AN EXPERIMENTAL STUDY ON THE INFLUENCE OF TICK INFESTATIONS ON NESTLING PERFORMANCE IN GREAT TITS (*PARUS MAJOR*)

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Abstract.—Parasite defense is costly, and the detrimental effects of parasites, which can be measured in terms of parasite virulence, are thought to be influenced by the resources available to the host and, ultimately, by environmental conditions. Hence, if conditions are good, hosts can tolerate a certain number of parasites without suffering severe effects. In addition, the presence of other parasites can influence the virulence of a focal parasite either positively or negatively. We tested whether an experimental tick infestation reduced nestling performance in Great Tits (*Parus major*) and whether the effect was altered by a maternal response induced by Hen Fleas (*Ceratophyllus gallinae*) that is known to protect nestlings against flea infestations (i.e., we tested whether one parasite can alter the virulence of another parasite across host generations). We induced the maternal effect by experimentally infesting half the birds' nests with fleas during egg laying. After hatching, nestlings were cross-fostered into broods that then contained both nestlings with and without the maternal effect. Half of these broods were infested with five tick larvae per nestling. This resulted in tick infestation levels similar to levels found in natural populations. The tick infestation did not affect nestling mass, tarsus length, or time until fledging. Thus, an effect of the flea-induced maternal effect on tick virulence was not detectable. From these results, we concluded that either tick larvae do not affect nestlings, or nestlings or their parents can compensate for the negative consequences of tick infestations in numbers similar to those that occur in nature. *Received 18 October 2007, accepted 11 April 2008.*

Key words: *Ceratophyllus gallinae*, cross-species resistance, Great Tit, *Ixodes ricinus*, maternal effect, *Parus major*.

Étude expérimentale de l'influence des infestations de tiques sur la performance des oisillons de *Parus major*

Résumé.—La défense contre les parasites est coûteuse et les effets néfastes des parasites, qui peuvent être mesurés en termes de virulence des parasites, seraient influencés par les ressources disponibles pour l'hôte et, en définitive, par les conditions environnementales. Ainsi, si les conditions sont bonnes, les hôtes peuvent tolérer un certain nombre de parasites sans subir de graves séquelles. De plus, la présence d'autres parasites peut influer de façon positive ou négative sur la virulence parasite d'intérêt. Nous avons testé si une infestation expérimentale de tiques réduit la performance des oisillons de *Parus major* et si les séquelles sont modifiées par un effet maternel induit par *Ceratophyllus gallinae* qui protège les oisillons contre les infestations de puces (i.e., nous avons testé si un parasite peut modifier la virulence d'un autre parasite chez les différentes générations de l'hôte). Nous avons induit l'effet maternel en infestant expérimentalement la moitié des nids avec des puces au cours de la ponte. Après l'éclosion, les oisillons étaient intervertis dans des couvées qui contenaient des oisillons avec et sans effet maternel. La moitié de ces couvées étaient infestées de cinq larves de tiques par oisillon. Ceci a résulté en des niveaux d'infestation de tiques similaires aux niveaux observés dans des populations naturelles. L'infestation de tiques n'a pas affecté la masse des oisillons, la longueur du tarse ou l'âge à l'envol. Par conséquent, l'effet du effet maternel induit par les puces sur la virulence des tiques n'était pas détectable. À partir de ces résultats, nous avons conclu que soit les larves de tiques n'affectent pas les oisillons, soit les oisillons ou leurs parents peuvent compenser pour les conséquences négatives des infestations de tiques en nombres semblables à ceux observés en nature.

By definition, parasites negatively affect their hosts. The effects on a host's life history can include weakened body condition, decreased survival, and reduced reproductive success (Richner et al. 1993, Fitze et al. 2004). However, hosts have evolved defense strategies such as immune responses (Zuk and Stoehr 2002) and grooming (Mooring et al. 2004) to counter parasite infestation, and these mechanisms may reduce the parasites' influence on the host. Yet, because antiparasite responses are costly (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Bonneaud et al. 2003, Brommer 2004), the damage imposed by a parasite on its

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host (i.e., "parasite virulence") is expected to depend on the resources available to each individual and, therefore, also on environmental conditions, such as food abundance (Simon et al. 2004), climatic conditions (Dufva and Allander 1996), or time in the season (de Lope et al. 1993). Also, environmental conditions such as temperature are known to affect the life history of parasites (e.g., Krasnov et al. 2003) and may, therefore, affect parasite virulence. The presence of two or more cofeeding parasites could modulate the virulence of a focal parasite either positively or negatively. On one hand, parasite feeding can activate components of the host's nonspecific immune system (Wikel 1996), which can, in turn, protect the host against another parasite (Huang and Matsumoto 2000). On the other hand, parasites are also known to actively suppress hosts' immune systems (Wikel 1996) and may, therefore, facilitate cofeeding by other parasites. Parasites do not have to be present on the host at the same time to affect each other, because parasite-induced changes in a host's physiology can be long-lasting and may even be passed on across host generations via maternal effects (Mousseau and Fox 1998, Grindstaff et al. 2003). Parasites have long been known to induce maternal effects, mediated by the transfer of maternal antibodies (Gasparini et al. 2001, Buechler et al. 2002, Grindstaff et al. 2003) or hormones (Tschirren et al. 2004) that reduce parasite virulence for the offspring (Heeb et al. 1998). In addition, a parasite infestation can shape parental behavior and the investment in reproduction, which can also be expected to change the quality of offspring as hosts for other parasites. For example, an infestation in nestlings leads to increased parental feeding effort, which, in turn, reduces the parasite-induced damage in the offspring (Christe et al. 1996a, b; Tripet and Richner 1997a). In contrast to this, injection of an antigen used to simulate a parasite infestation in the parents, but not the offspring, reduced parental effort (e.g., feeding rates) and resulted in decreased nestling performance (Råberg et al. 2000, Bonneaud et al. 2003, Hanssen et al. 2004).

In a recent study of Great Tits (*Parus major*), Gallizzi et al. (2008a) found that prehatching maternal effects (i.e., maternal products transferred to the offspring) may induce cross-species resistance in offspring: a maternal effect induced by Hen Fleas (*Ceratophyllus gallinae*) reduced feeding times of European Castor Bean Ticks (*Ixodes ricinus*) on nestlings. However, it remains unclear whether reduction of tick feeding times can, in turn, reduce the damage inflicted on nestlings by ticks. Here, we present the results of an experiment conducted in the same year and on the same populations of Great Tits, fleas, and ticks, testing whether an ectoparasite-induced maternal effect has the potential to change the virulence of another ectoparasite. Hen Flea infestations of Great Tits during egg laying induce a well-studied maternal effect that reduces the influence of fleas on nestlings (Heeb et al. 1998); this effect has been proposed to originate from an increased amount of antibodies (Buechler et al. 2002) and a decreased amount of androgens (Tschirren et al. 2004) transferred to the nestling via the egg by flea-infested mothers. However, only a few studies exist on the intensity of tick infestation in Great Tit nests (Roulin et al. 2003) and on the effect of ticks on passerine nestlings (Szép and Møller 1999, Proctor and Owens 2000, Szép and Møller 2000). Because studies on the effects of ticks on Great Tit nestlings are scarce, it is important to establish whether and how nestlings are affected by ticks before addressing whether a Hen Flea-induced maternal effect can reduce tick virulence.

To our knowledge, all previous studies have investigated the influence of natural, unmanipulated tick loads on nestling performance, and not that of experimentally induced tick infestations. That approach has two major problems. First, it is possible that the ticks' natural infestation rates are nonrandom with respect to parent or nestling phenotype, which would lead to a bias in the infested group. Second, a significant proportion of ticks and birds in natural environments are infested with microparasites such as *Borrelia burgdorferi sensu lato* (Gasparini et al. 2001, Comstedt et al. 2006, Poupon et al. 2006). Therefore, it is not possible to distinguish the effects of the ectoparasite from those of the microparasite transmitted by the ticks. In our research, we avoid both these problems by experimentally infesting nests with a fixed number of laboratory-raised ticks that are not infected with any microparasites. In addition, the experimental induction of the flea-induced maternal effect and a cross-fostering design allowed us to investigate whether an experimental tick infestation in Great Tit nestlings influenced nestling development and whether this effect can be altered by the presence of another parasite during the egg-laying phase of the parents via a flea-induced maternal effect.

METHODS

We first surveyed the natural level of tick infestation in our population. We then infested a proportion of the Great Tit nests, during egg laying, with Hen Fleas to induce the maternal effect. Before nestlings hatched, all fleas were removed from the nests. Nestlings were then cross-fostered to produce broods that contained, at the same time, nestlings with and nestlings without the flea-induced maternal effect. To avoid the confounding effects of the flea infestation on posthatching parental behavior (Christe et al. 1996a, Råberg et al. 2000, Bonneaud et al. 2003, Gallizzi et al. 2008b), we then eliminated all nests with infested rearing parents from the experiments. Half of the experimental nests were later infested with five tick larvae per nestling. These intensities are similar to those found in the natural population and are, therefore, expected to show biologically relevant effects in hosts.

Estimation of the natural tick load per nestling.—Tick load was monitored on Great Tit nestlings in the breeding seasons (May until beginning of June) of 2005 and 2006. In 2005, Great Tit populations in the Spilwald and the Forst, two forests near Bern, Switzerland, were monitored, whereas in 2006 we examined tick loads in an area of the Forst that was not used for the tick infestation experiment. We counted the ticks (larvae and nymphs) on each nestling in the nest at the age of 5 days in the Spilwald and at the age of 9 days in the Forst to estimate the percentage of nests with infested nestlings, the mean number of ticks attached to nestlings per infested nest, and the mean number of ticks on each of the infested nestlings.

Experiments.—The experiments were performed in spring 2006 in the Forst and the Spilwald. Before the start of nest building, we cleaned the nest boxes to remove parasites still present from the previous year. Old nests were collected and stored in a climatic chamber at 5°C to serve as a stock of adult fleas to be used later for experimental infestations.

Induction of the maternal effect.—Nest boxes were visited regularly to determine the beginning of nest construction, the onset of egg laying, the start of incubation, and the hatching date of the first nestling (defined as day 1). As soon as the moss used

Cross-fostering design

Infested with fleas during egg laying
Maternal Effect

After cross-fostering

Removed from the experiment

Parasite-free during egg laying
No Maternal Effect
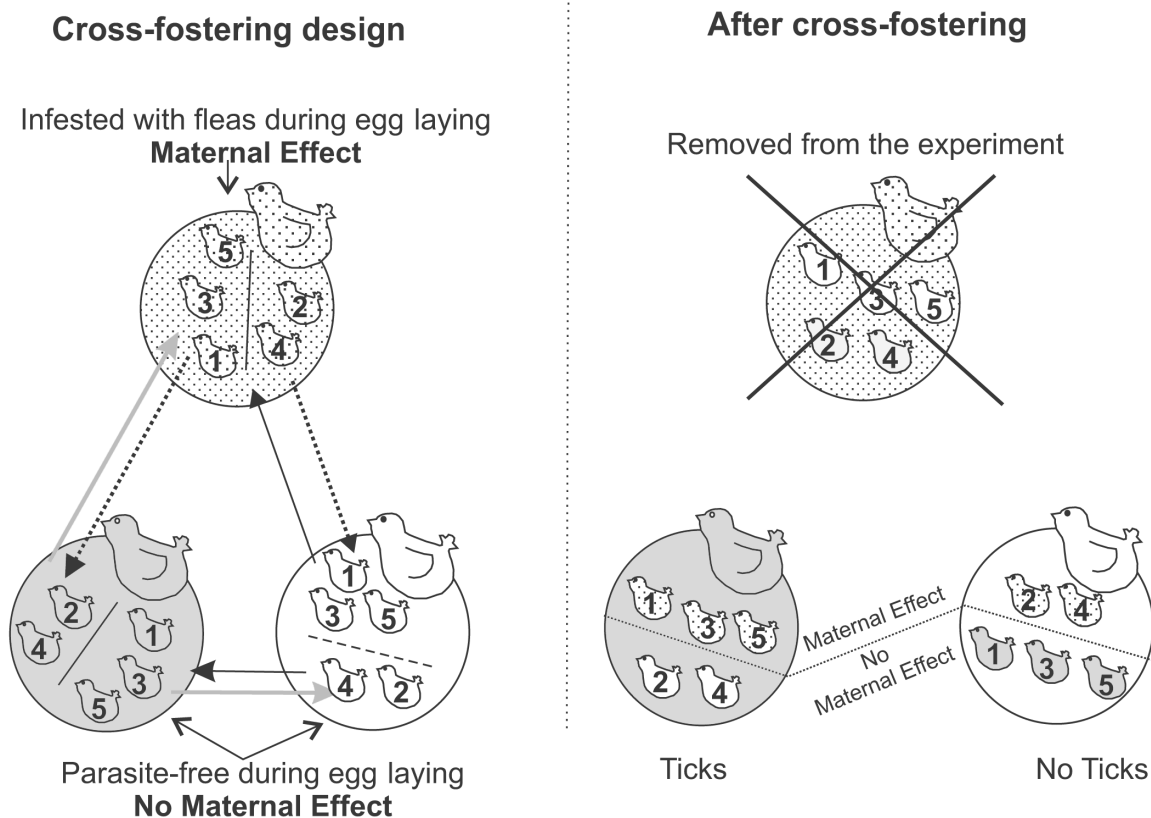
Ticks   No Ticks

FIG. 1. Cross-fostering design (left): one out of three nests was infested with fleas during egg laying (dotted) to induce a maternal effect, whereas parasites were removed from the other two nests. Before hatching, all fleas were removed from all nests. Three days after hatching, all nests were split in two halves containing either the odd or the even mass ranks (small numbers on nestlings), and each half was transported to a different acceptor nest (arrows, left figure). After cross-fostering (right), the nest that had been infested with fleas during egg laying (dotted) was removed from the experiment. The other two nests, where rearing parents had not been exposed to fleas, now contained nestlings originating from two different nests, one that had been infested with fleas during egg laying ("maternal effect") and one that was parasite-free during egg laying ("no maternal effect"). One of these two nests was infested with ticks when nestlings were 5 days old, whereas the other nest served as a tick-free control.

for nest construction covered the floor of the nest box, we heat-treated all nests with a microwave appliance (Richner et al. 1993) to remove all parasites from the nest. Nests were placed inside a plastic bag to prevent humidity loss and then microwaved for 3 min at 60% power. We used trial studies to confirm that this treatment effectively kills all fleas. After the treatment, the nests were sprayed with water to compensate for the water lost during the heat treatment. In a total of 28 sets of three nests, one nest in each set was randomly assigned to be infested with 40 fleas collected in the old nest material ("maternal effect" treatment), whereas the other two nests were kept free of parasites ("no maternal effect" treatment). This design allowed us later to impose cross-fostering among the three nests of each set (see below). The application of the flea-infestation treatment started on 4 April (i.e., after breeding territories had been established; Gosler 1993). Also, because nest construction had already begun when the treatment was applied, it is unlikely that birds changed the breeding site as a consequence of the flea infestation. Therefore, our treatment is not expected to bias the quality of the breeding pair. On the fourth day of incubation, all nests were heat-treated again to remove all

fleas from the nests. Therefore, one-third of the parents were infested with fleas during the egg-laying period and, thus, are expected to induce the maternal effect (Heeb et al. 1998, Buechler et al. 2002). Because flea immigration rates into the nests are very low (Heeb et al. 1996), all nestlings grew up in practically flea-free environments.

Cross-fostering.—When nestlings started to hatch, nests were visited daily, and newly hatched nestlings were weighed during each visit with an electronic portable scale to the nearest 0.01 g and individually marked by clipping some of their down feathers. These measures were used to determine the mass hierarchy within nests. Early in the morning three days after hatching (day 3), when all nestlings had hatched, nestlings were cross-fostered between the nests (Fig. 1). We used a split-brood cross-fostering design involving three nests, one "maternal effect" and two "no maternal effect" nests. The brood was split into two halves, one with the odd and one with the even ranks in the nestling size hierarchy, and the two halves were each transferred to a separate acceptor nest. All nestlings were moved, and brood size before and after cross-fostering was kept constant. After cross-fostering, each nest

TABLE 1. Natural tick infestation levels (tick larvae and nymphs) in the Spilwald and the Forst in 2005 and 2006, compared with infestation levels in experimentally infested nests in 2006 (tick larvae only). Values are means ± SD.

	Spilwald 2005	Forst 2005	Forst 2006	Experimental nests 2006
Nestling age	5 days	9 days	9 days	7 days
Total nests	124	149	85	32
Infested nests (%)	29.0	40.0	16.4	100.0
Infested nestlings per nest in infested nests (%)	22 ± 14	29 ± 20	24 ± 28	80 ± 18
Mean ticks per infested nestling	1.13 ± 0.57	1.16 ± 0.42	1.33 ± 0.65	3.81 ± 3.20
Total ticks per infested nest	1.84 ± 1.52	2.53 ± 2.79	2.50 ± 3.71	26.6 ± 11.9

contained nestlings of all size ranks that originated from two different donor nests (Fig. 1, right). To avoid an influence of flea infestation on the rearing parents during egg laying (Gallizzi et al. 2008b), we did not further consider the nest that had been infested with fleas during egg laying. Thus, all nestlings in the two remaining pairs of experimental nests ($n = 56$; i.e., 28 pairs) were raised by parents that had not been infested with fleas, and each nest contained, at the same time, nestlings with and without the flea-induced maternal effect.

Tick infestation.—In each pair of experimental nests, we infested one nest with *I. ricinus* larvae when nestlings were 5 days old (Fig. 1, right). The other nest in the pair was left tick-free and served as a control. Five larval ticks were applied on each nestling using forceps. Because nestlings touch each other inside the nest cup, ticks did not always stay on the nestling on which they had been applied, but moved around before attaching. Also, some ticks did not attach to nestlings at all; therefore, the mean number of ticks attached per nestling was <5 (Table 1). For the statistical analyses, all nestlings in the infested nests, including the rare ones on which no ticks had attached, were considered to belong to the "tick" group. For the infestation, we used ticks from a laboratory colony free of the most common tickborne pathogens, maintained at the Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland. The ticks were raised as described by Graf (1978). To ensure that the ticks were free of pathogens, they were regularly tested for *Borrelia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Babesia* spp., and *Theileria* spp. using reverse line blotting. Because only larvae and nymphs of *I. ricinus* are frequently found to infest birds (Papadopoulos et al. 2002) and because Great Tit nestlings are relatively small, we decided to use larval ticks for this experiment. When the ticks were applied, nestling mass did not differ between the treatment groups "maternal effect," "no maternal effect," "ticks," and "no ticks" (see below). Starting on day 7 after hatching, we checked nests daily and counted the number of ticks attached to each nestling until all ticks had detached. Control nests were also visited daily, and nestlings were handled similarly to the ones in the "ticks" nests. In the rare cases when we encountered a nestling with a tick in a control nest, the tick was removed with forceps to keep these nests tick-free. Also, the only nest in the population that was infested with *Protocalliphora* blow fly larvae was removed from the experiments to avoid interference by other parasites. Ticks fed for an average of three days before they dropped off. This estimate is conservative, because we considered a tick that was seen after three days but not after four days of feeding to have fed for only three days (Gallizzi et al. 2008a). For *I. ricinus* feeding on rodents, it was found that all ticks feeding for more than three days had fully engorged (Hughes and

Randolph 2001). Even though feeding times on birds may be different from those on rodents, it can be assumed that a considerable proportion of our ticks successfully finished their blood meal.

Nestlings were weighed with an electronic portable scale on days 5, 7, 9, and 14 after hatching. Additionally, on days 9 and 14 after hatching, we measured the length of the right tarsus with a caliper. On day 9 after hatching, nestlings were banded with small numbered aluminum rings (Swiss Ornithological Station, Sempach). On day 5, we took a small blood sample of ~5 µL for molecular sexing of the nestlings, using a previously described method (Griffiths et al. 1998). Because the amount of blood taken corresponds to ~0.001 of the nestlings' body mass and this method has been used for many years in our lab without complications, blood sampling was not expected to influence the outcome of our experiments.

Starting on day 18 of the nestling period, we visited nests daily to determine the date of fledging. Time until fledging was defined as the day in the nestling period when the last nestling had fledged.

Statistical procedure.—All analyses were performed in R (R Development Core Team 2007). Time until fledging was analyzed with a linear model (lm). For the analyses of nestling mass and tarsus length, we used general linear mixed-effect models with two random effects, nest of origin and nest of rearing. Nest of origin was partially crossed and partially nested within nest of rearing (Fig. 1; nestlings originating from the "maternal effect" nest were raised in two different experimental nests, whereas only half the nestlings of the "no maternal effect" nests were used for the experiment, and they were all raised in the same nest). The analyses were performed in LME4 (Bates and Sarkar 2006), which can handle complicated random-effect structures (Bates and DebRoy 2004). However, in such a complex situation, it is no longer possible to calculate exact F (and P) values, because the degrees of freedom cannot be estimated exactly (Hornik 2006). Hence, instead of using P values, we used Markov-chain Monte Carlo (MCMC) simulation-based, highest-posterior-density (HPD) 95% confidence intervals to estimate the significance of the fixed effects (i.e., the effect was considered significant if the confidence interval did not include zero). The chain length for MCMC sampling was fixed at 100,000. To make the confidence intervals easier to read, we estimated approximate P values by changing the confidence level of the confidence interval until the interval included zero (for significant terms) or no longer included zero (for nonsignificant terms). However, these values are not exact, and they have to be interpreted with care. We did not perform power analyses, because—as outlined by Hoenig and Heisey (2001)—confidence intervals contain more information about the parameter values

TABLE 2. Summary of the fixed effects influencing nestling mass at different ages. All models included "nest of origin" and "nest of rearing" as random effects. The variables not included in the table were not significant and, thus, were removed from the model. Markov-chain Monte Carlo (MCMC) confidence intervals (CI) and P values of the main effects were deduced from the model without the interaction term. P values were estimated by changing the confidence level of the CI until the interval did or did not include 0.

Response	Sample size	Variable	MCMC 95% CI	Approximate P
Mass, day 7	$n = 409$ (nestlings)	Mass, day 5	19.392 to 20.723	$P < 0.001$
	$n = 56$ (nest rearing)	Sex	−1.583 to −0.289	$0.03 < P < 0.04$
	$n = 73$ (nest origin)	Brood size	0.089 to 3.284	$0.004 < P < 0.005$
		Tick infestation	−2.479 to 0.397	$0.15 < P < 0.20$
		Maternal effect	−1.278 to 1.298	$P > 0.9$
		Tick infestation*maternal effect	−0.512 to 1.330	$0.35 < P < 0.40$
Mass, day 9	$n = 396$ (nestlings)	Mass, day 5	25.479 to 28.100	$P < 0.001$
	$n = 55$ (nest rearing)	Sex	−5.512 to −3.017	$P < 0.001$
	$n = 71$ (nest origin)	Tick infestation	−5.008 to 1.423	$0.25 < P < 0.30$
		Maternal effect	−3.454 to 2.173	$0.60 < P < 0.65$
		Tick infestation*maternal effect	−1.480 to 2.454	$0.60 < P < 0.65$
Mass, day 14	$n = 372$ (nestlings)	Mass, day 5	11.695 to 17.061	$P < 0.001$
	$n = 45$ (nest rearing)	Sex	−11.908 to −7.141	$P < 0.001$
	$n = 67$ (nest origin)	Hatching date	−18.869 to −7.451	$P < 0.001$
		Tick infestation	−5.744 to 11.274	$0.45 < P < 0.50$
		Maternal effect	−8.118 to 7.467	$0.95 < P < 0.90$
		Tick infestation*maternal effect	−3.368 to 6.543	$0.55 < P < 0.60$

that are supported by the data. Maximum-likelihood tests were used to test for the importance of the random effects (Pinheiro and Bates 2004). If one of the random effects did not help to explain the variance in the data ($P > 0.5$), it was eliminated, and the data were further analyzed with general linear mixed-effect models in NLME (Pinheiro et al. 2006). Initial fixed effects were maternal effect treatment, tick infestation, nestling sex, brood size at hatching, time of measurements, hatching date, and all interactions between maternal effect treatment, tick infestation, and nestling sex. In the analyses of nestling mass, we also added nestling mass on day 5 (i.e., day when ticks were applied). This covariate corrects for potential mass differences before the ticks were applied. Because parents had already fed the nestlings for a few days, nestling mass on day 5 depends on feeding rates and food quality and can also be viewed as a proxy for habitat, parental quality, or both. Hence, the analyses of nestling mass reflect the mass increase between the application of the ticks and the day of measurement.

Fixed effects were reduced with a backward stepwise procedure, and only significant terms were kept in the model. Comparison of the reduced models with the full models showed that the significant variables retained in the reduced model were also significant in the full model. The only exception to this rule was clutch size in the analyses of nestling mass at day 5 and day 9, which was only marginally significant in the full model. This indicates that the models were robust. Model assumptions were checked as proposed by Pinheiro and Bates (2004), and data were transformed to achieve the requirements by square transformation for nestling mass on days 5, 7, 9, and 14 and for tarsus length on day 9.

RESULTS

Natural compared with experimental tick infestation.—Natural tick infestation levels were relatively low in both forests and in both years (Table 1). Between 16% and 40% of all nests were infested with ticks, and in the infested nests only 22–29% of all nestlings

were infested. This resulted in total tick numbers per naturally infested nest between 1.8 and 2.5. In the experimentally infested nests, 3× more nestlings were infested, with ~3× more ticks per nestling (Table 1).

Effects of experimental tick infestation.—Nestling mass on day 5 after hatching (i.e., before the application of ticks) was smaller in larger broods (MCMC interval: −4.519 to −0.624; $0.009 < P < 0.010$) but was unaffected by the flea-induced maternal effect (MCMC interval: −2.567 to 0.654; $0.20 < P < 0.25$). On day 5, nestling mass was balanced between the two groups of the tick treatment (MCMC interval: −1.827 to 1.712).

Neither the maternal effect nor the tick infestation, nor their interaction, had an influence on increase of nestling mass between application of ticks on day 5 and day 7, day 5 and day 9, or day 5 and day 14 after hatching (Table 2). Mass increase in male nestlings was always larger than that in females, mass increase between days 5 and 7 after hatching was significantly larger for larger brood sizes, and nestling mass increase between days 5 and 14 after hatching was significantly lower toward the end of the season.

The random effect "nest of rearing" did not explain a significant proportion of the variation in tarsus length on day 9 after hatching (maximum likelihood test: $\chi^2 = 0.149$, df = 1, $P = 0.700$); therefore, tarsus length on day 9 after hatching was analyzed with a mixed model with only "nest of origin" as a random effect. Nestling tarsus length on day 9 after hatching was significantly smaller in females than in males ($F = 10.8$, df = 1 and 331, $P = 0.0011$), and it decreased during the breeding season ($F = 6.39$, df = 1 and 331, $P = 0.0119$). However, we did not detect an influence of tick infestation ($F = 0.247$, df = 1 and 331, $P = 0.619$), of the flea-induced maternal effect ($F = 1.86$, df = 1 and 71, $P = 0.176$), or of the interaction of the two ($F = 0.121$, df = 1 and 330, $P = 0.728$) on tarsus length on day 9 after hatching.

On day 14 after hatching, nestlings with the flea-induced maternal effect had a significantly shorter tarsus than controls (MCMC interval: −0.187 to −0.013; $0.02 < P < 0.03$; $n = 337$ [nestlings], $n = 50$ [nest rearing], $n = 65$ [nest origin]). Tick infestation, however,

did not have an effect on tarsus length (MCMC interval: −0.107 to 0.101; $P > 0.9$), and neither did the interaction of tick infestation with the maternal effect (MCMC interval: −0.019 to 0.118; $0.15 < P < 0.20$). Also, tarsus length was shorter for females than for males (MCMC interval: −0.266 to −0.162; $P < 0.001$) and shorter late in the season (MCMC interval: −0.106 to −0.018; $0.005 < P < 0.006$).

The time until fledging in the Forst was longer than that in the Spilwald ($F = 9.74$, df = 1 and 56, $P = 0.0029$), and there was a trend for an increased time until fledging in large broods ($F = 3.22$, df = 1 and 56, $P = 0.078$). Tick infestation of nests had no influence on duration of the nestling period (i.e., time until fledging; $F = 0.84$, df = 1 and 55, $P = 0.36$).

Discussion

Effect of tick infestation on nestlings.—To our knowledge, the present study is the first to investigate the effect of an experimental tick infestation on bird nestlings. The experimental infestation, within the natural range of tick intensities in our study population and other populations (see below), did not significantly affect nestling growth or the duration of the nestling period. Our experimental tick loads per nestling (Table 1) are comparable to levels found in other studies of noncolonial bird species. In a Great Tit population studied by Roulin et al. (2003), 63% of all nests contained ticks, with a mean load of 2.45 ticks nest⁻¹. In an Algerian population of Blue Tits (*Cyanistes caeruleus*), average tick loads per nest were 25.3, or 3.8 ticks nestling⁻¹ (Bouslama et al. 2001). The average tick load in four Eurasian Blackbird (*Turdus merula*) populations in France (nestlings and adults) was 7.1 ticks individual⁻¹ (Gregoire et al. 2002). Surveys of tick infestation on migratory birds found an average tick load per infested individual of 1.94–2.32 in Switzerland (Poupon et al. 2006) and 2.1–2.6 in Sweden (Comstedt et al. 2006). Only the last two studies differentiated between the different stages of ticks. They found 87% larvae, 13% nymphs, and zero adults (Poupon et al. 2006), and 54% larvae, 46% nymphs, and 0.6% adults (Comstedt et al. 2006). Hence, the experimental tick loads used in the present study are close to natural infestation levels in our study population and other passerine populations, and such infestation levels have been shown to impair nestling performance in other species (Szép and Møller 1999, Proctor and Owens 2000, Szép and Møller 2000, Hoodless et al. 2003).

The fact that the tick infestation in our experiment did not affect nestling performance does not necessarily imply that ticks do not, in general, have an effect on Great Tits. Under favorable conditions, for example, hosts can compensate for the detrimental effects of parasite infestations (Pacejka et al. 1998, Bouslama et al. 2001). Ticks in our study attached preferentially to the heaviest nestlings, and this preference was independent of the maternal effect treatment (Gallizzi et al. 2008a). It is likely that nestlings in good condition can overcome the negative effects of an infestation. In addition, parents could increase the rate of food provisioning and, thus, compensate for the resources drawn from the nestlings by the parasite (Tripet and Richner 1997b, Bouslama et al. 2002). Thus, our tick infestations either had no effect on nestlings or were compensated for.

Also, we used only tick larvae in our experiments, whereas both larvae and nymphs infest nestlings in the wild. It could be argued that we did not find an effect of the infestation because larvae extract less blood than nymphs. The amount of blood extracted by both larvae and nymphs of *I. ricinus* is small. Engorgement weights of larvae are ~0.5 mg (Hughes and Randolph 2001), and those of nymphs are ~10× larger (Dusbábek 1996). If it is assumed that blood concentration leads to a blood meal that is 2× larger than the engorgement weight (Magano et al. 2000, Kaufman 2007), this means that larvae extract 1 mg and nymphs 10 mg of blood, which corresponds to 0.02% and 0.20% of the nestlings' body mass on day 5. Although the amounts of blood extracted are small, nymphs extract ~10× more blood than larvae and, thus, it is possible that we would have found an effect if we had experimentally infested nestlings with nymphs.

Alternatively, wild ticks are often infested with microparasites such as *Borrelia* spp. (Gasparini et al. 2001, Comstedt et al. 2006, Poupon et al. 2006), whereas our laboratory-raised ticks were free of the usual pathogens, such as *Borrelia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Babesia* spp., and *Theileria* spp. This suggests that effects of tick infestations that are usually attributed to the ticks themselves (Szép and Møller 1999, 2000; Proctor and Owens 2000; Hoodless et al. 2003) may actually be attributable to the microparasites transferred by these ticks or to the combined effect arising from a challenge by both the tick and the tickborne pathogen. Finally, the laboratory-raised ticks could have been less virulent *per se* than ticks from the natural habitat.

Ticks were applied to the nestlings, but not all of them attached. Therefore, some of the ticks we applied may have infested the parents instead of the offspring. Yet, because we did not find an effect of the tick infestation on the nestlings, we can conclude that the possible infestation of the parents did not affect their performance and, therefore, did not interfere with our results.

Maternal effect.—The interaction between tick infestation and the presence or absence of the flea-induced maternal effect did not affect nestling performance. This finding, however, does not allow us to make inferences about cross-resistance to ticks arising from the flea-induced maternal effect, given that tick infestation *per se* did not affect nestling performance. Therefore, we could not test whether the reduced tick feeding times on the nestlings of flea-exposed mothers, which we found previously (Gallizzi et al. 2008a), reduces the negative effects of ticks on nestlings.

The flea-induced maternal effect *per se* had a negative influence on nestling tarsus length on day 14 after hatching. This is in contrast to our results from a study conducted in 2005 in the same population (Gallizzi et al. 2008b), where we showed that in flea-free nests, the maternal effect positively affected nestling performance under relaxed rearing conditions, whereas it had no effect under harsh rearing conditions. However, in 2005, we cross-fostered whole clutches, whereas a split-brood design was used here; thus, in 2005, the nests contained only one type of nestling, whereas in the present study, nestlings with and without maternal effect were raised in the same brood and competed with each other for access to parental feeding. The results of the two experiments suggest that the maternal effect is a disadvantage if the nestlings must compete for the same resources with nestlings that are not exposed to the maternal effect. It is known that immune-challenged mothers in general, and mothers with a flea-induced maternal response in particular, reduce the level of androgens deposited into the eggs (Tschirren et al. 2004, Gil et al. 2006). It has also been shown that reduced androgen levels decrease nestling begging

behavior (Eising and Groothuis 2003) and that nestlings hatched from testosterone-injected eggs are heavier than untreated controls (Tschirren et al. 2005; that study employed a split-brood design similar to the one used here). Hence, one possible explanation for our results is that the nestlings with the flea-induced maternal effect received reduced amounts of testosterone, leading to decreased begging activity compared with controls. The reduced begging activity could, then, have resulted in a decreased rate of food provisioning by the parents (Bengtsson and Rydén 1983, Kölliker et al. 2000) and, thus, in reduced tarsus growth. Regarding the mechanisms, it is also possible that the reduced testosterone levels in the eggs of the maternal effect nestlings (Tschirren et al. 2004) directly influenced nestling tarsus growth and that this effect depends on environmental conditions, such as food availability. Further investigations are needed to test this hypothesis.

Contrary to our expectation, we did not find a negative effect of a tick infestation on Great Tit nestlings, whereas the experimental infestation of parents with fleas during egg laying reduced nestling tarsus growth. These results suggest that parasites can influence their hosts across generations and that these effects can even be stronger than the consequences of a direct infestation of an individual.

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