On the Equivalence of Host Local Adaptation and Parasite Maladaptation: An Experimental Test

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ABSTRACT: In spatiotemporally varying environments, host-parasite coevolution may lead to either host or parasite local adaptation. Using reciprocal infestations over 11 pairs of plots, we tested local adaptation in the hen flea and its main host, the great tit. Flea reproductive success (number of adults at host fledging) was lower on host individuals from the same plot compared with foreign hosts (from another plot), revealing flea local maladaptation. Host reproductive success (number of fledged young) for nests infested by foreign fleas was lower compared with the reproductive success of controls, with an intermediate success for nests infested by local fleas. This suggests host local adaptation although the absence of local adaptation could not be excluded. However, fledglings were heavier and larger when reared with foreign fleas than when reared with local fleas, which could also indicate host local maladaptation if the fitness gain in offspring size offsets the potential cost in offspring number. Our results therefore challenge the traditional view that parasite local maladaptation is equivalent to host local adaptation. The differences in fledgling morphology between nests infested with local fleas and those with foreign fleas suggest that flea origin affects host resource allocation strategy between nestling growth and defense against parasites. Therefore, determining the mechanisms that underlie these local adaptation patterns requires the identification of the relevant fitness measures and life-history trade-offs in both species.

Keywords: host-parasite coevolution, resistance, individual investment strategies, dispersal rate, specialization, reproductive success, accelerated growth.

Introduction

In spatially heterogeneous environments, evolution may lead to local adaptation, defined by a higher average fitness of individuals in their local environment compared with fitness of those in a foreign environment (Ebert 1994;

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Galloway and Fenster 2000; Gómez et al. 2009). In host-parasite systems, parasites have traditionally been thought to be ahead in the coevolutionary arms race and therefore to be locally adapted to their hosts (Gandon et al. 1996; Gandon and Michalakis 2002). This is mainly due to parasites' shorter generation times and higher reproductive rates compared with those of their hosts (Gandon and Michalakis 2002). However, experimental tests of local adaptation using reciprocal transplants in natural host-parasite systems have failed to detect parasite local adaptation in nearly half of the cases (34 out of 70 studies, i.e., 49%; reviewed in Greischar and Koskella 2007; Hoeksema and Forde 2008; and app. A, available online). They often have even shown parasite local maladaptation (8 out of 70 studies, i.e., 11%).

Theoretical studies have revealed that this discrepancy between predictions and experimental observations could be explained by the introduction of genetic novelty via gene flow, recombination, or mutation. The introduction of genetic novelty has been considered to put a brake on local adaptation by counteracting gene frequency changes resulting from selection (Gandon and Slatkin 1995; Lively 1999; Michalakis 2002; Thompson et al. 2002). However, it could also allow novel resistance and/or virulence alleles to enter populations, favor asymmetry in coevolutionary processes by increasing the evolutionary potential of one interacting species only, promote asynchrony in coevolutionary dynamics across populations, and thereby facilitate the establishment of local adaptation (Slatkin 1995; Gandon et al. 1996; Gandon and Michalakis 2002; Garant et al. 2007; Gandon and Nuismer 2009). Consequently, genetic novelty could give an advantage to either the parasite or the host, and hosts could also be predicted to be locally adapted to parasites (Gandon et al. 1996; Gandon and Michalakis 2002; but see Ridenhour and Nuismer 2007).

The potential for local adaptation in both hosts and

parasites may be determined by their relative dispersal rates (Gandon et al. 1996; Gandon and Michalakis 2002): the species with the higher dispersal rate should be locally adapted to the other one, and no local adaptation could be expected when dispersal rates of hosts and parasites are equal. Reviews of experimental transplants support a link between relative dispersal rate and local adaptation: parasite local adaptation was more likely to be detected when parasites had a higher dispersal rate compared with that of their hosts (60% of studies, i.e., 18 out of 30) than a lower dispersal rate (28% of studies, i.e., 9 out of 33; Greischar and Koskella 2007; Hoeksema and Forde 2008). However parasite local maladaptation was not related to a lower parasite dispersal rate compared with that of their hosts (Greischar and Koskella 2007; Hoeksema and Forde 2008). Thus, the conditions favoring parasite and host local adaptation or maladaptation remain poorly understood and deserve further experimental work (Greischar and Koskella 2007; Hoeksema and Forde 2008).

In particular, the strong taxonomic bias observed in experimental tests of local adaptation could affect our understanding of host-parasite coevolution: the vast majority of experimental studies were conducted on plant and invertebrate species rather than on vertebrate host species (64 and 7 studies, respectively; reviewed in Greischar and Koskella 2007; app. A). Strikingly, the proportion of studies showing parasite maladaptation was strongly taxonomically biased: 43% in systems involving vertebrate hosts against 8% and 5% in plant and invertebrate hosts, respectively ($N = 75$ host-parasite systems, $\chi_2^2 = 7.61$, $P = .02$; data from Greischar and Koskella 2007 and app. A). Furthermore, while studies in plant hosts usually rely on fitness measures of both the host and the parasite, studies in animal hosts often measure parasite fitness only and may therefore underestimate host tolerance (Råberg et al. 2009; Svensson and Råberg 2010). A full understanding of local adaptation patterns in vertebrate host-parasite systems therefore requires measuring both host and parasite fitness, and such studies are still scarce, with only three studies to date (fish: Ballabeni and Ward 1993; birds: Dufva 1996; McCoy et al. 2002).

In this study, we used reciprocal infestations to investigate local adaptation between the hen flea *Ceratophyllus gallinae*, an ectoparasite living in bird nest material, and the great tit *Parus major*. In this well-studied vertebrate host-ectoparasite interaction, the relative dispersal ability of the parasite compared to that of its host is unknown. The rapid infestation of deparasitized nests separated by a few dozen meters from infested nests suggests high short-distance dispersal ability of fleas (Heeb et al. 1996), while great tits may be expected to have higher dispersal ability at larger spatial scales (a few kilometers to tens of kilometers; see details in "Study Species and Study Site"). Con-

sequently, flea local maladaptation and great tit local adaptation may be expected. In line with this prediction, great tit recruits from experimentally infested nests dispersed shorter distance than uninfested controls did, and their breeding success decreased with increasing dispersal distance (Heeb et al. 1999; Tschirren et al. 2007), providing indirect evidence of great tit local adaptation to hen fleas at a fine scale. A previous reciprocal infestation experiment in these species revealed no local adaptation of either tits or fleas (Dufva 1996). However, this study involved one continental and one island population separated by a large distance (300 km), while local adaptation is more likely to occur at the spatial scale of host and parasite dispersal and gene flow (Thrall et al. 2002). Here, we used 11 pairs of discrete plots separated by 14 km on average and tested local adaptation by comparing host and parasite reproductive success when hosts bred with fleas from the same or the paired plot. By recording different measures of host success, in particular offspring number and size, we also aimed at providing insight into host strategies to cope with parasites of different origins.

Material and Methods

Study Species and Study Site

The great tit is a cavity-nesting passerine bird and is one of the main hosts of the ectoparasitic hen flea (Tripet and Richner 1997*a*), a common blood-feeding arthropod living in host nest material. Adult fleas feed intermittently on the host (on incubating females and later on nestlings) for short periods of time, while larvae feed on host organic material and undigested blood from adult flea feces. After a first blood meal, females lay eggs into the nest material. Adult fleas always produce at least one new adult generation during one host breeding cycle (Tripet and Richner 1999*b*). This first offspring cohort also lays eggs. The possibility for the second larvae generation to reach the adult stage should be highly dependent on ambient temperature and has been documented only in blue tit nests so far (Tripet and Richner 1999*b*). Between the start of incubation and fledging (the so-called warm period; Tripet and Richner 1999*b*), the parasite developmental cycle takes 23 days on average (see Tripet and Richner 1997*a*; Tripet et al. 2002 for more details).

Fleas strongly affect many fitness components in great tits (current and future breeding success, adult and juvenile survival, including carryover effects; Richner et al. 1993; Fitze et al. 2004). In response to the presence of fleas, great tits can efficiently reduce flea impact through behavioral and physiological defense mechanisms (maternal effects, adaptive changes in offspring care, etc.; e.g.,

Christe et al. 1996; Buechler et al. 2002; Tschirren et al. 2004).

The experiment was conducted in 2007 in a patchy great tit population breeding on the island of Gotland, Sweden (57°10′N, 18°20′E). The study area comprised 22 discrete wood plots of different size and breeding density, interspersed with habitat lacking both natural and artificial (nest boxes) breeding sites (i.e., mostly fields and pastures) and in which great tit breeding density was therefore strongly limited. In this population, great tit natal dispersal distance, that is, the distance between the site of birth and the site of first reproduction, is $1{,}212 \pm 1{,}178$ m (mean $\pm$ 1 SD; minimum 51, maximum 5,666) for females ($N = 50$) and $969 \pm 1{,}077$ m (minimum: 0, maximum: 5,052) for males ($N = 60$). These are close to dispersal distances found in other populations (McCleery et al. 2004; Tinbergen 2005; Doligez et al. 2008). The study area covered approximately 50 km along a north-south axis, and the distance between paired plots ranged from 3.8 to 28.5 km, that is, up to five times the observed maximal natal dispersal distance (app. B, available online).

Experimental Reciprocal Flea Infestations; Host and Parasite Reproductive Data

The 11 pairs of plots were formed by matching vegetation type (deciduous vs. mixed forests) and breeding density (number of great tit pairs in the year preceding the experiment) in order to account for environmental variation and to balance sample sizes within pairs of plots. Before the start of the breeding season, hen fleas were collected from old great tit nest material stored by plot (mixing fleas from different nests of same plot). Reciprocal flea infestations were performed within each pair of plots. Before infestation, all nests were heat treated using a microwave oven (Richner et al. 1993) to kill all naturally occurring ectoparasites. During the treatment, eggs were kept in a separate box. This procedure has been used in many previous studies on great tit–hen flea interactions (e.g., Richner et al. 1993; Heeb et al. 1999; Buechler et al. 2002; Fitze et al. 2004; Tschirren and Richner 2006) and has no effect on nest desertion or hatching rates. On the second day after the last egg was laid (to ensure clutch completion), each experimental clutch within each plot was assigned to receive one of the three following treatments using a randomization-by-block procedure (with blocks of three nests of the same date of clutch completion in order to ensure a homogeneous distribution of treatments over time): (i) heat treatment to eliminate fleas alone (controls), (ii) heat treatment followed by infestation with 40 fleas from the same plot (local fleas), and (iii) heat treatment followed by infestation with 40 fleas from the other plot of the pair (foreign fleas).

Laying date, clutch size, incubation starting date, hatching date, and number of hatchlings were determined by regular visits (at least one per week) of occupied nest boxes throughout the season. Nestlings were individually marked, and blood was sampled for molecular sexing (following Griffiths et al. 1998) when 2 days old; they were ringed when 9 days old and measured when 14 days old to record tarsus length (to the nearest 0.1 mm), body mass (to the nearest 0.1g), and length of the eighth primary wing feather (i.e., the longest one, to the nearest mm). When nestlings were aged 5 to 14 days, parents were caught in nest boxes and weighed and measured.

Nests were checked daily from the day when nestlings were 16 days old to record final number of fledged young and fledging date and to collect nest material of successful nests (i.e., that fledged at least one young) just after fledging. Nest material was stored for 2 days at 16°C before being frozen at −18°C over 48 h. Nests were brought back to the laboratory after the field season, placed in a freezer again for 2 days at −80°C and subsequently stored at 4°C. Adult fleas were counted from a subset of successful nests ($N = 91$) as follows: first, nests were cut in four equal parts. Each quarter was ripped up and fleas were searched for at least 10 min. When more than 50 adult fleas were counted in a quarter, it was inspected for an additional 10 minutes. Consequently each nest was inspected for at least 40 min. All counts were performed blindly with respect to experimental treatment. The number of adult fleas present in control nests was an estimate of the reproductive success of immigrant fleas after the treatment took place, while the number of adult fleas in infested nests was an estimate of the reproductive success of fleas that were added experimentally plus those that immigrated naturally. The number of adult fleas at the end of the host breeding attempt was used as the estimate of average flea reproductive success. This estimate integrates both flea adult fecundity and larvae survival, which can be affected by population dynamic processes such as larval competition (Tripet and Richner 1999a). Adult body mass and size, which have been shown to be correlated with fecundity in insects (Honěk 1993), could not be used here as measures of flea fitness because flea bodies appeared deteriorated after nest material had been frozen and transported. Finally, the number of flea larvae could also measure flea reproductive success. However, it was not possible to obtain an accurate estimate of the number of flea larvae and adults simultaneously because flea larvae need to be extracted alive from nest material while mobile adult fleas need to be extracted dead from nest material after freezing.

A total of 217 great tit nests were manipulated (70 control nests, 71 nests with local flea, and 76 nests with foreign fleas; app. B). Nests were randomized among experimental groups with respect to laying date ($F_{2,214} = 0.43$, $P =$

.65) and clutch size ($F_{2,214} = 1.49$, $P = .23$). Similarly, parental body mass, tarsus, and wing length did not differ between experimental groups ($F_{2,247} = 1.34$, $P = .26$; $F_{2,250} = 1.11$, $P = .33$; and $F_{2,250} = 0.85$, $P = .43$, respectively; accounting for sex: all $P < .001$). For the subsample of nests where adult fleas were counted, clutch size, laying date, and hatching date did not differ among experimental groups ($F_{2,88} = 2.42$, $P = .10$; $F_{2,88} = 1.55$, $P = .22$; $F_{2,88} = 0.45$, $P = .64$, respectively). In the year following the manipulation (2008), breeding attempts were monitored in the same plots to detect great tit offspring from experimental nests recruiting into the population (five experimental plots in 2007 were, however, not monitored in 2008).

Statistical Analyses of Variables Measuring Host and Parasite Reproductive Success

The number of adult fleas was log transformed to meet the assumptions of homoscedasticity and normality of residuals. Continuous variables (i.e., log-transformed number of adult fleas; number of great tit nestlings at day 2 in nests where at least one young hatched and survived until day 2; number of fledged young in successful nests [i.e., where at least one young fledged]; duration of incubation and nestling period; nestling mass gain from day 2 to day 8 and from day 8 to day 14; fledgling morphological measurements) were analyzed with general linear mixed models (GLMM). Binary variables (overall failure probability, including desertion before hatching and entire brood failure after hatching; hatching probability, i.e., the number of hatchlings divided by clutch size; offspring mortality from day 2 until fledging and from fledging until recruitment) were analyzed using generalized linear mixed models with binomial error and logit link function. Overall failure probability (success vs. failure) and fledging success in successful nests were analyzed separately (i) because they often represent different biological processes with different individual responses (e.g., in dispersal or reproductive investment decisions), (ii) to meet the assumptions of statistical tests for data with a large fraction of zero values (overall, 25% of nests failed), and finally, (iii) to fully compare host and parasite reproductive success since flea success could be assessed in successful nests only, where fleas could complete their breeding cycle.

All initial models included treatment as a fixed effect and plot identity and pair of plots as random effects. Models for fledgling morphological measurements also included brood size at day 14, laying date, and fledgling sex as covariates, as well as their two-way interactions with treatment, and brood identity as a random effect to correct for common rearing environment and parental effects. All interactions were nonsignificant and did not change the

results for main effects; they were therefore removed from final models. In a second step, we included parasite load (flea number at the end of the nestling period) as a covariate in models for fledgling morphological measurements, for the subset of infested broods for which fleas had been counted ($N = 59$ in 19 plots). We tested the interaction between treatment and parasite load; that is, we tested for differences between treatments in the relation between parasite load and fledgling morphological measurements, which could reflect differences in tolerance level between treatments (Råberg et al. 2009). In models for fledgling morphological measurements, a variance function structure allowing different variances for each treatment level was used to correct for heteroscedasticity among treatments ("varIdent" function of the "nlme" package; Pinheiro and Bates 2000). Overall, the variance explained by pairs of plots was low (between 0% and 3% for the different models). Therefore, we excluded this effect from final models, but retaining it did not qualitatively change the results. Similarly, because we found only two sibling pairs among recruits, we excluded brood identity from models for morphological measurements of recruits, but retaining it did not qualitatively change the results.

All statistical analyses were performed using R software (R Development Core Team 2010). Model selection was done using maximum likelihood while parameter estimates of mixed models were obtained via restricted maximum likelihood (Pinheiro and Bates 2000). We used a stepwise backward deletion procedure to remove nonsignificant effects ($P > .10$), starting with interactions. Fixed effects were tested for significance using two-tailed, Type II F-tests or χ^2 tests. Sample sizes varied among analyses because not all adults or nestlings could be measured.

Results

Flea Reproductive Success

The number of adult fleas in nests after great tit fledging strongly differed between experimental treatments ($F_{2,69} = 9.07$, $P < .001$; fig. 1*A*). Nests infested with local and foreign fleas respectively harbored twice and four times as many adult fleas compared with controls, and nests infested with foreign fleas harbored twice as many adult fleas compared with nests infested with local fleas (fig. 1*A*; table 1A).

Host General Reproductive Success

Neither desertion (before hatching) nor brood failure (after hatching) probabilities were influenced by the experimental treatment ($\chi^2_2 = 0.49$, $P = .78$ and $\chi^2_2 = 0.34$, $P = .84$, respectively). Therefore, the overall probability
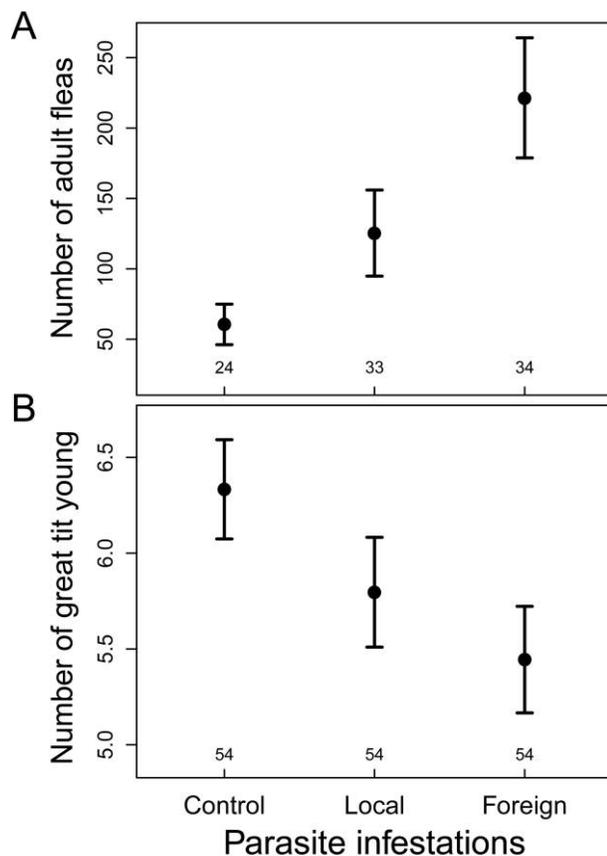
Figure 1: Reproductive success of parasites (hen fleas) and hosts (great tits) according to experimental treatment. *A*, Final number of adult fleas in great tit nests after fledging (mean ± 1 SEM); and *B*, number of fledged great tit young in successful nests (i.e., where at least one young fledged; mean ± 1 SEM). Control: no infestation; local: infestation by 40 adult fleas of local origin (i.e., from the same plot); foreign: infestation by 40 adult fleas of foreign origin (i.e., from a different plot). Numbers indicate sample size.

of reproductive failure was not influenced by the experimental treatment ($\chi^2_2 = 0.83$, $P = .66$; percentage of failed nests: 29%, 24%, and 23% for nests with foreign fleas, local fleas, and control nests, respectively).

Hatching probability in nests where at least one young hatched marginally differed between experimental treatments ($\chi^2_2 = 5.79$, $P = .055$). Fewer young hatched in nests infested with foreign fleas than in control nests (90% and 95%, respectively; post hoc z value $= 2.36$, $P = .02$). Hatching success in nests infested with local fleas was intermediate (93%) and did not differ from either control nests (post hoc z value $= 1.32$, $P = .19$) or nests infested with foreign fleas (post hoc z value $= 1.09$, $P = .28$). As a consequence, in nests where at least one young hatched and survived until day 2, brood size at day 2 differed between experimental treatments ($F_{2,156} = 3.54$, $P = .03$).

Brood size at day 2 in nests infested with foreign fleas (6.85 ± 0.23, $N = 62$) was marginally lower than nests infested with local fleas (7.42 ± 0.20, $N = 59$) and significantly lower than control nests (7.68 ± 0.25, $N = 59$; table 1B). The number of fledged young in successful nests (i.e., that fledged at least one young) tended to differ among experimental treatments ($F_{2,138} = 2.91$, $P = .058$; fig. 1B). Control nests fledged more young than nests infested with foreign fleas (fig. 1B; table 1C); the number of fledged young in nests infested with local fleas was intermediate but did not significantly differ from either control nests or nests infested with foreign fleas (fig. 1B; table 1C).

Duration of Incubation and Nestling Period, Nestling Mortality, and Mass Gain

The duration of incubation and nestling period did not differ between experimental treatments ($F_{2,137} = 0.64$, $P = .53$ and $F_{2,138} = 0.63$, $P = .53$, respectively). Nestling mortality from day 2 to fledging was not affected by the experimental treatment ($\chi^2_2 = 1.21$, $P = .55$; with no effect of nestling sex: $\chi^2_1 = 0.78$, $P = .38$). For nestlings surviving until fledging, mass gain from day 2 to day 8 was affected by the treatment ($F_{2,137} = 3.47$, $P = .03$) after accounting for nestling sex ($F_{1,817} = 52.86$, $P < .001$). Nestlings had a higher early mass gain when reared with foreign fleas (9.50 ± 0.08, $N = 300$) compared with rearing with local fleas (8.88 ± 0.10, $N = 322$) or in control nests (9.02 ± 0.09, $N = 357$; table 1D). Nestling mass gain from day 8 to day 14 did not differ between treatments ($F_{2,137} = 0.88$, $P = .41$) after accounting for nestling sex ($F_{1,818} = 13.41$, $P < .001$). In both cases, male nestlings gained more mass than female nestlings (0.44 ± 0.06 g for early mass gain and 0.25 ± 0.07 g for late mass gain).

Fledgling Size and Body Mass at Day 14

After accounting for brood size at day 14, that is, for a relation between offspring quantity and quality, and for laying date and fledgling sex, fledgling morphological measurements were influenced by the experimental treatment (table 2; fig. 2). Fledglings reared with foreign fleas were heavier and had significantly longer tarsus and wing length than fledglings reared with local fleas (table 1E–1G; fig. 2). When compared with control fledglings, fledglings reared with local fleas had marginally smaller tarsus length but similar wing length and body mass, while fledglings reared with foreign fleas had longer wing length but similar tarsus length and body mass (table 1E–1G; fig. 2). The effect of brood size remained unchanged when excluding treatment from the models ($F_{1,136} = 10.28$, $P = .002$; $F_{1,137} = 0.04$, $P = .84$; $F_{1,136} = 14.61$, $P < .001$ on fledgling

Table 1: Pairwise comparisons (contrasts) of measures of parasite (no. adult fleas) and host reproductive success between treatment groups

Pairwise comparison	Difference ± SE	df	t	P
A. No. adult fleas (log transformed):				
Foreign fleas vs. local fleas	.58 ± .28	69	2.10	.04
Foreign fleas vs. control	1.27 ± .30	69	4.26	<.001
Control vs. local fleas	.70 ± .31	69	2.27	.03
B. No. nestlings at day 2:				
Foreign fleas vs. local fleas	.57 ± .31	156	1.83	.07
Foreign fleas vs. control	.79 ± .31	156	2.58	.01
Control vs. local fleas	.22 ± .31	156	.71	.48
C. No. fledged young in successful nests:				
Foreign fleas vs. local fleas	.41 ± .35	138	1.18	.24
Foreign fleas vs. control	.84 ± .35	138	2.45	.02
Control vs. local fleas	.43 ± .35	138	1.20	.23
D. Early nestling mass gain (g; between days 2 and 8):				
Foreign fleas vs. local fleas	.58 ± .26	137	2.24	.03
Foreign fleas vs. control	.57 ± .26	137	2.18	.03
Control vs. local fleas	.001 ± .26	137	.006	.99
E. Fledgling tarsus length (mm) at day 14:				
Foreign fleas vs. local fleas	.45 ± .14	134	3.41	<.001
Foreign fleas vs. control	.20 ± .14	134	1.55	.12
Control vs. local fleas	.25 ± .14	134	1.88	.06
F. Fledgling body mass (g) at day 14:				
Foreign fleas vs. local fleas	.90 ± .32	137	2.88	.005
Foreign fleas vs. control	.42 ± .31	137	1.33	.18
Control vs. local fleas	.48 ± .32	137	1.51	.13
G. Fledgling wing length (mm) at day 14:				
Foreign fleas vs. local fleas	1.88 ± .67	134	2.79	.006
Foreign fleas vs. control	1.36 ± .66	134	2.05	.04
Control vs. local fleas	.52 ± .67	134	.78	.43

Note: A, log-transformed number of adult fleas in nests after fledging; B, no. nestlings at day 2 in nests where at least one young hatched and survived.

tarsus length, body mass and wing length respectively), showing that the effect of treatment was not due to differences in fledgling number. Furthermore, all interactions between brood size and treatment were nonsignificant ($F_{2,132} = 0.45$, $P = .64$; $F_{2,133} = 1.04$, $P = .36$; and $F_{2,132} = 1.07$, $P = .35$ on fledgling tarsus length, body mass, and wing length, respectively), showing that the treatment did not alter the relationship between offspring quality and quantity (table 2).

The interaction between treatment and parasite load was significant for none of the fledgling morphological measurements ($N = 348$ fledglings in 59 nests; $F_{1,35} = 0.04$, $P = .84$; $F_{1,35} = 0.01$, $P = .94$; and $F_{1,35} = 0.01$, $P = .93$ for fledgling tarsus length, body mass, and wing length, respectively), suggesting no difference in tolerance level between hosts infested with local and foreign fleas. Furthermore, parasite load alone did not affect fledgling morphological measurements either ($F_{1,36} = 1.35$, $P = .25$; $F_{1,36} = 0.18$, $P = .68$; $F_{1,36} = 0.80$, $P = .38$ for fledgling

tarsus length, body mass, and wing length, respectively), showing that the differences in fledgling morphological measurements between treatments were not due to different final parasite load.

Offspring Local Recruitment

Out of 765 fledged young, a total of 18 breeding recruits (i.e., 2.3%) from 16 experimental nests were caught; six came from control nests, five had been reared with local fleas, and seven with foreign fleas. Recruitment probability was not influenced by the experimental treatment ($\chi_2^2 = 0.50$, $P = .78$), fledgling sex ($\chi_1^2 = 1.45$, $P = .23$), or fledgling body mass ($\chi_2^2 = 0.02$, $P = .90$; no significant interaction with sex or treatment). All recruits were caught in their natal plot, except two that dispersed between plots close by (~1 km).

Table 2: Influence of the experimental treatment and covariates on fledgling morphological measurements

| Variable	Ndf, ddf	F	P
Fledgling tarsus length (mm):			
Brood size at day 14	1, 134	13.27	<.001
Laying date	1, 134	3.50	.06
Sex	1, 817	190.80	<.001
Treatment	2, 134	5.96	.003
Fledgling body mass (g):			
Brood size at day 14	1, 135	.003	.96
Laying date	1, 136	.10	.76
Sex	1, 818	85.27	<.001
Treatment	1, 137	4.20	.02
Fledgling wing length (mm):			
Brood size at day 14	1, 134	18.14	<.001
Laying date	1, 134	14.06	<.001
Sex	1, 817	1.42	.23
Treatment	2, 134	4.28	.02

Note: Fledgling measurements shown as GLMM with normal distribution. Covariates are brood size at day 14, laying date, fledgling sex. Brood and plot identities were included as random factors to account for common rearing environment and common parental effects. Ndf and ddf are numerator and denominator degrees of freedom, respectively. Fledgling tarsus length and body mass showed sexual dimorphism: males had longer tarsi and were heavier at fledgling than females (mean $\pm$ 1 SEM for tarsus length [mm] and body mass [g], respectively: 19.76 $\pm$ 0.04 and 16.96 $\pm$ 0.09 for males, 19.22 $\pm$ 0.04 and 16.24 $\pm$ 0.09 for females). Fledgling wing length increased with increasing laying date (partial regression coefficient: 0.23 $\pm$ 0.06). Fledgling tarsus and wing length increased with increasing brood size (partial regression coefficients: 0.17 $\pm$ 0.03 and 0.68 $\pm$ 0.16, respectively).

Discussion

Using reciprocal infestations in paired host and parasite populations, we investigated local adaptation between hen fleas and great tits. Parasite reproductive success (measured as the number of adult fleas) at the end of tit breeding was higher for foreign than local fleas, revealing parasite local maladaptation. Host reproductive success (measured as the number of fledged young) was lower for nests infested by foreign fleas compared with controls, with nests infested by local fleas in an intermediate position. This suggests host local adaptation. However, host reproductive success was not significantly higher for nests infested with local fleas compared with foreign fleas, thus, the absence of local adaptation cannot be excluded. Interestingly, fledglings were significantly larger and heavier when reared with foreign fleas compared with those reared with local fleas, revealing different investment strategies by great tits. The consequences of these different strategies on long-term host fitness, however, depend on the link between offspring morphology and future fitness, which needs to be investigated.

Parasite Local Maladaptation

Because treatments were randomly distributed in space within plots, the higher number of adult fleas in nest material after tit fledging when nests were infested with for-
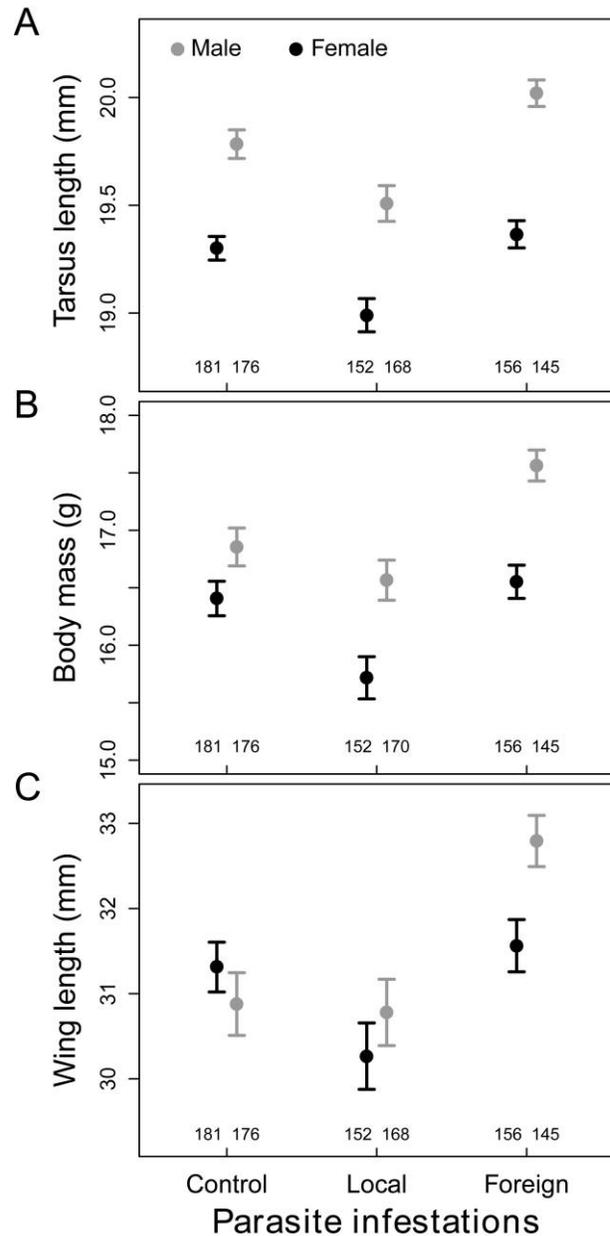
Figure 2: Fledgling morphological measurements (mean $\pm$ 1 SEM) according to experimental treatment and sex. *A*, Tarsus length; *B*, body mass; and *C*, wing length. Control: no infestation; local: infestation by 40 adult fleas of local origin (i.e., from the same plot); foreign: infestation by 40 adult fleas of foreign origin (i.e., from a different plot). Gray dots: males; black dots: females. Numbers indicate sample size.

eign fleas compared to nests with local fleas is unlikely to be explained by differential flea immigration, even if immigration was higher here than previously found in this system (Heeb et al. 1996). Thus, our results show that fleas were less successful in exploiting local hosts compared to exploiting foreign hosts, demonstrating parasite local maladaptation. Because a large number of paired host and parasite populations was manipulated here, the observed pattern is unlikely to result from cyclic oscillations or rare parasite genotypes exploiting more efficiently some host populations (Thrall et al. 2002). Locally adapted and maladapted populations of both hosts and parasites may occur here, reflecting a pattern consistent with a geographic mosaic of coevolution within this host-parasite interaction (Thompson 2005). However, the limited number of nests in our experimental plots (app. B) did not allow us to test for between-plot differences in parasite and host performance. Furthermore, in the absence of identified traits mediating host-parasite interactions here, a geographic mosaic coevolution pattern cannot be distinguished from locally frequency-dependent cycling dynamics of host-parasite antagonistic coevolution.

Flea local maladaptation could result here from a lower relative dispersal rate in parasites compared with the rate in hosts. Although the hen flea is an opportunist ectoparasite that could potentially disperse using other host species as a vehicle, flea dispersal is temporally constrained. Many fleas remain in the old host nest in immobile cocoons after the breeding season, with no access to hosts (Tripet et al. 2002). Flea dispersal should therefore occur either straight after host fledging or at the beginning of the next breeding season of their hosts, that is, at times when host movements should be spatially limited. Genetic data would appear to be necessary to obtain information about the relative dispersal ability of fleas compared with their hosts and test this hypothesis. Preliminary analyses of the genetic structure of hosts and parasites in the study population suggest a lower relative dispersal rate in fleas compared with that of great tits (M. Lemoine et al., unpublished results).

Parasite local maladaptation may also result from the exploitation of multiple hosts (Combes 1997; Thompson et al. 2002), affecting the relative intensity of selection acting on each species (Gandon and Nuismer 2009). A parasite with a broad host range should face diverse selective pressures imposed by different hosts and adaptations to one host may limit the range of possible adaptations to others (Lajeunesse and Forbes 2002). Thus, a host mainly infested (in terms of impact on host fitness rather than mere prevalence or intensity) by one parasite species that itself infests different hosts could be predicted to be ahead in the antagonist coevolution and thus locally adapted to its parasite. This could be the case here since

hen fleas are found in nests of numerous bird species (Tripet and Richner 1997*a*) and flea reproductive success did not differ on the main host and on an alternative host (collared flycatcher *Ficedula albicollis*) in this population (Lemoine et al. 2011). Nevertheless, local (mal)adaptation in a host-parasite system should depend on the asymmetry of specialization of both species rather than the degree of parasite specialization. Regrettably, little information is available on the parasitic community exploiting great tits. In particular, the relative importance of fleas as a selective pressure compared with that of other parasite species and/or cross responses to different parasites are poorly known (e.g., Gallizzi et al. 2008). Such information would be needed to make inferences regarding the origin of the flea local maladaptation observed here.

Host Local Adaptation or Differential Strategies according to Flea Origin?

Fleas had a detrimental effect on great tit success as shown by the decrease in number of fledged young in infested nests compared with the number in control nests, regardless of flea origin ($F_{1,139} = 4.45$, $P = .04$; 5.62 ± 0.20 and 6.33 ± 0.26 young for infested and control broods, respectively). This confirmed the detrimental effect of fleas shown by previous studies (Richner et al. 1993; Fitze et al. 2004). However, the effect of flea origin here was unclear because the number of fledged young in nests infested with local fleas did not statistically differ from either controls or nests infested with foreign fleas. Therefore, hosts could have been more successful when infested with local fleas than when infested with foreign fleas, indicating host local adaptation, but our power to detect the difference was low. Alternatively, host success could have been similar on local and foreign fleas, and thus, the absence of host local adaptation cannot be excluded. The absence of a negative impact of parasites on fledging mortality could be explained first by parental compensation (increased feeding rate; Christe et al. 1996). A second explanation could be a low direct effect of ectoparasites on host mortality but a high pathogenic effect leading to increased host mortality only in unfavorable conditions (e.g., McCoy et al. 2002).

However, fledglings reared with foreign fleas were heavier and larger than fledglings reared with local fleas. These differences were not due to a classical trade-off between offspring quantity and quality because the effect of experimental treatments was observed after accounting for brood size. Furthermore, because the interaction between brood size and treatment was never significant, the relation between offspring quantity and quality itself was not affected by treatment. Offspring fitness may increase with body size and mass at fledging in great tits, at least up to

a given level (see Linden et al. 1992; Tilgar et al. 2010). Therefore, future fitness prospects of fledglings could be higher when reared with foreign fleas compared with those reared with local fleas. Individual recruitment probability did not differ between treatments; however, the power of this analysis was limited, especially as we were not able to detect dispersing fledglings. If the long-term fitness gain in offspring size offsets the potential cost in offspring number, our results would show no host local adaptation or even host maladaptation to fleas. Overall, fledglings had higher early mass gain and reached higher body mass and larger size when reared with foreign fleas compared with rearing with local fleas, but they had a higher final number of adult fleas in their nests. Taken together, these results suggest that great tits in nests with foreign fleas (over)compensated via accelerated growth (Metcalfe and Monaghan 2001; Barber et al. 2008) without resisting as shown by a high flea reproductive success. On the contrary, great tits in nests with local fleas resisted without compensating, with an immediate cost in terms of fledgling body mass and size. In other words, great tits could trade investment in resistance to limit parasite infection against investment in growth to limit the length of exposure to damages caused by parasite infection (in the nest and later), in that case paying the cost later (Metcalfe and Monaghan 2001). Faster growth (as reflected here by longer tarsus and wing and larger body mass) may allow nestlings to fledge earlier (Verhulst 1995) and/or disperse farther, although we could not detect this here. In the absence of estimates of long-term fitness effects of these different resource allocation strategies on individual fitness, it is difficult to draw conclusions about host local adaptation here. Importantly, such alternative strategies (involving accelerated growth) as well as trade-offs between size and number could also occur in the parasite (see Tripet et al. 2002; Lemoine et al. 2011) and could affect host responses, but they could not be investigated here.

Fleas may affect nestling resource allocation to resistance or growth both directly, by exploiting host resources (Richner et al. 1993; Christe et al. 1996), and indirectly, via the negative impact of the activation of the immune system on growth (Tschirren and Richner 2006). The impact of fleas on these physiological processes may depend on their geographical origin due to specific interactions between host and parasite genotypes, reflecting a genetic basis of local adaptation, and/or to phenotypic plasticity linked to, for example, parental epidemiological history (see Galloway and Etterson 2007; Wegner et al. 2009). However, information on previous ectoparasite exposure is generally difficult to assess in the wild and was not available here. Females in contact with fleas of different origin during winter or nest building could deposit different amount or

type of components into eggs, such as antibodies, hormones, antioxidants, and so forth (e.g., Buechler et al. 2002; Tschirren et al. 2004), affecting resource allocation strategies between nestling immune system and growth. In addition, parental investment after hatching may differ according to flea origin in response to differences in nestling behavior (e.g., begging; Christe et al. 1996; Tripet and Richner 1997*b*), possibly mediated by nestling immune response and/or maternal effects. Thus, parents may use both their own interaction with fleas and nestling behavior as cues to discriminate between local and foreign fleas and adjust their investment accordingly.

Importantly, however, our results do not necessarily imply differential parental investment according to flea origin. Parental investment could increase similarly in broods infested with both local and foreign fleas, but the effect of this increase on nestling growth may differ according to flea origin, depending on nestling investment in immune response. Nestlings reared with foreign fleas could allocate all resources to growth as a result of a reduced efficiency of their immune response and, as a consequence, reach larger body size and mass than nestlings reared with local fleas and control nestlings, at the expense of a higher parasite reproductive success. To distinguish between these alternative mechanisms, it would be necessary to measure parental investment and nestling immune response directly in nests infested with local and foreign fleas to test whether parental investment is higher in broods infested with foreign compared with local fleas and/or whether nestling immune response is higher towards local compared with foreign fleas.

Assessing Host and Parasite Local (Mal)Adaptation: Which Measures Should Be Used?

Under asymmetric antagonistic coevolution, host and parasite fitness measures are predicted to be negatively correlated, leading to the generally accepted assumption that host local adaptation and parasite local maladaptation (and vice versa) should occur simultaneously (Gandon and Michalakis 2002). Studies on local adaptation in host-parasite interactions generally consider parasite infectivity (i.e., the ability of parasites to infest hosts) to be a good estimate of parasite fitness and thus the target of selection (Hoeksema and Forde 2008). Parasite infectivity is by definition the inverse of host resistance (see Råberg et al. 2007). Therefore, many theoretical and empirical studies have neglected host local adaptation and measured only parasite local adaptation via host resistance, or they measured host local adaptation via parasite fitness (Gandon et al. 1996; Gandon and Michalakis 2002; Hatcher et al. 2005; Morgan et al. 2005).

However, the strong assumption of co-occurrence of

host local adaptation and parasite local maladaptation (and vice versa) can be challenged. First, host fitness may not be directly related to host resistance, as suggested by our results. The existence of a negative correlation between host and parasite fitness measures could depend on the cost of expressing resistance in the host, which could generate a nonlinear (Stjernman et al. 2008) or conditional (McCoy et al. 2002) relationship. Second, immunological processes after parasite encounter are often considered to drive host resistance (Råberg et al. 2009). However, different strategies may allow hosts to reduce parasite fitness (see Roy and Kirchner 2000), including the alteration of host life-history traits (e.g., body size, fecundity, age at reproduction) or behavior (e.g., dispersal, self-grooming, or mating preferences; reviewed in Agnew et al. 2000; Gandon et al. 2002). Such changes in the hosts could in turn affect life-history or behavioral traits of parasites and ultimately local adaptation. Life-history and behavioral changes have, however, received little attention in either theoretical or experimental studies (Agnew et al. 2000; Gandon et al. 2002). The co-occurrence of host local adaptation and parasite local maladaptation (and vice versa) could therefore be affected by restricting host overall resistance to immune resistance and measuring it via parasite reproductive success after infection, ignoring changes in other traits. Finally, local adaptation results from divergent selection when genotype × environment interactions shape fitness (Kawecki and Ebert 2004). Antagonist coevolution is therefore not a prerequisite for local adaptation in general and more particularly in host-parasite systems (Kawecki and Ebert 2004). In this case, host and parasite fitness could be decoupled and a context-dependent expression of tolerance, for instance, could lead to host local adaptation.

Understanding the mechanisms of local adaptation in this and other host-parasite systems requires identifying the relevant fitness measures of both species in time and space and investigating trade-offs in host and parasite life-history and behavioral traits. In host-parasite studies, the questions of which traits could (or not) lead to patterns of local adaptation due to reciprocal coevolution and should therefore be measured (e.g., parasite infectivity, infection intensity or virulence; host resistance, tolerance, mortality, or growth), and how to define the traits (e.g., fixed trait or reaction norm, on the short- or long-term, at the individual or population level), are under debate (Dybdahl and Storfer 2003; Greischar and Koskella 2007; Råberg et al. 2007; Hoeksema and Forde 2008; Laine 2008; Råberg et al. 2009; Svensson and Råberg 2010). More work is therefore needed before we can predict whether and when host local adaptation and parasite local maladaptation (and vice versa) are equivalent.

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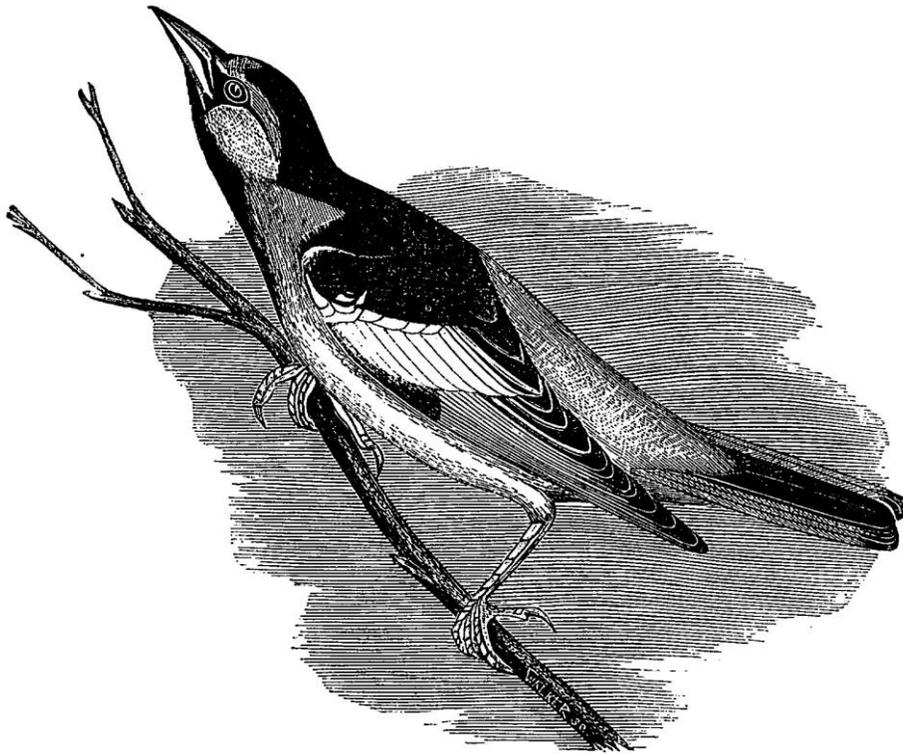
Bullock's Oriole. "Little time passes before the modestly attired females, rambling silently through the verdure, are singled out and attended each by her impetuous consort, who sings his choicest songs and displays the prowess she admires most." From "Bullock's Oriole," by Elliott Coues (*American Naturalist*, 1871, 5:678–682).