[1] ALAIN JACOT, AND HEINZ RICHNER

Division of Evolutionary Ecology, Zoology Department, University of Bern, CH-3032 Hinterkappelen, Switzerland

Abstract. Dispersal has profound effects on parasite populations. Understanding the dispersal behavior of parasites is fundamental to our appreciation of their virulence, epidemiology, and host specificity. Very few host–parasite systems, however, allow for studying how parasites optimize their transmission rates. Here, we investigated the dispersal behavior of a common ectoparasite of European passerine birds, the flea *Ceratophyllus gallinae*. This flea primarily infests hole-nesting species and breeds during its host's breeding season. Once the host leaves the nest, flea larvae build cocoons, pupate, and remain dormant before initiating their host search. There is considerable variation in the time at which they hatch and disperse from the nest boxes. Some offspring disperse before the hosts choose their nest sites at the beginning of the next breeding period, while others wait until after that stage to disperse. By experimentally manipulating the density of fleas in the nests of their breeding hosts we were able to investigate density-dependent processes that would later affect the dispersal behavior of flea offspring. We found that the density of offspring in the nests was negatively correlated with the proportion of early-dispersing individuals and negatively affected the phenotypic quality of dispersers. Flea offspring of poor phenotypic quality in terms of body size dispersed earlier and had lower potential fecundity than bigger individuals. In a laboratory experiment, we found that the intensity of larval competition strongly affected offspring development, body size at maturity, and overwintering capacity. Thus, in order to maximize their chance of transmission, *C. gallinae* individuals adjust their dispersal behavior according to their phenotypic quality. In this species, dispersal in time may be explained by the carryover effects of variation in the amount of competition experienced at the larval stage.

Key words: Avian ectoparasite; *best-of-a-bad job strategy; body size;* Ceratophyllus gallinae; *competition; dispersal; fecundity; fleas;* Parus caeruleus.

INTRODUCTION

Dispersal may be defined as the process by which individuals escape from their natal environment and move to other locations where they reproduce (Begon et al. 1990, Futuyma 1998). Dispersal is a fundamental aspect of an organism's life history because of its effect on the dynamics and genetic structure of its populations. Virtually all species disperse but organisms that live in temporary habitats are typically more dispersive than others (Begon et al. 1990). In this regard, parasites and other infectious diseases whose reproductive rates depend on their offspring's ability to successfully locate and infest a host (May and Anderson 1983) are usually highly dispersive. Describing dispersal and understanding the factors determining those patterns bears special significance in parasites since their mode of transmission, vertical, horizontal, or a combination of the two, may affect their virulence (Ewald 1983, Clayton and Tompkins 1994), may have important implications for their epidemiology (Anderson and May 1982), and may influence their host specificity (Price 1980, Thompson 1994). Identifying the selection pressures shaping the dispersal behavior of parasites would also give us important insights on coevolutionary processes with their hosts. Few studies, however, have investigated dispersal patterns probably because dispersal is extremely difficult to quantify (Begon et al. 1990). This is especially true for parasites whose reduced size makes them difficult to track.

The following study investigates density-dependent factors influencing the timing of host search in an avian ectoparasite, the flea *Ceratophyllus gallinae* (Schrank), commonly infesting European passerine birds (Traub et al. 1983, Tripet and Richner 1997*a*). Blue Tits, *Parus caeruleus*, and Great Tits, *P. major*, are the main hosts (Rothschild and Clay 1952) and the majority of the flea population reproduces in their nest holes (Tripet and Richner 1997*a*). *C. gallinae* requires a nest occupied by breeding hosts in order to breed. Adult fleas draw blood from laying and incubating female birds and later from the chicks until these fledge. The flea larvae develop in the nest material and feed on organic material from the nest cup and from a constant supply of undigested blood produced by adult fleas as liquid feces (Marshall 1981*a*, Lehane 1991). Up to two generations

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[1] Present address: Vector Genetics Lab, WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, Texas 77555-0609 USA.
E-mail: frtripet@utmb.edu

are completed within the six- to seven-week long bird-nesting period (Tripet and Richner 1999*a*). Soon after all young have fledged, the larvae build cocoons at the bottom of the deserted nest while adult fleas disperse (Humphries 1967*a*, Tripet and Richner 1999*a*). The larvae then undergo the larval–pupal and pupal–imaginal molts within the cocoons (Humphries 1967*a*).

In the hen flea, the newly hatched imagos are the free-living infective stage responsible for finding a new host and infesting its nest. The most obvious sign of dispersal in this species occurs in early March of the following year as Blue and Great Tits start inspecting tree holes and other cavities in search of a potential nesting site. At this stage, the nests may be reused by tits for another breeding season and the freshly emerged fleas simply remain in the nest material. If the nest remains unused, however, fleas will come out of the nest hole and wait at the nest entrance. They are sometimes so numerous that they form a conspicuous dark ring around entrance holes (F. Tripet, *personal observation*). Next, they progress upward on higher tree branches in order to jump on passing birds (Humphries 1968). It is believed that the majority of imagos remain quiescent in the cocoons unless stimulated by increasing temperatures and the vibrations indicating the presence of a potential host (Humphries 1967*a*). However, reports indicate that a significant proportion of new generation offspring hatch and disperse earlier than the next bird-nesting period, in the fall and winter (Bates 1962, Du Feu 1987). The adaptive significance of early flea dispersal is not obvious because finding a non-breeding host requires a hazardous host search, does not offer any flea breeding opportunities, and fleas will need dangerous opportunistic feeds in order to survive to the next bird-breeding event.

There are some indications that the density of flea offspring in the nest might explain some of the observed variation in the timing of their dispersal. In a previous study conducted on the same nests during the previous year's nesting season, we found that larval competition negatively affected the reproductive rates of fleas within Blue Tit nests (Tripet and Richner 1999*b*). Thus competition might also negatively affect the phenotypic quality of flea offspring and this could in turn influence their overwintering capability and optimal timing of dispersal. Early dispersal would in this case reflect a strategy of individuals of lower phenotypic quality optimizing their chance of transmission. There is also experimental evidence that tits avoid visiting, roosting, and breeding in heavily infested nests (Du Feu 1992, Christe et al. 1994, Oppliger et al. 1994, Merilä and Allander 1995). Thus, density-dependent dispersal could also arise because of a totally different process. In crowded nests, dispersal in time (Begon et al. 1990) might maximize transmission rates because of the high probability that birds will avoid such nest sites and that newly hatched offspring will eventually have to disperse anyway in search of a host. Host nest-site choice may therefore select for the evolution of a density-dependent phenotypic plastic response to crowding. Early dispersal would in this case reflect the optimal dispersal behavior at high density while flea offspring should remain in the nests at low densities. Phenotypic plasticity in dispersal behavior of adult fleas could be induced in response to density-dependent cues gathered during the larval stages as in some cases of wing morph determination (Harrison 1980, Dixon 1985, Denno and Peterson 1995, Zera and Denno 1997).

These two hypotheses were evaluated in a forest in Northern Switzerland by randomly infesting Blue Tit nests at the beginning of their breeding cycle with three densities of founder fleas. We investigated the relation between flea density and the proportion of early-dispersing offspring, the timing of the offspring host-search, and their body size. Since it could be argued that body size alone may not be a reliable estimate of phenotypic quality in this organism, we also investigated the relationship between female body size and potential fecundity. Finally, in an experiment conducted in the laboratory, we directly tested the effects of competition for food on larval development, adult size at maturity, and adult overwintering capacity.

Material and Methods

Experiment 1 of this study was conducted in 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 nestboxes and natural cavities. Previous researchers working on this population on other topics reported high natural infestation rates by *Ceratophyllus gallinae* (H. Zandt, *personal communication*). Between 1990 and 1994, prior to experiment 1, both the bird and the flea population were left unmanipulated. Experiment 2 of this study was conducted in 1998 in the laboratory at the Ethological Station of the Zoology Department of the University of Bern, Switzerland using flea material collected from the study area described above.

Experiment 1

In January 1994 we replaced the old nest boxes in the study area with new ones (model ''Varia'' built by the Swiss Ornithological Institute, Sempach, Switzerland). Any cracks and holes visible in the inside of the new boxes were sealed with glue so that the fleas present in the nests during the experiment could only hide within the material of the nest itself. The nests from old nest boxes that contained fleas were stored in plastic bags for later use. At the beginning of the Blue Tits' breeding period we visited the nest boxes daily and recorded the onset of laying, number of eggs laid, start of incubation, and the first day of hatching (referred as day 0 of the nestling period). Variation in nest mass is known to influence flea development (Eeva et al. 1994,

discussed in Heeb et al. 1996). Therefore, we standardized nest size during the bird laying period to an average height of 8 ± 1 cm, the mean nest size in 1994, by adding or removing material from the column of moss present under the nest cup.

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 there were sprayed with 4 mL of water after the heat treatment. The nests were infested with 6, 20, or 50 adult fleas on the second day of incubation. In order to avoid biases due to seasonal effects, we sequentially assigned the nests to the treatment groups as we detected the start of their occupant's egg-laying period. We opted for the following randomization procedure, which ensured that the mean flea sex ratio did not differ among treatment groups and, in case of genetic polymorphism, that a mixture of genotypes was represented. Nests were collected in January within the study area and stored in plastic bags. Before picking fleas for infestation, three to five 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 fleas were collected from the nest material. A large number of fleas were then collected in a small pile, and either 6, 20, or 50 fleas picked at random. The mean sex ratio at infestation was 0.41 male to 1 female.

At the end of the bird breeding period, after the young fledged, we sealed all cracks and holes visible on the outside of the nest boxes with sticky tape and set a flea trap on the front hole of the nest box. The flea traps were built following Bates (1962). Additional new nest boxes were installed in the forest to compensate for those closed by flea traps thus ensuring that birds had enough roosting and nesting sites for the next breeding season. We collected dispersing fleas on the 15th of each month from mid-June 1994 to mid-March 1995. At mid-March, Blue Tits in our study area had chosen their new nest sites among the nest boxes that were not closed by flea traps and they were building their nests. Throughout the paper, fleas that dispersed before the bird nest-site choice (equivalent here to mid-March) will be referred to as ''early dispersers'' as opposed to fleas that did not initiate dispersal before the bird's nest site choice referred to as ''fleas remaining in the nests.'' The nests were stored in plastic bags and sampled for adult fleas in summer 1995. The sampling procedure was the following: We first microwaved the nests in order to kill fleas that were still alive. We then thoroughly mixed the nest material above a metal mesh on top of a plastic dish until no more dust fell from it. The fine fraction of nest material was then mixed, spread on the plastic dish, and divided into eight sectors (two rows of four sectors). Since it was difficult to spread the material totally homogeneously, we counted adult flea bodies from the sector of the first row that appeared to contain the highest amount of dust and from the sector of the second row that contained the least amount of material. We took the mean of those two values and multiplied it by four to obtain the estimated number of fleas from the fine fraction of material. Next, the entire coarser fraction of the nest material was discarded and carefully inspected visually. The total number of offspring produced during the bird breeding period was calculated as the sum of fleas caught in the traps (early dispersers) and the fleas remaining in the nests counted from the nest material. In order not to count adult fleas present in the nest during the nestling period as offspring, we excluded from our counts adults dispersing within two weeks after fledging.

We calculated the per nest monthly sex ratios of early-dispersing fleas as male percentages based on all collected individuals. For late dispersers, we calculated the sex-ratio from a sample of 50 individuals per nest. We also measured body size as the length of the tibia of the right jumping leg using a measuring table (Leitz, Glattbrugg, Germany) fitted to a dissecting scope (Wild Heerbrugg, Bensheim, Switzerland). Tibia length was chosen as a measure of body size because it was previously found to highly correlate with dry mass ($n = 15$, $r = 0.695$, $P = 0.004$) and body length ($n = 15$, $r = 0.820$, $P < 0.001$) in newly hatched adults. It allows comparing the size of live and dead fleas regardless of their fat or water content. It is also highly repeatable ($n = 15$, $r = 0.858$, $P < 0.001$). The mean tibia length of the rear legs of each sex was calculated per nest per month on the basis of measurements made on 10 individuals picked at random. When more than 10 individuals of a given sex dispersed, the mean was based on the number of individuals available.

Original sample size was 49 nests. Three bird pairs deserted their nests before the end of incubation and one brood entirely failed just after hatching. 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 flea offspring produced could therefore only be recorded from 41 nests.

In the winter 1994–1995, we collected three nests from our study area, and extracted adult female fleas from cocoons in order to dissect their ovaries. We visually picked 20 females so as to cover a wide range of body sizes and limit the number of dissections. The ovaries from freshly killed females were extracted under a dissecting scope using micro-pins. They were then spread on a micro-slide, colored with methyl blue and covered with a cover slide. We counted the number of developed ovarioles under the microscope (magnification 100×). Under higher magnification (400–1000×), we also counted the number of oocytes present in the germarium and vitellarium of the biggest and the smallest ovarioles. This count was made twice per ovar-

iole so as to increase the precision of this rough estimation. From the counts of ovarioles and oocytes, we calculated a mean number of oocytes per ovariole.

Experiment 2

Nest material was collected at the end of May 1998 from Blue Tit nests in which nestlings had recently fledged. In order to separate the larvae from the nest material, we mixed and shook the material above a metal grid. The resulting mixture of dust and larvae was spread on the most elevated half of an inclined plastic tray. The larvae crawled down to the lower part of the tray while the dust remained in place. We then separated visually first and small second instar larvae from older ones (see Cotton 1970) using fine flexible entomological tweezers.

Fifty larvae were picked at random and put in a 12 × 4 cm (diameter by height) petri dish with 6 g of VERMEX granules as substrate (granulometry 0.3–0.8 mm, Vermica AG, Boezen, Switzerland). The dishes were then assigned to a group with 1 g of food, referred as high food treatment and food ad libitum, or to a group with 0.1 g of food referred to as low food. The food was a mixture of dry yeast, dry beef blood, and pulverized dry dog chow in the respective weight proportions 8%, 12% and 80% (Silverman and Rust 1983). Each treatment had 20 replicates. To insure equal climatic conditions in the petri dishes, we cut out a piece of the lid and replaced it with nylon mesh. To provide sufficient humidity to the food and the substrate, we sprayed 3mL of water in each petri dish before the start of the experiment. All 40 petri dishes were then installed in a climate chamber at 24°C, 78% relative humidity, and a 12-h photoperiod. The dishes were positioned so as to alternate the low and high food treatments and to avoid biases linked to their position in the climate chamber.

From the start of the experiment, we controlled each box every second day in order to detect fourth instar larvae also called white larvae (see Cotton 1970). Fourth instar larvae were taken out, weighed, and placed singly in an individually marked plastic tube with a perforated lid allowing air exchanges. When we had missed the white larva stage and the larvae had already spun cocoons, we proceeded in a similar way but with the cocoon. The majority of white larvae spun cocoons within a week. When all larvae reached the cocoon stage, we calculated the mean larval survival per box and the developmental time of larvae from the beginning of the experiment till the stage white larvae or freshly spun cocoon. Every white larvae or cocoon from the two food-level groups was then sequentially assigned to two subgroups and placed in a climatic box at 15°C and 30–40% relative humidity with a photoperiod of 12 h.

In the first experimental subgroup we trigger hatched the fleas 2 mo later. Fleas were activated by shaking the tube in which they were kept or, when this was not sufficient to induce hatching, by gently rupturing their cocoon with fine tweezers. The empty cocoons were removed from the tubes so as to prevent fleas from using them as shelter. The tubes were then placed in a second climatic box at 25°C, 30–40% relative humidity, and 12-h photoperiod. All these tubes were checked every second day, and the number of dead fleas recorded. Body size was measured as the length of the tibia of the right jumping leg as previously described. This procedure was continued until the last flea died. In the second subgroup, fleas were trigger hatched 4 mo later than in the first group. They were then set in the same climatic chamber that had been used for the first group also at 25°C and 30–40% relative humidity. All tubes were checked every second day, and the number of dead fleas recorded.

Statistical analyses were performed using the Systat Statistical Package (Wilkinson et al. 1992) and JMP software (Sall and Lehman 1996). Data were checked for normality and heterogeneity of variances. Values are reported ± standard errors throughout the text.

RESULTS

Experiment 1

Effects of founder density on offspring number, dispersal, and sex-ratios.—The number of offspring produced per founder flea per nest (log-transformed) significantly decreased with increasing founder density (ANOVA: $F_{2,38} = 30.9$, $P < 0.001$). Because of this density-dependent population growth, the final number of offspring per nest was almost equal across the two experimental groups infested with 20 and 50 founder fleas, but fewer fleas were produced in nests originally infested with 6 founders (ANOVA: $F_{2,38} = 4.02$, $P = 0.026$). Detailed analyses of density-dependent processes and time–temperature effects affecting flea demography within the same nests are presented elsewhere (Tripet and Richner 1999*a*, *b*). Founder density had no significant effects on the variables examined in this study and we therefore pooled the data set for further analysis. There were no significant relationships between the mean, standard deviation, kurtosis, and skewness of the size distributions of offspring and the number of offspring produced per nest when all measured offspring were considered (Table 1). Female offspring were significantly larger (tibia length: 0.42 ± 0.002 mm) than males (0.38 ± 0.002 mm) (Table 1). On average 16.7 ± 1.7% of the offspring dispersed before mid-March. This percentage significantly decreased with increasing number of offspring present in the nest at the end of the breeding period ($r = 0.390$, $n = 41$, $P = 0.012$) (Fig. 1). The mean sex ratio (percentage of males) was 49.9 ± 1.1% for early-dispersing fleas and 48.5 ± 1.3% for fleas remaining in the nests (paired *t* test: $T = 4.7$, df = 40, $P < 0.001$). Offspring density had no significant effects on the per-nest sex ratio of early dispersers ($r = 0.084$, $n = 41$, $P = 0.601$)

TABLE 1. Summary of *F* values from a general linear model with repeated measures for effects of sex and offspring number (continuous variable) on the mean, standard deviation, kurtosis, and skewness of per nest flea offspring size distributions.

Source	*df*	Mean	SD	Kurtosis	Skewness
Offspring no.	1, 39	0.20	1.17	0.54	0.08
Sex	1, 39	146.42***	6.77*	0.30	1.32
Sex × Offspring no.	1, 39	1.97	1.25	0.09	0.79

* $P < 0.05$; *** $P < 0.001$.

or on the sex ratio of fleas remaining in the nests ($r = 0.032$, $n = 41$, $P = 0.842$).

Relationships between body size, timing of dispersal, and density.—The number of dispersing fleas varied significantly from one month to another (repeated-measures ANOVA: $F_{9, 353} = 43.13$, $P < 0.001$) and overall the mean number of dispersers increased with the season ($r = 0.632$, $n = 10$, $P = 0.050$) (Fig. 2). Females and males dispersing from June to September were significantly bimodally distributed with regard to their body size as revealed by cluster analysis (Fig. 3a, b and Table 2). Note that the distributions in Fig. 3 do not reflect the total frequencies of offspring sizes because a maximum of 10 individuals of each sex was measured per nest per month (see *Methods*). There was a significant linear relationship between body size and the timing of dispersal in females ($r = 0.506$, $n = 3054$, $P < 0.001$) and in males ($r = 0.447$, $n = 2748$, $P < 0.001$). The early dispersal of bigger morphs was in sharp contrast with the general pattern of dispersal and might have reflected a different biological process (see *Discussion*). Thus we conducted separate analyses, first on the data set excluding the cluster of large morphs dispersing early, and next on the cluster of large fleas itself. Removing the cluster of large and early-dispersing morphs considerably improved the fit of the linear relationship between body size and timing of dispersal in females ($r = 0.740$, $n = 2814$, $P < 0.001$) and males ($r = 0.757$, $n = 2476$, $P < 0.001$). The same correlation was found when considering the average of mean per-nest body sizes of dispersing adults. Females were significantly larger than males and the seasonal increase in body size of migrating fleas was significantly stronger in females than in males (repeated-measures ANCOVA: sex, $F_{1,9} = 15.3$, $P = 0.004$; month, $F_{1,9} = 39.0$, $P < 0.001$; sex × month, $F_{1,9} = 14.7$, $P = 0.004$) (Fig. 4). There was a significant negative relationship between the mean tibia length of early-dispersing females and males offspring and the density of offspring in the nests (repeated-measure ANCOVA: sex, $F_{1,39} = 235.9$, $P < 0.001$; offspring, $F_{1,39} = 11.9$, $P = 0.001$; sex × offspring: $F_{1,39} = 5.3$, $P = 0.026$) (Fig. 5).

In the case of early-dispersing large fleas, there was no significant seasonal effect on the body size of females or males within the 4-mo period during which they dispersed (repeated-measures ANCOVA: sex, $F_{1,2} = 58.2$, $P = 0.017$; month, $F_{1,2} = 0.4$, $P = 0.595$; sex × month, $F_{1,2} = 0.5$, $P = 0.552$). There was also no significant effect of offspring density on their body size (repeated-measures ANCOVA: sex, $F_{1,31} = 66.5$, $P < 0.001$; offspring, $F_{1,31} = 0.03$, $P = 0.864$; sex × offspring: $F_{1,31} = 5.5$, $P = 0.026$).

Offspring density also had no significant effect on the body size of females and males remaining in the nests at mid-March 1995 (repeated-measures ANCOVA: sex, $F_{1,39} = 76.2$, $P < 0.001$; offspring, $F_{1,39} = 0.6$, $P = 0.426$; sex × offspring, $F_{1,39} = 0.4$, $P = 0.523$).

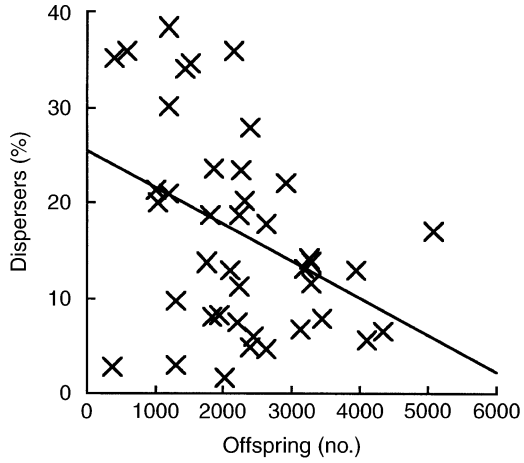

FIG. 1. Percentage of dispersing offspring (June 1994 to mid-March 1995) in relation to the number of offspring produced during the breeding period.

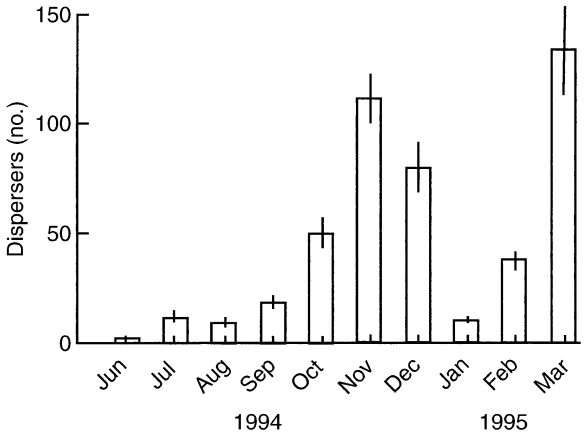

FIG. 2. Number of dispersing fleas collected monthly in the flea traps. Vertical bars are standard error of estimates.

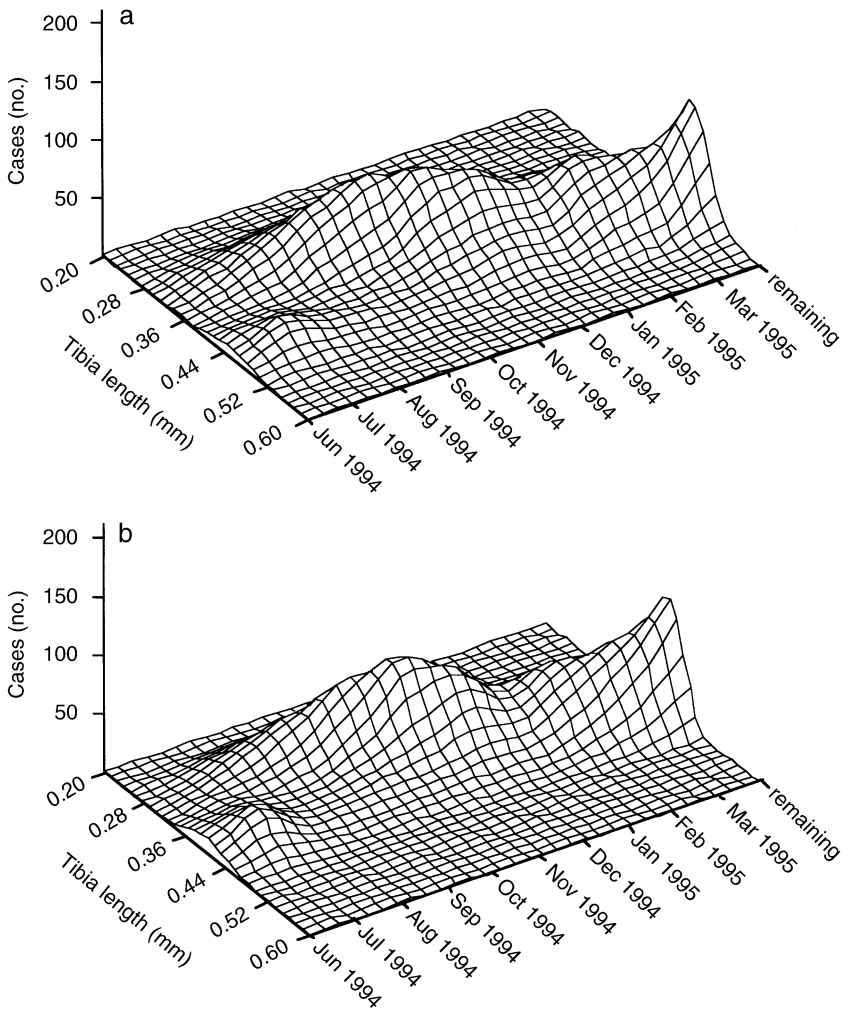

FIG. 3. Size (tibia length) distribution of the sampled fleas in relation to their timing of dispersal for (a) females (n = 3054) and (b) males (n = 2748).

Relationship between body size and potential fecundity.—There was a significant linear relationship between the body size of female offspring and the number of developed ovarioles ($r = 0.798$, $n = 20$, $P < 0.001$), but the number of oocytes per ovarioles did not correlate significantly with body size ($r = 0.062$, $n = 20$, $P = 0.290$) (Fig. 6a, b). The product of those two variables, or potential fecundity, was significantly linearly related to the body size of female offspring ($r = 0.656$, $n = 20$, $P < 0.001$) (Fig. 6c).

Experiment 2

Effect of food availability on larval and pupal development.—Food availability had a significant effect on the number of larvae that reached the white larva stage (or fresh cocoon stage). There were fewer white

TABLE 2. Summary statistics for the two modes of the bimodal flea size distributions of fleas dispersing between June and September.

Cluster	Mode	Min	Max	Mean	SD	Cases (no.)	df	F ratio
Females	1	0.41	0.54	0.46	0.03	240	1, 671	2563***
	2	0.22	0.40	0.34	0.03	433		
Males	1	0.37	0.50	0.42	0.03	272	1, 559	2470***
	2	0.22	0.36	0.31	0.03	289		

Notes: The distributions were partitioned by cluster analysis.
*** $P < 0.001$.

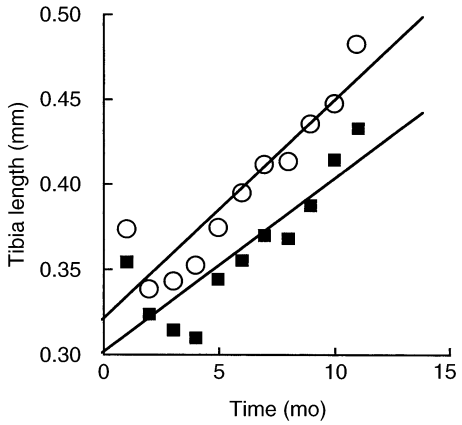

FIG. 4. Average size (tibia length) of female (circles) and male (squares) fleas in relation to their timing of dispersal.

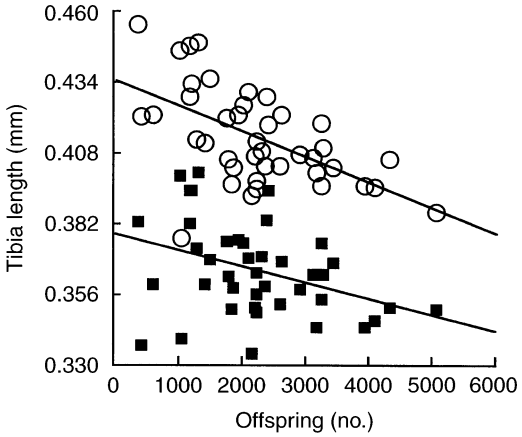

FIG. 5. Mean female (circles) and male (squares) dispersers' size in relation to the number of offspring produced during the breeding period.

larvae at low food level and they also had significantly lower body mass (Table 3). The amount of food had no significant effect on the number of days taken by the larvae to reach the white larvae stage and start spinning cocoons (Table 3). There was no statistical difference in mortality from the cocoon or white larvae stage to the imaginal stage between the two groups (Table 4). At high food availability, there tended to be more adults produced and the mean sex ratio of imagos was significantly lower than at low food level (Table 4). Food availability significantly affected the body size of adult fleas (Table 4). The same analysis performed on the data split by sex, showed that females were significantly larger (tibia length: high food 0.442 ± 0.002 mm, low food 0.400 ± 0.004 mm) than males (high food 0.397 ± 0.003 mm, low food 0.356 ± 0.004 mm), but food availability did not significantly affect the body size of one sex more than the other (repeated-measures ANOVA: food level, $F_{1,38} = 140.8$, $P < 0.001$; sex, $F_{1,38} = 107.3$, $P < 0.001$; interaction, $F_{1,38} = 0.1$, $P = 0.757$).

Effect of food availability on survival.—In the low food availability group 23.04 ± 2.70% of the fleas hatched and died before artificial activation of the cocoon (see *Methods*) and 20.46 ± 3.22% in the high food group (*t* test: $t = 0.38$, $n = 40$, $P = 0.544$). The mean survival of adult fleas artificially hatched from cocoons 2 and 6 mo after pupation was significantly affected by food level and by the amount of time spent in the cocoon (repeated-measures ANOVA: food level, $F_{1,38} = 113.0$, $P < 0.001$; hatching time, $F_{1,38} = 1057.3$, $P < 0.001$; interaction, $F_{1,38} = 17.9$, $P < 0.001$) (Fig. 7). Adult fleas activated 2 mo after pupation survived on average 23.6% longer in the high food availability group than in the low food group. Those activated after 6 mo lasted 25.0% longer (Fig. 8). The same analysis conducted per sex (group artificially hatched after 2 mo only), showed that females survived significantly longer (high food 34.3 ± 1.3 d, low food 23.4 ± 1.0 d) than males (high food 30.5 ± 1.3 d, low food 21.5 ± 0.7 d) but food availability did not significantly affect the survival of one sex more than the other (repeated-measures ANOVA: food level, $F_{1,38} = 96.0$, $P < 0.001$; sex, $F_{1,38} = 8.7$, $P = 0.005$; interaction, $F_{1,38} = 0.1$, $P = 0.334$).

DISCUSSION

General patterns of dispersal

We found that an average 16.7% of offspring dispersed before mid-March thus confirming Bates' (1962) and Du Feu's (1987) observations that a significant proportion of new generation offspring disperse before the start of the next bird nesting period in the fall and winter. In fact we found fleas dispersing as early as June, July, and August. Dispersal overall increased with season but it seems that the low temperatures in December, January, and February negatively affected flea propensity to disperse. The 82.3%

TABLE 3. The mean number and mass of larvae that reached the white larva stage (or fresh-cocoon stage) at high and low food availability, and the mean duration of their development from the start of the experiment until they spun cocoons.

Variable	Low food	High food	*t*	*P*
Larvae (no.)	36.05 ± 1.20	39.65 ± 0.97	2.33	0.025
Larvae weight (mg)	57.44 ± 1.17	67.84 ± 0.70	7.68	<0.001
Development (d)	7.56 ± 0.24	8.02 ± 0.22	1.39	0.171

Notes: Values are presented ± SE, and sample size was 20 in each group.

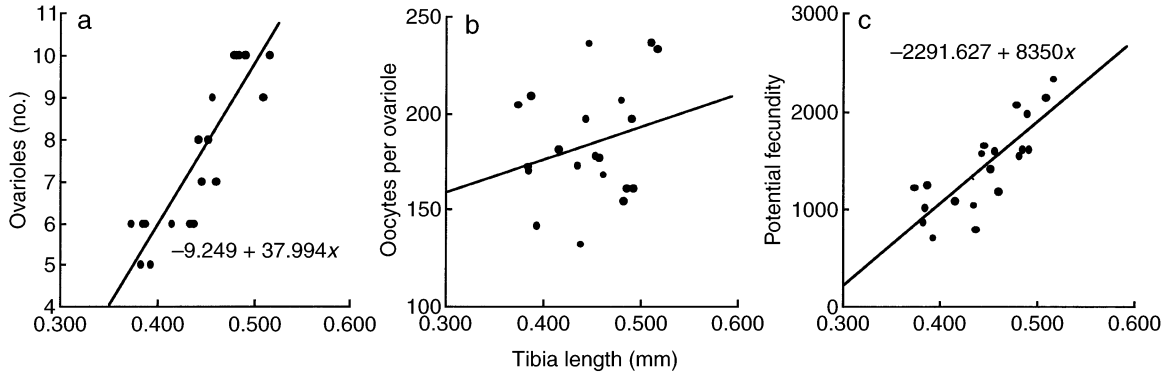

FIG. 6. Relationship between size at maturity (tibia length) and measures of fecundity in female fleas: (a) number of developed ovarioles in the ovaries; (b) mean number of oocytes per ovariole; and (c) potential fecundity.

of imagos that remained undisturbed inside our nest boxes seem to have followed a ''sit-and-wait'' strategy, which consists of remaining protected from desiccation in the cocoons until stimulated by an increase in temperatures and the vibrations associated with the presence of a potential host (Humphries 1967*a*). Since flea traps prevented birds from roosting in and inspecting nest boxes, it is likely that in a natural situation a higher proportion of fleas would have been stimulated to hatch and initiate their host search by mid-March.

Contrary to our predictions, we did not find a significant positive relationship between the density of offspring in the nests and the proportion of offspring dispersing from it. We found a significant negative relationship instead. This result clearly rules out the possibility of a density-dependent phenotypic plastic response to crowding selected through host nest-site choice. It also suggests that some nests offer better conditions both for the developmental stages during the reproductive period and for the survival of preemerged imagos overwintering inside the cocoons. Humidity level is known to affect both the development of eggs and larvae (Baccot 1914, Rothschild and Clay 1952, Heeb et al. 2000), and the survival of overwintering adults (Humphries 1967*a*, Silverman and Rust 1983). Variation in humidity within the nests depends on abiotic and biotic factors inside nest boxes (Heeb et al. 2000), and on properties of the microhabitat surrounding nest boxes. Variations in these factors through their effect on humidity in the nest could result in the observed correlation between flea reproductive rates and flea overwintering capacity.

Dispersal in time as a consequence of larval competition

Competition at the larval stage had costs in terms of offspring size at maturity and the offspring's ability to overwinter but it affected only a small fraction of larvae, here the 16.7% of early dispersers in our field experiment. The explanation to this pattern may lie in characteristics of their host breeding cycle that induce changes in the resources available to flea larvae. The larvae's two main food sources are proteins, from hair, feathers, and epidermal scales brought in and produced by the host, and blood feces produced by adult fleas after feeding on the host (Rothschild and Clay 1952). The analysis of density-dependent processes taking place during the bird incubation period suggest that first generation flea larvae compete for the fixed amount of proteinic material available from the nest cup in addition to the supply of blood feces provided by the adults. This could explain the strong density-dependent larval production at that stage (Tripet and Richner 1999*b*). During the nestling period, on the other hand, larvae rely mainly on blood feces for their subsistence and the number of offspring produced was found to be proportional to the number of adult fleas (Tripet and Richner 1999*b*). In the absence of density-dependent competition, the size of offspring is not likely to be affected. However, this situation is expected to change

TABLE 4. The mean number and size of imagos produced, as well as the sex ratio and mortality rate during the cocoon stage (pupal and imaginal molts) in groups of larvae that were raised at high and low food availability.

Variable	Low food	High food	*t*	*P*
Imagos (no.)	27.10 ± 1.53	30.85 ± 1.45	1.78	0.084
Tibia length (mm)	0.380 ± 0.032	0.426 ± 0.020	12.2	<0.001
Sex ratio (% male)	44.80 ± 3.59	35.03 ± 2.81	2.14	0.039
Mortality cocoon stage (%)	17.90 ± 1.64	17.60 ± 2.71	0.095	0.925

Notes: Values are presented ± SE, and sample size was 20 in each group.

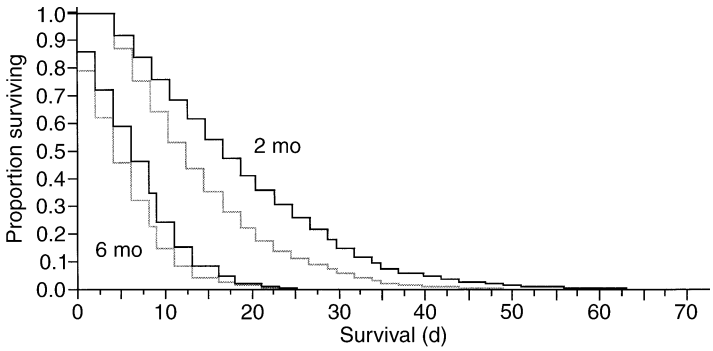

FIG. 7. Survival curves of adults fleas artificially hatched two and six months after they spun cocoons. The larval stages were either raised with food ad libitum (black lines) or with little food available (gray lines).

once the nestlings fledge and adult fleas disperse. Without the blood feces supplied by adult fleas, flea larvae that are not fully grown are likely to compete with older larvae for the little proteinic material remaining in the nests in order to complete their development. This fraction of larvae may therefore exhibit a density-dependent survival and development to the pre-pupal stage. Thus the negative correlations between the number and size of early-dispersing individuals, and offspring density is understandable if we assume that early-dispersing offspring finished their larval development under the highly competitive conditions that follow host departure.

Our second, laboratory experiment confirms the causal relationship between resource availability, size at maturity, and overwintering capacity. Poorly fed individuals stored less metabolites as shown by their reduced survival following trigger hatching. They also used up their energy reserves at a faster rate as shown by the significant interactive effects of food availability at the larval stage and timing of artificial hatching on survival. The simplest rationale behind this phenomenon is that small individuals are less resistant to desiccation because of their higher surface-to-volume ratio resulting in lower fat and water storage capacity (e.g., Ribeiro 1996). As another consequence of that allometry, larvae of lower pre-pupal mass might lack enough metabolite reserves to spin a cocoon of sufficient quality to protect the imago from desiccation (Silverman and Rust 1985). Flea offspring should initiate their host search before their diminishing water or energetic reserves compromise their survival within the cocoon or their capacity to break the cocoon wall (Humphries 1967*a*, Silverman and Rust 1985). In fact, hatching should be triggered at a level of metabolic reserves sufficient to allow for the complete host-finding process. Environmental conditions such as temperature and humidity are probably the most important determinants of the rate at which overwintering fleas will use up their reserve of metabolites inside the cocoons. Thus, environmental factors interact with the phenotypic quality of fleas to determine their optimal dispersal time.

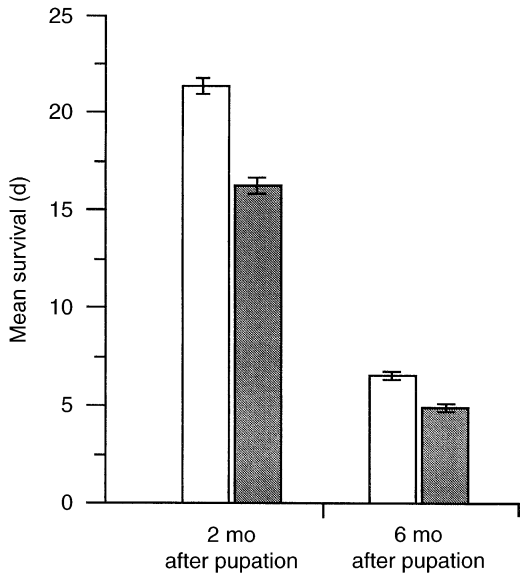

FIG. 8. Mean duration (expressed in days ± 1 SE) of survival of adults fleas artificially hatched two and six months after they spun cocoons. The larval stages were either raised with food ad libitum (white bars) or with little food available (gray bars).

Given their small body size, it might be argued that smaller phenotypes might be better adapted to feed on temporary hosts outside the nesting season than larger individuals. If such is the case, body size might not be a reliable estimate of phenotypic quality. The strong decrease in potential fecundity associated with smaller female body size suggests however that these individuals are individuals of lower phenotypic quality with poor survival prospect and potential fecundity. Thus it may well be that individuals of sub optimal sizes follow a ''best-of-a-bad job strategy'' (e.g., Roff 1992). A positive relationship between body size and fecundity is common to many insect species and has been reported in all major insect orders (reviewed in Kindlmann et al. 1992, Honèk 1993) but, to our knowledge, not yet in the order Siphonaptera. Important counter-selection pressures, such as those imposed by host preening, the constraints imposed by host body size (Kirk 1991), and possibly feather structure (Humphries 1967*b*), as well as selection for short flea generation

time, are expected to select against a further increase in body size in *C. gallinae*.

Sex-dependent constraints on development and dispersal

We found that the sex ratio of individuals dispersing before March was more biased toward females than the sex ratio of nondispersers. Female predominance in secondary sex ratios is not uncommon in Siphonaptera and is usually attributed to males being less resistant to adverse conditions (Marshall 1981*b*). Assuming a balanced sex ratio at the beginning of our laboratory experiment, the results suggest that males survive less well than females at high food level but survive proportionally better with little food. Female offspring are larger than males in this species (Traub et al. 1983) and might suffer more from harsh competition at the larval stages because they need to achieve a higher prepupal mass before metamorphosis. If proportionally more females fail to achieve an optimal body size, this could result (as observed in our field experiment) in a stronger female bias in the sex ratio of early-dispersing individuals.

Early dispersal of large morphs

We observed two types of large fleas dispersing in the summer. The first dispersers were adults from the first generation of fleas that developed during the bird incubation period and gave rise to the second generation offspring whose dispersal pattern is investigated in this study (for details on the flea life cycle see Tripet and Richner 1999*a*). These first generation fleas dispersed either on the fledglings themselves or crawled out of the nest boxes shortly after fledging (F. Tripet, *personal observation*). They were deliberately excluded from our study of the dispersal of second generation offspring (see *Methods*). By dispersing immediately after the fledging of their hosts' young they may be able to reach the nests of bird species whose breeding periods extend into summer. The same rationale may apply to the large second generation offspring dispersing from our nest boxes in June and July. By August and September, however, these fleas are very unlikely to find any bird species breeding at all. It may be that fleas use birds for phoresis in order to reach new patches of forest. Very little is known of the behavior of fleas outside the nest boxes and on their survival on birds outside the breeding season. The size of large early-dispersing fleas did not significantly depend on the density of offspring in their natal nest. Thus, as opposed to the majority of dispersers, their dispersal seemed to be independent of phenotypic quality and it could be influenced by genetic factors. Genetic polymorphism could occur through frequency-dependent selection for two morphs of fleas differing in the timing of their dispersal (Ridley 1996, Futuyma 1998). We would rather favor the explanation that genetic variation in the threshold level of vibrations required for triggering emergence from the cocoon led to the wrong timing of dispersal of these few large individuals. Erroneous emergence might have been enhanced by the use of hanging nest boxes rather than the fixed ones previously used in the area. It might also have been enhanced by the vibrations associated with our inspections of the flea traps.

Conclusions and perspectives

Although our results suggest that early-dispersing individuals are less fit than fleas remaining in the nests, the question will only be settled by an experiment yielding data on the transmission rates and reproductive rates of fleas of different sizes. Further studies should also aim at understanding how fleas of high phenotypic quality optimize their host search. These fleas, when remaining in unused nests, eventually emerge and climb up in the trees (Humphries 1968; F. Tripet, *personal observation*), probably in order to reach the foraging patches of their main hosts. Preliminary data even suggest that they might match their location in the trees to that of caterpillars, the main food source of Blue and Great Tits (H. Richner, *personal communication*). When reaching a nest, these late immigrants are at a disadvantage compared to fleas already present in the nests because their larvae will compete with older ones, and might not be able to complete two generations within the nesting period of the host (Tripet and Richner 1999*a*). Again, nothing is known of their transmission rate thus making it difficult to access their reproductive rate. The proportion of unused nest, itself, is dependent on the size of the bird population. Thus it would be interesting to compare the dispersal of fleas in forest habitats that differ in the proportion of nest sites available per host. Further study is also needed to assess how the host population fluctuates in relation to changes in the flea population. Titmice avoid infested nest sites (Oppliger et al. 1994) and their reproductive success and/or survival decrease in the presence of fleas (Richner et al. 1993, Tripet and Richner 1997*b*, Richner and Tripet 1999). It has also been shown that there is a genetic component to the immunocompetence of infested tits (Brinkhof et al. 1999). Hence, fluctuations in the flea population size potentially affect the size of bird populations, the genetic structure of these populations within habitat, and the dispersal of hosts between habitats.

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