What Makes a Host Profitable? Parasites Balance Host Nutritive Resources against Immunity

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ABSTRACT: Numerous host qualities can modulate parasite fitness, and among these, host nutritive resources and immunity are of prime importance. Indeed, parasite fitness increases with the amount of nutritive resources extracted from the host body and decreases with host immune response. To maximize fitness, parasites have therefore to balance these two host components. Yet, because host nutritive resources and immunity both increase with host body condition, it is unclear whether parasites perform better on hosts in prime, intermediate, or poor condition. We investigated blood meal size and survival of the ectoparasitic louse fly *Crataerina melbae* in relation to body condition and cutaneous immune response of their Alpine swift (*Apus melba*) nestling hosts. Louse flies took a smaller blood meal and lived a shorter period of time when feeding on nestlings that were experimentally food deprived or had their cutaneous immune response boosted with methionine. Consistent with these results, louse fly survival was the highest when feeding on nonexperimental nestlings in intermediate body condition. Our findings emphasize that although hosts in poor condition had a reduced immunocompetence, parasites may have avoided them because individuals in poor condition did not provide adequate resources. These findings highlight the fact that giving host immunocompetence primary consideration can result in a biased appraisal of host-parasite interactions.

Keywords: host-parasite interaction, immunocompetence, nutrition, parasite fitness, phytohemagglutinin (PHA) skin test.

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Host-parasite relationships are usually described by the harm caused by parasites to their hosts (i.e., virulence), which is partly due to the direct competition for resources between hosts and parasites (Bedhomme et al. 2005), and by the defense mechanisms developed by hosts to resist parasite exploitation (Combes 2001). From this description, it follows that two major forces shape host-parasite relationships. First, in immunoecology, conventional wisdom holds that immunocompetence is the most important host phenotypic characteristic for determining parasite resistance (Wakelin 1996) and that thus, parasites preferentially attack hosts that are in poor condition and immunodeficient (Christe et al. 1998; Roberts et al. 2004). Although host immune resistance has a genetic basis (Wakelin and Apanius 1997; Roulin et al. 2007), individuals in poor condition have fewer resources to allocate to costly defense mechanisms (Sheldon and Verhulst 1996; Norris and Evans 2000; Alonso-Alvarez and Tella 2001; Martin et al. 2006). Therefore, individuals in poor condition are thought to be phenotypically less resistant than their genotype might otherwise suggest (Agnew and Koella 1999; Lambrechts et al. 2006; but see Krist et al. 2004).

Second, parasites are limited not only by host immunocompetence but also by numerous other host qualities (e.g., host body temperature, skin thickness, and grooming behavior; Clayton et al. 1999; Elliot et al. 2002), among which the amount of high-quality resources parasites can extract from the host body is a prime additional aspect (Nayar and Sauerman 1975; Canyon et al. 1999; Lehane 2005). Host resources available to parasites can be related to the size per se of their host and/or to the nutritional quality of the host and by extension to host body condition. Accordingly, several studies have reported that large hosts are more intensely parasitized (Valera et al. 2004; Ezenwa et al. 2006) and that parasites have a higher reproductive success when raised on hosts experimentally supplemented with food (Christe et al. 2003; Tseng 2006; Tschirren et al. 2007).

However, the key issue in host-parasite interaction is that the host is able to fight back (Hanley et al. 1996;

Combes 2001), and therefore to maximize fitness, parasites have to balance various host qualities, which in most systems are likely to be host immunity against nutritive resources. Host immunity and nutritive resources, however, both improve with host body condition (and probably also with host body size, since size is often linked to dominance and access to food). Hence, it is unclear where the balance is and, for that reason, what makes a host profitable. If parasite fitness is mainly affected by resources that parasites can extract from their host, parasites should achieve a greater fitness on hosts in good condition. Conversely, if parasite fitness is primarily affected by host immune responses, parasites should perform better on immunodeficient hosts in poor condition. However, as highlighted above, it is likely that parasites balance host nutritive resources against host immunity (or, more generally, resistance strategies), and thus parasites may achieve maximal fitness returns on hosts in intermediate body condition. That is, parasites may avoid hosts in low condition even if they are immunodeficient because such hosts may not provide adequate food resources. Furthermore, they may avoid hosts in prime condition even if such hosts provide high-quality resources because these hosts are likely to fight back with an efficient immune system (and/or any other condition-dependent resistance strategies, such as grooming; Giorgi et al. 2001). To the best of our knowledge, two studies have shown that parasites exploit, and in turn achieve a higher fitness, when feeding on hosts that have access to intermediate food levels and in turn that have an intermediate body condition (Bedhomme et al. 2004; Lambrechts et al. 2006). These two studies, however, did not explicitly identify and experimentally test whether the forces behind this nonlinear relationship between host condition and parasite fitness was caused by the opposite relationships linking host body condition, immunocompetence, and host nutritive resources available to parasites.

Blood-sucking insects are ideal for testing whether parasites maximize fitness by balancing the amount of high-quality resources they can extract against host immunity. Indeed, the size and nutritional quality of the last blood meal determine the survival of parasites (Nayar and Sauerman 1975; Canyon et al. 1999; Lehane 2005), parameters that are also negatively affected by the strength of host immune defenses (Wikel 1996; Lehane 2005). We therefore measured blood meal size and survival in the blood-sucking louse fly *Crataerina melbae* (Hippoboscidae; Diptera) in relation to the nutritional status and immunity of nestling Alpine swift (*Apus melba*) hosts.

Crataerina melbae are found in high numbers exclusively on adults and nestlings of *A. melba* (Tella et al. 1998) and impose significant reproductive costs on their host (Bize et al. 2003*b*, 2004). Both sexes feed on blood on a daily basis and can live for several weeks on the body of their host (Bequaert 1953; P. Bize, personal observation). One larva develops at a time in the maternal abdomen (viviparity) until the prepupal stage, when it is released near or inside the host nests and then pupates immediately. *Crataerina melbae* females produce several pupae in succession throughout the nestling period, and pupae do not hatch before the following year (P. Bize, personal observation). *Crataerina melbae* is flightless but can rapidly switch hosts and nests on foot (Bize et al. 2003*a*). As an index of nestling immunity, we measured cutaneous immunity in nestlings using the phytohemagglutinin (PHA) skin test. This immunological assay consists of injecting young subcutaneously with the mitogen PHA and measuring the local proliferation of T lymphocytes and granulocytes at the site of injection 24 h later (Smits et al. 1999; Martin et al. 2006). Host cutaneous immunity (i.e., adaptive and innate immunity) renders the biting site unfavorable for the feeding process in blood-sucking insects (Wikel 1996; Kamhawi et al. 2000; Lehane 2005). Host cutaneous immunity as measured by the PHA skin test has been previously demonstrated to rely on host nutritive resources, with hosts in better condition mounting a stronger immune response against PHA (Alonso-Alvarez and Tella 2001; Martin et al. 2006).

To investigate performance (and by extension fitness) of the louse fly *C. melbae* in relation to condition and immunity of nestling Alpine swifts, we measured the survival of 1,288 louse flies collected on 217 nonexperimental nestlings. This observational data set was used as a first step to explore whether the reaction norm of louse fly survival in relation to nestling body condition follows a bell curve. Indeed, we predicted that if louse flies feeding on nestlings in poor condition do not have access to adequate resources and if nestlings in good condition fight back to limit louse flies' food intake, parasite survival should be maximal on hosts in intermediate condition. In a second step, we carried out two experiments to test the effects of host nutritive resources and immunity on louse fly meal size and survival. To demonstrate experimentally that a poor nestling body condition negatively affects blood meal size and in turn survival of louse flies, we used neck collars (Dyrcz and Flinks 2003) to reduce nestling food supply and body condition. We predicted that louse flies would take a smaller blood meal and have a shorter life span on food-deprived nestlings than on control nestlings. To demonstrate that a potent cutaneous immune response of Alpine swift nestlings negatively affects blood meal size and in turn survival of louse flies, we supplemented nestlings early in their development with either a solution of methionine or water (to obtain a control group). Methionine is an essential amino acid that can be catabolized to cysteine and incorporated into glutathione, and glutathione is known to improve the production and acti-

vation of T cells (Dröge et al. 1986) and ultimately to lead to stronger PHA responses, as recently highlighted in three wild bird species (magpie *Pica pica*: Soler et al. 2003; blue tit *Parus caeruleus*: Brommer 2004; great tit *Parus major*: Tschirren and Richner 2006). We predicted that nestlings fed with methionine would have a higher cell-mediated immune response (and in turn a higher cutaneous immune response) than control nestlings fed with water and that louse flies would take a smaller blood meal and be shorter lived on methionine- than on water-supplemented nestlings.

Methods

Observational Data: Relationships among Host Body Condition, Host Cutaneous Immunity, and Louse Fly Survival

In 2004 we collected 739 female louse flies on 131 nestlings from 55 broods in Bienne, Switzerland, and 549 female louse flies on 86 nestlings from 36 broods in Solothurn, Switzerland. This was done when young were 30 days of age, which is the time when the population of louse flies is at its peak (Bize et al. 2003*b*). On average, eight (mean ± SE = 6.92 ± 0.15 louse flies per nestling; range: 1–9 louse flies) louse flies collected on the same nestling were kept together in a 50-mL Falcon tube pierced with small holes. Tubes were stored at 24° ± 1°C in a dark room and checked every 12 h at 0800 and 2000 hours to record mean louse fly survival per tube. We measured louse fly survival in females rather than in males because females were producing pupae (Bequaert 1953), and thus female louse fly performance is likely to be more related to host quality than male performance. Female louse fly survival was not significantly related to the number of individuals stored per tube (Pearson correlation: $r = 0.09$, $P = .22$, $n = 186$ nestlings/tubes). We calculated nestling body condition as the second axis (PC2) of a principal components analysis with body mass (eigenvector: 0.85), wing length (−0.35), and sternum size (−0.39) as factor loadings. High PC2 values indicate that nestlings were heavy for their size. A PC2 index provides similar information as residuals extracted from a linear regression of body mass on wing length and sternum size (Pearson correlation between PC2 and residual body mass, $r > 0.95$), and Ardia (2005) has shown in nestling European starlings (*Sturnus vulgaris*) that residual body mass is an accurate predictor of lipid reserves. The first axis (PC1) of this principal components analysis was an indicator of nestling body size (eigenvectors for body mass: 0.52; wing length: 0.60; sternum: 0.60; Bize et al. 2006*b*). The PC1 and PC2 axes explained 80.8% and 15.2% of the variance, respectively. We assessed the ability of 30-day-old nestlings to mount a cutaneous immune response by injecting them subcutaneously in the wing web with 0.1 mg PHA (Sigma, code L1668) dissolved in 0.02 mL of phosphate-buffered saline and by subsequently measuring wing web swelling at the site of injection 24 ± 1 h later (Smits et al. 1999; Martin et al. 2006). The same trained person (P. Bize) measured wing web swelling of each nestling with a micrometer (Mitotuyo, ref. 2046FB-60) to the nearest 0.01mm; it has been demonstrated elsewhere that our method of assessing nestling immune response against PHA is reliable (Bize et al. 2005).

Experiment 1: Food Deprivation

In 2005 in Bienne, we deprived 12 nestlings of food using neck collars (Dyrcz and Flinks 2003) for 7 h/day between 1100 and 1800 hours during three consecutive days. In brief, nestlings were fitted with a neck collar that prevented them from swallowing the food bolus brought by their parents, and we visited nests at least every hour between 1100 and 1800 hours to remove food boluses from the mouths of nestlings. Food deprivation occurs naturally, with nestling Alpine swifts being able to fast during several consecutive days in periods of inclement weather (Bize et al. 2006*a*). As a control, we applied the same procedure on 10 additional nestlings, except that they were hand fed with the food brought by their parents. At the start of the experiment, there was no difference in age (mean ± SE = 32.6 ± 1.3 days), body size, and mass between food-deprived and control nestlings (Student's *t*-tests, all $P > .60$). Swifts forage exclusively on aerial insects, and therefore we used neck collars during periods of prime weather condition to ensure that control nestlings were adequately provisioned. At the end of the neck-collaring experiment (i.e., nestlings were 35.6 ± 1.3 days old), we measured louse fly blood intake using the following procedure. Experimental nestlings were deparasitized and individually placed in a closed 4.5-L box, and then eight female louse flies were gently placed on the plumage of each nestling and allowed to feed for 20 min. The eight female louse flies used to measure blood intake were collected 1 day before in randomly chosen nonexperimental nests, and thus louse flies fasted for 24 h before their blood intake was measured. Mean blood intake was calculated as the difference in body mass of the eight louse flies weighed together to the nearest 0.01 g just before they were put on nestlings and 20 min later. We examined whether the size of the blood meal was a determinant of louse fly survival by storing them in a 50-mL Falcon tube at 24° ± 1°C in a dark room as explained above. Host body condition indexes were calculated as previously described in the observational data set.

Experiment 2: Methionine Supplementation

In 2005 in Solothurn, we supplemented 27 nestlings from 12 broods at the age of 6, 8, 10, 12, 14, 16, 18, 20, 23, 26, and 30 days with a solution of 0.1 g DL-methionine (Sigma, code M-9500)/mL of water. The dose of methionine was based on administering 1 mg methionine/g nestling body mass, which is similar to the dosage provided to poultry chickens (Subcommittee on Poultry Nutrition 1994). As a control treatment, we supplemented 32 nestlings from 14 other broods with a water dose. There was no difference in hatching date, clutch size, and brood size at hatching between treatments (Wilcoxon signed-rank test, all $P > .29$). At 30 days after hatching, nestlings supplemented with methionine or water were similar in body size and mass (all $P > .56$). We measured nestling cutaneous immunity with the PHA skin test at day 30 after hatching as described in the observational data set. Louse fly blood intake and survival were measured on the senior offspring of each experimental brood at day 31 after hatching, using the same protocol as described in experiment 1. Host body condition indexes were calculated as previously described in the observational data set.

Ethical Note

All procedures described were approved by the veterinary offices of Bern and Solothurn cantons. All food-deprived nestlings regained body mass in the days following the neck-collaring period (body mass of food-deprived nestlings vs. control nestlings at 50 days of age: 90.2 ± 5.2 vs. 92.2 ± 4.2 g; Student's t-test: $t = 0.28$, $P = .79$, $n = 9$ out of the 18 experimental birds were weighed at 50 days), and ultimately, they all successfully fledged. Methionine supplementation had no apparent negative effects on the health of nestlings: we found no significant difference between methionine- and water-supplemented nestlings in the growth of body mass, wing length, and sternum and in survival up to fledging (all $P > .26$).

Statistics

Observational data set. When analyzing louse fly survival, we statistically controlled for the nonindependence of louse flies collected on the same nestling by calculating a mean louse fly survival per nestling, and we statistically controlled for the nonindependence of louse flies collected on nestlings sharing a same nest by entering the nest of rearing as a random factor. In the same vein, when analyzing nestling cutaneous immune response, we statistically controlled for the nonindependence of nestlings sharing a same nest by entering the nest of rearing as a random factor. We included host squared body condition and squared body size in our statistical model to test for bell-shape relationships with louse fly survival and host cutaneous immune response.

Experiment 1. When analyzing the effect of host food deprivation on louse fly blood intake, we statistically controlled for the nonindependence of louse flies collected on the same nestling by calculating a mean louse fly meal size per nestling, and we statistically controlled for the number of louse flies that fed with certainty on nestlings by including the proportion of louse flies with blood in their guts (i.e., red abdomen) as a first covariate. The mean body mass of a louse fly just before a blood meal was entered as a second covariate because lighter louse flies are probably hungrier. When analyzing louse fly survival, we statistically controlled for the nonindependence of louse flies that fed on the same nestling by calculating a mean louse fly survival per nestling.

Experiment 2. When analyzing the effect of host cutaneous immunity on louse fly blood intake, the same statistical procedure as in the experiment 1 was applied, with the exception that indexes of host body size (PC1 and squared PC1) and body condition (PC2 and squared PC2) were also included in our starting statistical models to test for potential interactive effects of host immune responses and conditions on louse fly performance.

Analyses were performed using JMP 6 (SAS Institute, Cary, NC); tests were two tailed, and P values $<.05$ were considered significant. Preliminary analyses were conducted with a full model; nonsignificant terms, starting with nonsignificant interactions, were then backward dropped from our final models. All the variables entered in the starting models are reported in the "Results": for each analysis, significant variables kept in the final model are reported first, followed by nonsignificant variables dropped from the final model. In the mixed models, the variance matrix of the fixed effects was always modified to include a Kackar-Harville correction, and denominator degrees of freedom were approximated by the Satterthwaite method (JMP Statistics and Graphics Guide, release 6). Note that we performed analyses on mean louse fly survival per nestling rather than individual louse fly survival values (i.e., with nestling host as an additional nested stratum in the statistical models). This choice was constrained by the fact that we measured mean rather than individual louse fly meal size per nestling, and thus we had to calculate mean louse fly survival per nestling to analyze the consequences of "mean" meal size on "mean" survival. The use of mean louse fly survival per nestling allowed us to keep a statistical homogeneity throughout the article, and hence to increase the readability and understandability of our analyses.

Results

Observational Data: Relationships between Host Body Condition and Cell-Mediated Immunity and Louse Fly Survival

Louse fly survival was the highest when feeding on nestling Alpine swifts in intermediate body condition (three-way mixed-model ANCOVA; body condition: $F = 6.44$, df $= 1, 180.8$, $P = .012$; squared body condition: $F = 5.54$, df $= 1, 171.9$, $P = .020$; fig. 1*a*). On average, louse flies collected in Solothurn survived for a longer period than louse flies collected in Bienne (least squares means [± 1 SE] louse fly survival in Solothurn versus Bienne: 3.14 ± 0.08 days vs. 2.90 ± 0.07 days; $F = 5.04$, df $= 1, 80.1$, $P = .028$). Louse fly survival was not explained by variation in body size (body size: $F = 0.06$, df $= 1, 172.3$, $P = .80$; squared body size: $F = 0.09$, df $= 1, 121.7$, $P = .76$; these two terms were dropped from the final model). The final mixed model explained 77% of the variation in louse fly survival.

The PHA immune challenge in nestling Alpine swifts showed that individuals that were large and in prime condition just before the injection and that increased in body mass in the following 24 h to a large extent had the most potent cell-mediated immune response (three-way mixed-model ANCOVA where we controlled for the nonindependence of nestlings reared in the same nest by including the nest of rearing as a random factor; body size before injection: $F = 19.62$, df $= 1, 145.8$, $P < .0001$; body condition before injection: $F = 17.80$, df $= 1, 127.3$, $P < .0001$; change in body condition in the next 24 h: $F = 13.09$, df $= 1, 145.9$, $P = .0004$; fig. 2). These relationships were linear, as illustrated by the nonsignificant effects of the terms "squared body size" ($F = 3.20$, df $= 1, 143.4$, $P = .08$), "squared body condition" ($F = 2.54$, df $= 1, 142.9$, $P = .11$), and "squared change in body condition" ($F = 0.40$, df $= 1, 141.4$, $P = .53$). Nestlings in Bienne and Solothurn mounted a similar response against PHA ($F = 0.04$, df $= 1, 65.1$, $P = .85$). These four nonsignificant terms (i.e., squared body size, squared body condition, squared change in body condition, and colony) were dropped from the final model presented above. The final mixed model explained 60% of the variation in nestling response against PHA.

To examine the relative contribution of host body condition and cutaneous immunity on louse fly survival, we reran analyses where both indexes of host body size and body condition as well as measures of host cutaneous immunity were entered as explanatory variables in our starting model. This analysis showed, in agreement with our predictions, that the decrease in mean louse fly survival from hosts in intermediate to prime condition (i.e., right side of fig. 1*a*) was significantly associated with variation in host cutaneous immunity (three-way mixed-model ANCOVA: $F = 5.52$, df $= 1, 140.7$, $P = .020$; fig. 1*b*) and not with variation in host body condition ($F = 2.42$, df $= 1,141$, $P = .12$; this variable was dropped from the final model). The decrease in mean louse fly survival from hosts in intermediate to poor condition (i.e., left side of fig. 1*a*) was explained by host variation in squared body condition ($F = 4.06$, df $= 1, 141.9$, $P = .046$). This model also showed that louse flies lived longer when collected on highly infested hosts (i.e., positive correlation between louse fly survival and abundance; $F = 5.06$, df $= 1, 138.1$, $P = .026$). There were no significant effects of host body size ($F = 0.38$, df $= 1, 141$, $P = .54$), host

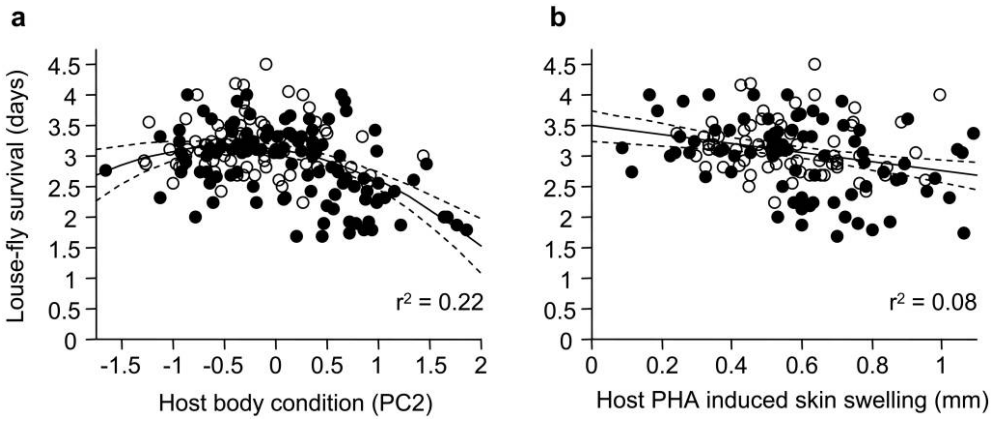

Figure 1: Louse fly survival in relation to body condition (*a*) and phytohemagglutinin (PHA)-induced skin swelling (*b*) in nonmanipulated nestlings in the colonies located in Solothurn (*open circles*) and Bienne (*filled circles*). Regression lines are shown with 95% confidence intervals; the proportion of the variance around the mean explained by the regression line (r^2) is reported. Note that the figures show regression lines on raw data, whereas statistics in the "Results" are based on mixed models.

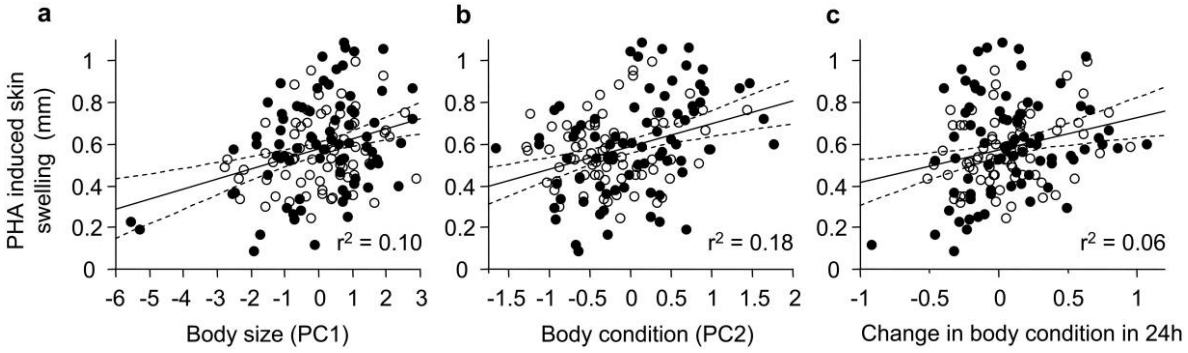

Figure 2: Phytohemagglutinin (PHA)-induced skin swelling in relation to body size (*a*), body condition just before injection (*b*), and change in body condition in the 24 h following injection (*c*) of nonmanipulated nestlings in the colonies located in Solothurn (*open circles*) and Bienne (*filled circles*). Linear regressions are shown with 95% confidence intervals; proportion of the variance around the mean explained by the model (r^2) is reported. Note that the figures show regression lines on raw data, whereas statistics in the "Results" are based on mixed models.

squared body size ($F = 0.13$, df $= 1, 138.1$, $P = .72$), and colony ($F = 1.68$, df $= 1, 65.5$, $P = .20$) on louse fly survival, and thus these three terms were dropped from the final model. The final mixed model explained 62% of the variation in louse fly survival.

Experiment 1: Effect of Host Body Condition on Louse Fly Meal Size and Survival

Food-deprivation experiments significantly impaired body condition of food-deprived nestlings compared with control nestlings (Student's *t*-test: $t = 5.13$, df $= 20$, $P < .0001$; fig. 3*a*), and as predicted, louse flies took a 21.6% smaller blood meal on food-deprived nestlings (three-way ANCOVA: $F = 7.35$, df $= 1, 18$, $P = .014$; fig. 3*b*) after the proportion of louse flies that took a blood meal ($F = 31.59$, df $= 1, 18$, $P < .0001$) and louse fly mean mass before blood meal ($F = 6.82$, df $= 1, 18$, $P = .018$; lighter ectoparasites took a larger blood meal) were taken into account. Louse flies that took a larger blood meal survived for a longer period of time (two-way ANCOVA: $F = 7.97$, df $= 1, 20$, $P = .011$) independent of whether they fed on food-deprived or control nestlings ($F = 0.01$, df $= 1, 18$, $P = .95$; treatment by blood meal size interaction: $F = 0.01$, df $= 1, 18$, $P = .94$; this variable was dropped from the final model), indicating that blood meal size per se determines survival of this ectoparasite. Note that body condition of food-deprived (and control) nestlings in 2005 remained within the natural range of variation in nestling body condition observed in 2004 (fig. 4), and thus our results were not an experimental artifact due to the use of unsuitable hosts. The final mixed models explained 68% of the variation in louse fly meal size and 29% of the variation in louse fly survival.

Experiment 2: Effect of Host Cutaneous Immunity on Louse Fly Meal Size and Survival

Supplementation of methionine significantly improved nestling ability to mount an immune response against PHA by 17.7% (Student's *t*-test: $t = 2.68$, df $= 24$, $P = .013$; fig. 5*a*), and louse flies significantly reduced by 16.3% the quantity of blood taken on methionine-supplemented nestlings compared with water-supplemented nestlings (five-way ANCOVA; treatment: $F = 4.58$, df $= 1, 18$, $P = .046$; controlling for the proportion of louse flies that took a blood meal: $F = 68.52$, df $= 1, 18$, $P < .0001$; fig. 5*b*). The reduction in blood meal size was more pronounced in heavier louse flies feeding on methionine-supplemented nestlings compared with those feeding on water-supplemented nestlings (interaction between louse fly mean body mass before blood meal and treatment: $F = 7.70$, df $= 1, 18$, $P = .013$; fig. 6*a*) and in louse flies feeding on methionine-supplemented nestlings compared with those feeding on water-supplemented nestlings that had gained mass during the previous 24 h (interaction between nestling body mass change and treatment: $F = 5.25$, df $= 1, 18$, $P = .034$; fig. 6*b*). This shows that louse flies in good condition restrained from feeding on nestlings with experimentally improved immune systems and on nestlings that naturally increased in body mass, which should positively affect their immunocompetence (as shown above; see also Alonso-Alvarez and Tella 2001). In the same statistical model, we also took into account nestling squared body condition ($F = 5.48$, df $= 1, 18$, $P = .031$), as we previously showed that louse flies extract a lower amount of blood from hosts in poorer condition. As already shown above, louse fly survival increased with the size of the blood meal (two-way ANCOVA: $F = 7.31$, df $= 1, 24$, $P = .012$) independent

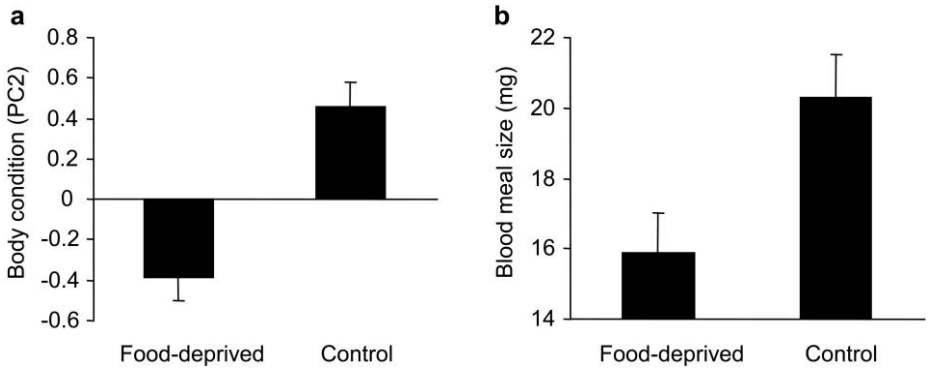

Figure 3: Body condition in experimentally food-deprived and control nestlings (*a*) and blood meal size of louse flies that fed on food-deprived and control nestlings (*b*). Least squares (LS) means ± 1 SE are presented; models from which LS means were extracted are described in "Results."

of treatment ($F = 0.50$, df $= 1, 22$, $P = .49$; treatment by blood meal size interaction: $F = 1.08$, df $= 1, 22$, $P = .31$), confirming the claim that blood meal size causally affects survival. The final mixed models explained 85% of the variation in louse fly meal size and 23% of the variation in louse fly survival.

Discussion

Understanding Parasite Strategies

This study builds on the predictions that parasite fitness is negatively affected by host immune response and is positively affected by the amount of nutritive resources extracted from the host body. Therefore, if parasites balance these two host components to maximize fitness, the most profitable hosts to parasites may be those in intermediate condition rather than those in poor condition, as a host-centered approach would predict (Christe et al. 1998). Accordingly, our findings show that louse flies lived longer when collected on nonexperimental nestling hosts in intermediate condition, and louse flies took a smaller blood meal and lived a shorter period of time when feeding on nestlings that were experimentally food deprived or had their cutaneous immune response boosted with methionine.

The biology of blood sucking in ectoparasites is complex (Wikel 1996; Lehane 2005), and thus several nonexclusive mechanisms can account for these results. Impaired performance of louse flies feeding on food-deprived nestlings can be attributed to the fact that louse flies take a smaller meal when feeding on a low-quality diet because costs associated with blood feeding are greater than benefits derived from the blood meal. Alternatively, variation, for instance, in host blood pressure, osmolarity, or viscosity may constrain the amount of blood louse flies are able to suck from their host (Lehane 2005). Similarly, impaired performance of louse flies feeding on methionine-supplemented nestlings can arise either because louse flies adjust their blood meal in relation to the benefits they will extract from the meal or because nestlings supplemented with methionine had not only an enhanced cutaneous immunity but also a higher amount of resources to allocate to alternative resistance strategies such as grooming (Clayton et al. 1999). Altogether, and whatever the exact mechanisms, our results suggest that louse flies achieved their best performance on nestlings in intermediate condition because nestlings in poor condition do not provide adequate resources (Nayar and Sauerman 1975; Canyon et al. 1999; Lehane 2005) and because nestlings in good condition are able to resist parasite exploitation (Wikel 1996; Lehane 2005; Tschirren et al. 2007). The fact that louse fly survival was related to the size of the last blood meal

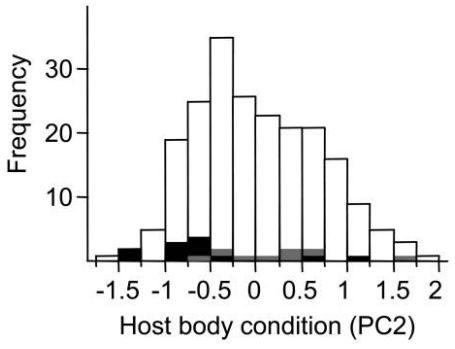

Figure 4: Body condition of experimentally food-deprived (*black bars*) and control (*gray bars*) nestlings in 2005 and of nonexperimental nestlings (*open bars*) in 2004. Nestling body condition was extracted from the second axis of a principal component analysis (see "Methods") after pooling the data collected in 2004 and 2005.

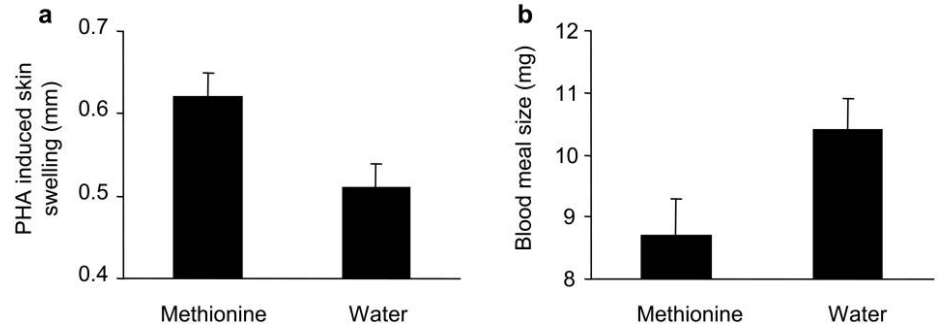

Figure 5: Phytohemagglutinin (PHA)-induced skin swelling of nestlings experimentally supplemented with methionine or water (*a*) and blood meal size of louse flies that fed on nestlings supplemented with methionine or water (*b*). Least squares (LS) means ± 1 SE are presented; models from which LS means were extracted are described in the "Results."

(this study and P. Bize, unpublished results) further indicates that in this system, parasite survival can be used as an accurate surrogate of host exploitation rate. In this context, it is interesting to notice that the absolute values of louse fly mean blood meal size (figs. 3*b*, 5*b*) and mean survival (fig. 1*a*) differed between colonies and years, which agrees with the idea that host exploitation rate is a dynamic process that can fluctuate over space and time (Lively 1999). Although the importance of incorporating measures of parasite performance (and in turn fitness) in host-parasite coevolutionary scenarios has been stressed in recent studies (Sukhdeo and Bansemir 1996; Poulin and Combes 1999; Thomas et al. 2002), we still know little about the relative effects of host immunocompetence, nutritional status, and other phenotypic traits on parasite fitness (e.g., Clayton et al. 1999; Jokela et al. 1999; Møller 2000; Roulin et al. 2001; Blanford et al. 2003; Pulkkinen and Ebert 2004; Krasnov et al. 2005; De Bellocq et al. 2006; Tseng 2006) in comparison to our knowledge of the effects of parasites on host fitness (Combes 2001). However, the present study is of importance because it highlights that the understanding of parasite strategies is a necessary step toward an appraisal of the outcome and evolution of host-parasite interactions.

Understanding Host Resistance Strategies

Hosts have evolved a wide array of resistance mechanisms among which the immune system is viewed as the most efficient one but probably also as the most costly one (Sheldon and Verhulst 1996; Wakelin 1996; Norris and Evans 2000). This study shows, in agreement with previous experimental studies (Alonso-Alvarez and Tella 2001; Martin et al. 2006), that the magnitude of the cutaneous immune response against PHA is condition dependent: young that were large, that were in prime condition, and that gained in condition in the 24 h following injection were the most immunocompetent. It also reveals that nestling PHA response explained louse fly meal size and survival, which indicates that this immune assay can provide valuable information on the ability of hosts to resist parasitic attack in the wild by blood-sucking insects (Tschirren et al. 2007; but see De Bellocq et al. 2006). The central point of our study, however, is that single measures of host resistance mechanisms (such as cutaneous immune response) are not sufficient to predict parasite fitness because louse flies had reduced life span both on nestlings in prime condition with a potent immune response and on nestlings in low condition with a weak immune response (see also Roulin et al. 2007). In other words, it shows that considering mainly host immunocompetence as the key determinant of parasite fitness, as is usually done in immunoecology, can give a biased appraisal of the outcome and evolution of host-parasite interactions. The fact that louse flies took a smaller blood meal and lived a shorter period of time when feeding on food-deprived nestlings suggests that facultative anorexia might be another host strategy to resist parasites. Indeed, facultative anorexia is a classical symptom of infection in numerous animals, and it has been suggested that it plays an important role in fighting back parasites and pathogens (Exton 1997). Yet in the Alpine swift, the occurrence of nestlings in poor condition may reflect a constraint rather than a facultative strategic response against louse fly infestation. Indeed, the Alpine swift is an aerial insectivorous bird, and nestlings frequently face periods of starvation in periods of inclement weather (Bize et al. 2006*a*). To survive these periods of starvation, nestlings build large reserves of energy that are unlikely to be trade-offs against parasite resistance. Accordingly, previous experimental manipulation of louse fly load in nests of the Alpine swift revealed no difference in feeding rates (Bize et al. 2004) and nestling body mass

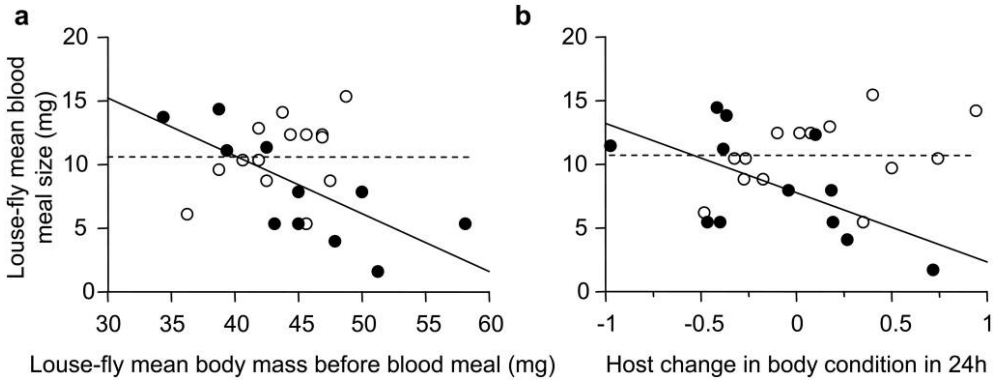

Figure 6: Louse fly mean blood meal size in relation to louse fly mean body mass before blood meal (*a*) and to host change in body condition in the 24 h immediately before blood meal (*b*). Louse flies were feeding either on methionine-supplemented nestlings (*filled circles*, *solid lines*) or on water-supplemented nestlings (*open circles*, *dashed lines*).

(Bize et al. 2003*b*) between parasitized and deparasitized treatments. Furthermore, figure 2*b* indicates that louse flies' performance can be ordered in three classes, where nestling swifts in intermediate condition are the most profitable, followed by nestlings in poor condition and nestlings in prime condition. That is, in the present system, nestlings are expected to actively fight back louse flies through their ability to mount a potent cutaneous immune response (or any other antiparasite strategies positively associated with host condition), while it is unlikely that nestlings use facultative anorexia as an active strategy to resist parasites.

Perspectives

We emphasize that in order to understand the coevolutionary arms race between parasites and hosts, one important first step is to establish how parasites balance the costs and benefits of host exploitation in relation to host condition, and in turn to identify which are the most profitable hosts to parasites. However, because parasites (and hosts) greatly differ in their ecological requirements and life-history traits, one can expect that not all parasites will prefer hosts in intermediate condition and that the balance may be shifted toward hosts in good or in poor condition depending on the host-parasite system (Roulin et al. 2003). Apart from the present study, maximal parasite fitness on hosts in intermediate condition has been found in two endoparasite species, namely, microsporidian (Bedhomme et al. 2004) and malarial parasites (Lambrechts et al. 2006) infesting mosquitoes. Although host condition is expected to have greater effects on the behavior of endoparasites because they cannot move from one host to another (Sukhdeo and Bansemir 1996; Poulin and Combes 1999; Thomas et al. 2002), the same can be true in ectoparasites, as shown here. The louse fly *Crataerina melbae* is flightless and has a vertical transmission in the colony due to nest reuse by their hosts, and prolonged periods of poor weather are the major factor modulating nestling body condition in the whole colony. Thus, as in endoparasites, there might be selection on louse flies to avoid overexploiting nestling hosts in poor condition and colony failure. Indeed, louse flies in the colony are probably genetically related because they cannot easily move between colonies, and louse flies will pass on genes to the next generation only if their hosts survive and return to the colony in the following years. If true, we can thus expect that parasite transmission mode (i.e., mobility) and feeding patterns (multiple vs. single feeding on the same host) should be key factors shaping the cost/benefit balance of parasite exploitation strategies. Information on louse fly fecundity is also required to test how blood meal size and fecundity co-vary, and thus how louse flies maximize lifetime reproductive success in relation to host body condition and associated qualities such as host nutritional status and immunity.

A second important point that has to be considered in future studies is what component of host resources is maximized by parasites: absolute or relative levels? Here, we found that louse fly performance was associated with relative levels of host resources (i.e., body condition) but not with absolute levels (i.e., body size), at least, in the limited range of host age and size used in this study. Because host body condition but not body size can fluctuate over short periods of time, one hypothesis is that parasites can evolve the ability to quickly adjust their performance, such as food intake, in response to short-term variations in host condition. Alternatively, we can expect that host body size should lead to long-term behavioral adjustment by parasites, such as settlement and dispersal decisions (Valera et al. 2004), when

hosts of different size provide different fitness returns over the long term. Interestingly, additional analyses on the observational data set, where louse fly load is entered as a dependent variable in place of louse fly survival, show that the number of louse flies per nestling reached an asymptote on hosts that were large (body size: $F = 118.64$, df $= 1, 184$, $P < .001$; squared body size: $F = 7.19$, df $= 1, 184$, $P = .008$) but in poor condition ($F = 4.35$, df $= 1, 184$, $P = .039$; see also fig. A1 and the "Results" in the appendix). Thus, louse flies (and parasites in general) may balance different host qualities when choosing, first, which host to go to/stay on, and second, how to exploit host resources. Altogether, this study suggests that parasites may show plastic and complex behavioral responses in the different steps of host-parasite interactions, hence emphasizing the need to take into account behavioral plasticity by parasites to appraise the whole diversity of host-parasite relationships (Sukhdeo and Bansemir 1996; Poulin and Combes 1999; Thomas et al. 2002).

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APPENDIX

Observational Data: Relationships between Host Body Condition and Body Size and Louse Fly Distribution

Statistics

When analyzing louse fly load, we used general linear models in SAS (proC GLIMMIX in SAS, ver. 9.1; SAS, Cary, NC) with an explicitly defined Poisson error structure (Wilson et al. 1996). We statistically controlled for the nonindependence of nestlings sharing a nest by entering the nest of rearing as a random factor. We included host squared body condition and squared body size in our starting statistical model to test for bell-shape relationships with louse fly load. Preliminary analyses were conducted with a full model; nonsignificant terms ($P > .05$), starting with nonsignificant interactions, were then backward dropped from our final model.

Results

Louse flies were found in higher numbers on nestling Alpine swifts that were large ($F = 118.64$, df $= 1, 184$, $P < .001$) but in poor condition ($F = 4.35$, df $= 1, 184$, $P = .039$). There was also a significant effect of nestling squared body size ($F = 7.19$, df $= 1, 184$, $P = .008$) on louse fly load, which is explained by the fact that the number of louse flies per nestling was reaching an asymptote (at ~31 louse flies/nestling) on large hosts. Louse fly load was not explained by the colony where the nestling was sampled ($F = 3.80$, df $= 1, 73.8$, $P = .06$) or by variation in squared nestling body condition ($F = 0.48$, df $= 1, 182$, $P = .49$); these two terms were dropped from the final model.

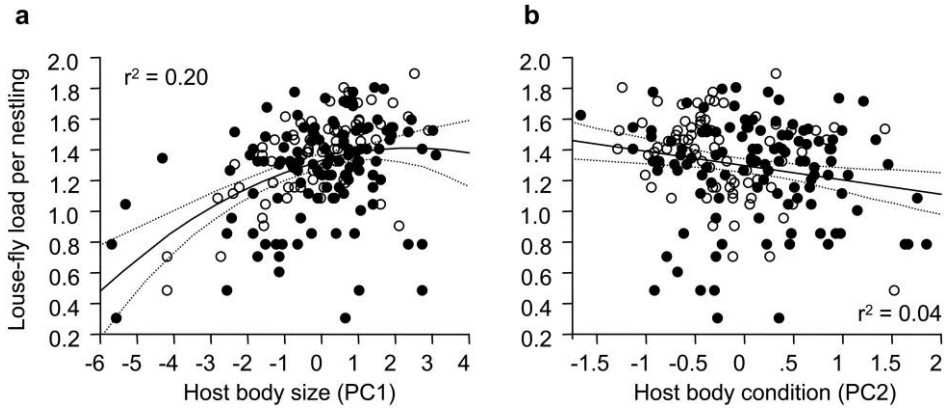

Figure A1: Louse fly load per nestling in relation to body size (*a*) and body condition (*b*) of nonmanipulated nestlings in the colonies located in Solothurn (*open circles*) and in Bienne (*filled circles*). Regression lines are shown with 95% confidence intervals; proportion of the variance around the mean explained by the model (r^2) is reported. Note that the figures show regression lines on log-transformed raw data, whereas statistics in the "Results" are based on general linear models with an explicitly defined Poisson error structure.

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