doi: 10.1111/j.1420-9101.2004.00774.x

Coevolutionary arms races: increased host immune defense promotes specialization by avian fleas

A. P. MØLLER,* P. CHRISTE† & L. Z. GARAMSZEGI*,‡

*Laboratoire de Parasitologie Evolutive, Université Pierre et Marie Curie, Paris, France
†Departement d'Ecologie et d'Evolution, Université de Lausanne, Lausanne, Switzerland
‡Department of Biology, University of Antwerp, Antwerp, Belgium

Keywords:

birds;
coevolution;
fleas;
immunity;
speciation;
T-cell mediated immune response.

Abstract

We investigated the relationship between host defense and specialization by parasites in comparative analyses of bird fleas and T-cell mediated immune response of their avian hosts, showing that fleas with few main host species exploited hosts with weak or strong immune defenses, whereas flea species that parasitized a large number of host species only exploited hosts with weak immune responses. Hosts with strong immune responses were exploited by a larger number of flea species than hosts with weak responses. A path analysis model with an effect of T-cell response on the number of host species, or a model with host coloniality directly affecting host T-cell response, which in turn affected the number of host species used by fleas, best explained the data. Therefore, parasite specialization may have evolved in response to strong host defenses.

Introduction

Hosts comprise the main environmental factor affecting the evolution of parasites, and given that parasites exploit limiting host resources, parasites have to adapt to their hosts in order to successfully survive and reproduce (Ehrlich & Raven, 1964; Gilbert & Raven, 1975; Thompson, 1994; Combes, 2001). Host specialization by parasites represents the reduction in number of host species on which a given parasite can successfully survive and reproduce, and such specialization implicitly implies that specialized parasites do better on their main host than on alternative hosts, whereas that is not the case for a generalist parasite exploiting several different hosts (Combes, 2001). This process gives rise to specialists with a small number of hosts and generalists with a large number of hosts. There are relatively few tests whether fitness of parasites is generally reduced on alternative hosts when a reduction in the number of host species has occurred (e.g. de Meeus *et al.*, 1990, 1995; Becnel & Andreadis, 2000; Kosoy *et al.*, 2000; Thresher *et al.*, 2000; Giorgi *et al.*, 2004), but we assume in the remaining part of this paper that a relatively higher fitness of an ectoparasite on a single host is more common when a parasite has few rather than many hosts. A reduction in the number of host species is thought to limit gene flow among parasite populations exploiting alternative hosts, thereby enhancing local adaptation and speciation (Price, 1980; Futuyma & Moreno, 1988; Thompson, 1994). Local adaptation occurs when there is geographical variation in the efficiency by which parasites exploit their hosts, depending on the balance between selection within and among host populations, and gene flow among host populations (Wright, 1978; Slatkin, 1987; Gandon *et al.*, 1996; Kaltz & Shykoff, 1998). Hosts are not passive victims of their parasites, but coevolve with these by producing efficient defenses that limit, reduce or eliminate the damage caused by parasites (Ehrlich & Raven, 1964; Gilbert & Raven, 1975; Thompson, 1994; Combes, 2001). The role of the host and its anti-parasite defenses in parasite specialization (the evolution of a small number of host species) largely remains undetermined, and the present study represents an attempt to address this problem using a comparative approach.

Correspondence: A. P. Møller, Laboratoire de Parasitologie Evolutive, CNRS UMR 7103, Université Pierre et Marie Curie, 7 quai St. Bernard, Case 237, F-75252 Paris Cedex 05, France.
Tel.: +33 144 272 594; fax: +33 144 273 516;
e-mail: amoller@snv.jussieu.fr

Here we suggest that host defenses play a crucial role in parasite specialization, and that adaptation by a given population of parasites to a given host and its defenses ultimately reduces the ability of that given parasite population to exploit other hosts. We use comparative analyses of bird fleas (Siphonaptera: Ceratophyllidae) and their avian hosts to unravel the relationship between parasite specialization and host defenses. Fleas of this monophyletic family are commonly found on birds and mammals, and the ability to infest birds has evolved relatively recently and independently within different flea genera (Traub *et al.*, 1983). Fleas suck blood from their hosts, and blood removal and the associated biochemicals injected into the host by the parasite are costly to the host (Marshall, 1981; Lehane, 1991). Fleas may cause severe fitness loss in their bird hosts (Brown & Brown, 1986; Richner *et al.*, 1993; Richner & Tripet, 1999), and there is thus intense selection on hosts to defend themselves against fleas (Christe *et al.*, 1996; Tripet & Richner, 1997; Tripet *et al.*, 2002a). It remains unknown whether host specialization by fleas causes a reduction in the reproductive performance of a specialist flea species on alternative hosts, although this would be expected to be the outcome of the specialization process. Previous work has shown that flea species parasitizing colonial birds have fewer host species than those infesting solitarily nesting hosts (Tripet *et al.*, 2002b). In addition, fleas of hosts with aggregated nests have lower mobility and a smaller geographical range (Tripet *et al.*, 2002a). Finally, colonial hosts harbour more flea species than solitary hosts, most of them specialists (Tripet *et al.*, 2002b). Therefore, fleas are known to specialize more frequently on colonial hosts (Tripet *et al.*, 2002a), and colonial hosts have stronger T-cell mediated immune responses than solitary hosts (Møller *et al.*, 2001). Although these findings are consistent with the suggestion that the spatial distribution of the host (or a factor correlated with host sociality) plays a major role for the evolution of specialization and speciation in fleas, the mechanism generating such an effect remains to be determined. We investigated different hypotheses linking host immune defense, host sociality and specialization by their flea parasites (see Materials and methods), using path analysis to identify the statistical model that explained most of the variance in the relationship among these three variables.

Avian hosts defend themselves against ectoparasites by behavioural, mechanical and immunological means (Marshall, 1981; Lehane, 1991). Host immune responses including T-cell mediated immune responses are an important component of defense against parasites including ectoparasites such as fleas (review in Wikel, 1996). Selection experiments on domesticated hosts or other means of increasing cell mediated immunity of hosts have resulted in reduced abundance of fleas (Wikel, 1996). We used a measure of T-cell mediated immunity (Goto *et al.*, 1978; McCorkle *et al.*, 1980; Abbas *et al.*, 1994), which is an important component of immunocompetence (National Research Council, 1992) that has evolved as a means of defense against ectoparasites. This response reflects the ability of individual birds to survive (Christe *et al.*, 1998; González *et al.*, 1999; Hörak *et al.*, 1999; Soler *et al.*, 1999; Merino *et al.*, 2000), and is a reliable indicator of the impact of parasites on nestling mortality across species of birds (Martin *et al.*, 2001). Furthermore, interspecific variation in this measure of T-cell mediated immunity is strongly positively correlated with the relative size of the thymus, which is the organ responsible for initial differentiation of T-cells (A. P. Møller and J. Erritzøe, unpublished data). Thus the magnitude of the T-cell mediated immune response reflects the magnitude of natural selection pressures from parasites. Finally, there is a significant additive genetic component of T-cell mediated immune response (Saino *et al.*, 1997; Brinkhof *et al.*, 1999; Christe *et al.*, 2000), providing the raw material for evolutionary changes in intensity of T-cell mediated immune response.

Materials and methods

Information on flea specialization

The abundance of flea species was quantified from information as reported in Traub *et al.* (1983), who provided all information on host taxa of flea species and subspecies. Hosts were described by Traub *et al.* (1983) as main hosts, if exploited commonly by a species or subspecies of flea, or as accidental hosts, if only occasionally exploited. As we did *not* make this distinction ourselves, this categorization of fleas cannot have been biased by our hypotheses or predictions. Tripet *et al.* (2002b) have shown that fleas of main hosts and accidental hosts differ significantly with respect to degree of host sociality, but also with respect to geographical range of fleas. We did find statistically significant correlations between some of the different variables describing flea specialization and abundance, these variables being number of main hosts, number of accidental hosts, and number of species of flea per host (log-transformed data: no. main hosts – no. accidental hosts: $r = 0.432$, $t = 3.25$, d.f. $= 46$, $P < 0.01$; no. main hosts – no. species of flea per host: $r = -0.551$, $t = 4.47$, d.f. $= 46$, $P < 0.0001$; no. accidental hosts – no. species of flea per host: $r = 0.241$, $t = 1.69$, d.f. $= 46$, $P < 0.05$). This shows that the three variables are not completely independent. However, the maximum amount of variance explained by these correlations was 30%, implying that 70% or more of the variance remained unexplained. For each flea species (or subspecies) we calculated mean nestling T-cell response of each of its hosts and mean number of species of flea per host species. For each host species we calculated the mean number of main hosts and the mean number of accidental hosts exploited by each of its species (or subspecies) of fleas.

Information on T-cell response

We used the T-cell mediated immune response of nestlings at an age of two-thirds of their nestling period, to standardize for rate of development, to a challenge with the mitogenic phytohemagglutinin, which is a standard estimate from the poultry literature of the ability to produce a T-cell mediated immune response (Goto *et al.*, 1978; McCorkle *et al.*, 1980). Injection with phytohemagglutinin results in local activation and mitogenic proliferation of T-cells, followed by local recruitment of inflammatory cells and major histocompatibility complex molecules (McCorkle *et al.*, 1980; Abbas *et al.*, 1994). Information on mean T-cell mediated immune response of nestlings was obtained from a number of other sources and ourselves. Exactly the same amounts of mitogen and the same instruments were used in this study and the studies by Møller *et al.* (2001), Moreno *et al.* (1999), J. Blount (personal communication), Johnsen *et al.* (2000), M. Soler, M. Martín-Vivaldi, J.M. Marín and A.P. Møller (personal communication), S. Schjørring (personal communication), K. McCoy (personal communication), Hoi-Leitner *et al.* (1999), whereas a slightly different instrument was used for measuring the response by Soler *et al.* (1999) and J. Fair (personal communication). It has been shown elsewhere that estimates of T-cell response from different sources are highly repeatable even when the amount of mitogen injected and the instrument used for measuring the response is not exactly the same (Tella *et al.*, 2002). The sources for the data are clearly listed in Appendix 2. During the breeding seasons 2000–2002 we spent large parts of April-June searching for nests of birds, in which nestlings could be tested for cell-mediated immune response. This was done in Switzerland for *Pyrrhocorax graculus* and in Northern Denmark by APM for the remaining species. We requested information on nests from a number of amateur ornithologists with a good knowledge of birds, and the nests recorded in this way were monitored until the test was being performed. Before injection we removed the feathers from a small spot of skin on the wing web (patagium) of the right and the left wings and marked the sites of injection with a permanent, water-resistant colour marker. We then measured the thickness of the skin to the nearest 0.01 mm with a pressure-sensitive caliper (Teclock SM112, Teclock, Japan). For each wing web we made three measurements to quantify measurement error. As in previous studies we found highly repeatable measurements, with repeatabilities above 0.95. This is similar to our previous studies of T-cell mediated immunity (e.g. Saino *et al.*, 1997; Soler *et al.*, 1999; Martin *et al.*, 2001; Merino *et al.* 2001). Subsequently, we injected 0.02 mg phytohemagglutinin dissolved in 0.04 mL physiological water in one wing web, and 0.04 mL physiological water in the other wing web. Approximately 24 h later we re-measured the thickness of the skin at the two sites of injection, as described above. The index of cell-mediated immune response was simply calculated as the difference in thickness of the wing web injected with phytohemagglutinin 24 h after and just before injection, minus the difference in thickness of the wing web injected with physiological water. Thus, the measure of response is expressed in mm. We calculated mean responses for each brood and then calculated an overall mean based on these brood mean values. For each flea species we obtained an estimate of T-cell response of its hosts by calculating the mean value of the mean T-cell responses for all host species for which we had information (as listed in Appendix 2). In many cases we only have T-cell mediated immune response for a few of the host species exploited by a given flea species. Hence, the number of host species exploited by a given flea species differs from the number of species for which we have obtained information on T-cell response.

We obtained information on body mass from our own field measurements, when available, or from Dunning (1993). In the comparative analyses of hosts, we adjusted for differences in T-cell mediated immune response among species of hosts because of differences in body mass by including mean body mass of hosts as an additional independent variable in the statistical analyses (see Comparative methods). We did not include body mass of hosts in the analyses of parasites because we considered that the main obstacle for parasites would be the immune defenses raised by their hosts independent of body size. We did redo all the analyses by including body mass of the host as an additional independent variable, but that did not change any of the conclusions.

Information on host coloniality

Host species were classified with respect to the most common spatial distribution of nest sites using the exact information given in Cramp *et al.* (1982–1994). Hosts were assigned a value of 1 for territorial birds (uniform distribution of nests), a value of 2 for birds nesting in loose mixed-species colonies (partial aggregation of nests), and a value of 3 for nesters commonly forming monospecific aggregates or colonies (fully aggregated nests, referred to as 'colonial nesters'). See Tripet *et al.* (2002a) for further information.

The two data sets on hosts and fleas are given in Appendices 1–2.

Comparative methods and phylogenetic information

We investigated the relationship between the evolution of host immune responses and species richness of fleas, although taking similarity because of common descent into account, using generalized least squares models as implemented in the program Continuous (Pagel, 1999). This approach allows tests for correlated evolution, and assessment of tempo and mode of evolution while taking

similarity due to common ancestry into account. In a first series of analyses, using hosts as the unit of analysis, we investigated whether flea species richness and specialization were related to host T-cell response. In a second series of analyses, using parasites as the unit of analysis, we investigated whether host T-cell response was related to flea species richness and specialization.

The phylogenetic approach relying on generalized least squares models characterizes evolutionary changes along each branch of a phylogenetic tree through the variance components of traits (Martins & Hansen, 1997; Pagel, 1997, 1999). Hypotheses are tested with likelihood ratio statistics. This compares the log-likelihood of the model corresponding to a null hypothesis (H_0) over the model for an alternative hypothesis (H_1), where the likelihood ratio $= -2 \log_e[H_0/H_1]$. The likelihood ratio statistic is asymptotically distributed as a chi-squared variate with degrees of freedom equal to the difference in the number of parameters between the two models. Models contain three scaling parameters that can be used to scale branch lengths in the tree (κ), scale total (root to tip) path in the tree (δ), and to assess the contribution of phylogeny (λ). We first assessed the contribution of scaling parameters, κ: branch length scaling factor, and λ: phylogeny scaling factor [recent simulations showed that the estimation of δ: overall path length scaling factor is biased (Freckleton *et al.*, 2002), and thus we avoided estimating this parameter]. We did not assess the contribution of the branch length scaling factor (κ) when we used the parasites as the unit of analysis, because the corresponding tree was ultrametric (Fig. 1). Once an appropriate model with adjusted scaling parameters had been

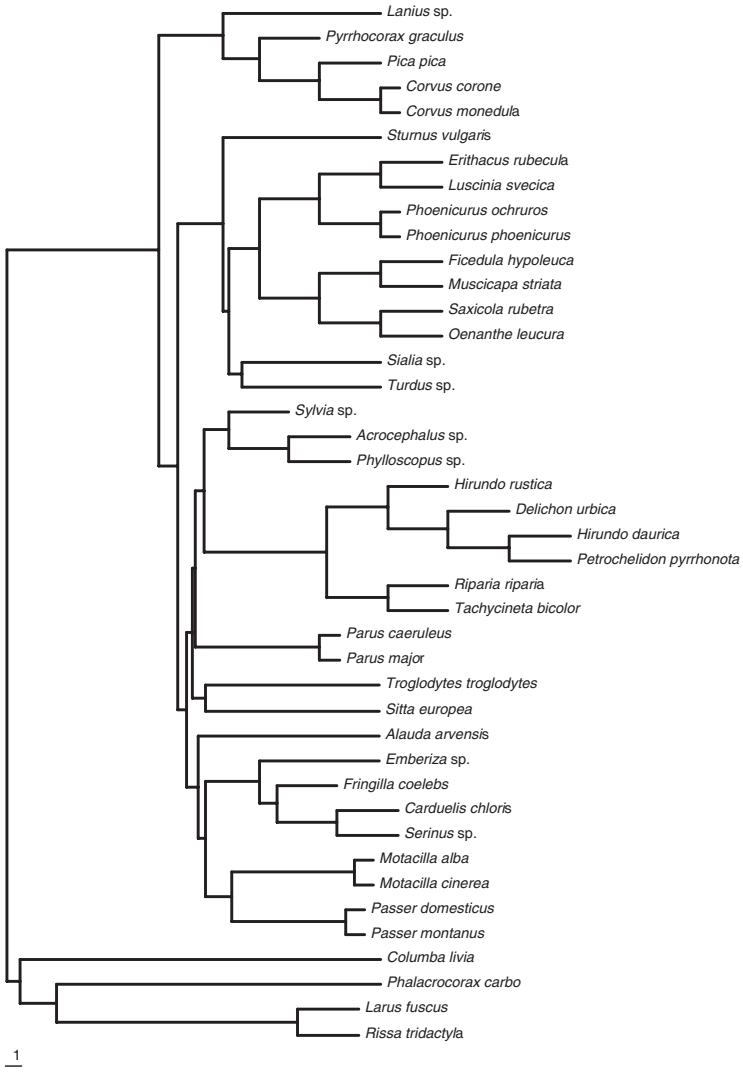

Fig. 1 A phylogeny of avian species used for the comparative analyses of host immune response, flea species richness and specialization. The phylogeny is based on that established by Sibley & Ahlquist (1990) from their analyses of DNA hybridization, and it is updated according to more recent literature (see Materials and methods). The scale for branch lengths is given in the bottom left.

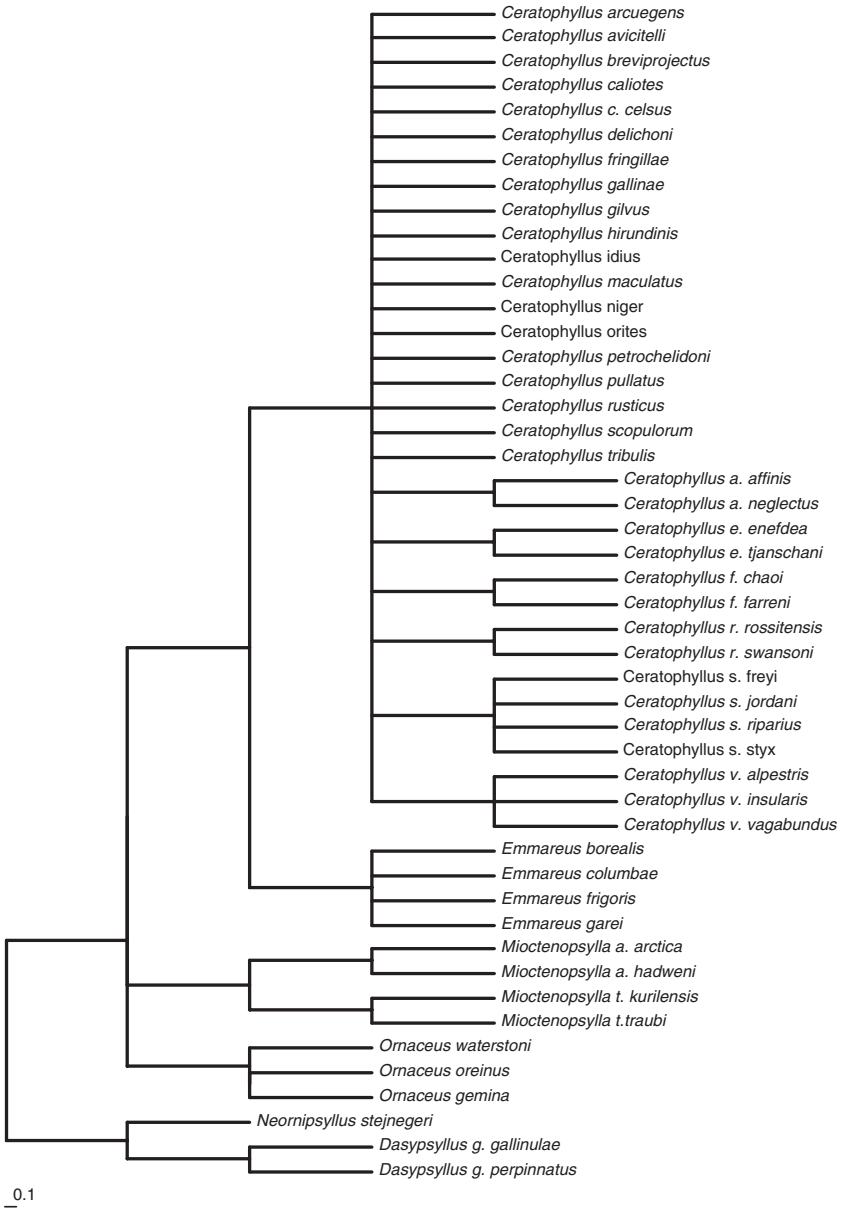

Fig. 2 A phylogeny of avian fleas of the Ceratophyllidae used for the comparative analyses of host immune response, flea species richness and specialization. The phylogeny follows Traub *et al.* (1983) and is based on morphological characters (see Materials and methods for details). Branch lengths are equal.

selected, we studied correlated evolution of traits of interest by comparing the goodness of fit of model H_0 fitted to the data by allowing only independent evolution with that alternative H_1 model that permits correlated evolution of the characters. The appropriate scaling parameters and the log-likelihood ratio statistics testing for correlated trait evolution are presented.

A phylogeny on avian hosts was constructed, based on Sibley & Ahlquist (1990) from analyses of DNA hybridization, and updated according to Sheldon & Winkler (1993) and Cibois & Pasquet (1999) (Fig. 1). A phylogeny on Ceratophyllid fleas was constructed following that in Traub *et al.* (1983), which is based on morphological characters, and assumes that the family is monophyletic (Fig. 2). This latter assumption was recently confirmed in a phylogenetic analysis of four genes from 160 genera of fleas (M. P. Whiting, personal communication). Traub *et al.*'s (1983) phylogeny is based on morphological characters that did not cause any circularity due to characters associated with specialization being used in both phylogeny reconstruction and analyses of comparative data. The use of the program Continuous requires that the phylogeny is rooted to achieve complete bifurcation, and we used the most likely root (M. P. Whiting and B. Lewis, personal communication). There are seven alternative ways of rooting the phylogeny, and analyses

Table 1 Generalized least squares analysis of the relationship between species richness of fleas and host T-cell response based on (a) the bird host phylogeny (Fig. 1; Sibley & Ahlquist, 1990) and (b) the flea phylogeny (Fig. 2; Traub *et al.*, 1983), using the program Continuous (Pagel, 1999). All calculations were based on transformed variables, as explained in the Methods. λ is the phylogeny scaling parameter and κ is the branch length scaling parameter. The likelihood ratio is the difference between the log-likelihood for a model with zero covariance and non-zero covariance. *P*-values indicate the significance of the log-likelihood ratio test for the correlated evolution of pairs of traits.

	κ	λ	Phylo-genetic correlation	LR	d.f.	*P*	*n*
(a) Analyses based on the bird host phylogeny, host T-cell immune response							
No. main hosts	0.419	0.832	−.613	9.904	1	<0.0001	42
No. accidental hosts	0.408	0.842	−.432	4.335	1	0.003	42
No. species of flea per host	1.000	0.691	0.278	1.688	1	0.066	42
(b) Analyses based on flea phylogeny, host T-cell immune response							
No. main hosts	1.000	0.318	0.557	8.909	1	<0.0001	48
No. accidental hosts	1.000	0.000	−.389	3.949	1	0.005	48
No. species of flea per host	1.000	0.000	0.382	3.776	1	0.006	48

with Continuous produced very similar results. We present the results that are based on the most likely phylogeny (Fig. 1), but computations relying on alternative rootings are given in Appendix 3. These computations revealed that the findings were independent of the exact way in which the phylogeny of fleas was rooted. Noteworthy, the low values for the phylogenetically scaling factor (λ) indicated that common ancestry does not confound the relationship between traits of interest (see Table 1 and Appendix 3). The phylogeny of fleas did not contain information on branch lengths, and thus we used equal branch lengths. For the phylogeny of birds we used the conventional approach applied by Bennett & Owens (2002). We applied branch lengths from the tapestry tree of Sibley & Ahlquist (1990) for higher taxonomic levels. Within families the distance between different genera was set to 3.4 **Δ**T50H units obtained from DNA-DNA hybridization, and between species within genera to 1.1 **Δ**T50H.

T-cell response and species richness and host distribution of fleas were $\log_{10}$-transformed before analyses. Møller *et al.* (2001) has shown that larger species of birds produce a stronger immune response, apparently because their larger circulatory system allowed accumulation of larger amounts of cells and other components of the immune response. In our data, based on the host phylogeny, there was no significant phylogenetic association between $\log_{10}$-transformed body mass and $\log_{10}$-transformed nestling T-cell immune response ($\kappa = 0.432$, $\lambda = 1.000$, LR = 0.394, phylogentic correlation: 0.136, $P = 0.375$). Although this relationship was significant when using the raw species data ($r = 0.393$, $P = 0.010$, $n = 42$), controlling for this allometry effect by calculating residuals from a regression of $\log_{10}$-transformed immune response on $\log_{10}$-transformed body mass did not change the conclusions (see Appendix 3). Similarly, estimates of species richness of parasites increase with study intensity (Walther *et al.*, 1995; Poulin, 1997). We used the number of studies published since 1945 on each bird as cited in the ISI Web of Science as a measure of study intensity (http://www.wos.isitrial.com/) (these data are listed in Appendices 1–2) We found that when using parasites as the units of analysis, measures of flea species richness and specialization were significantly related to study intensity (mean number of main hosts: $r = 0.588$, $P < 0.001$, mean number of accidental hosts: $r = 0.381$, $P < 0.01$, mean number of species of flea per host: $r = 0.582$, $P < 0.001$; $n = 48$). However, controlling statistically for this variable by calculating residuals from the regressions of $\log_{10}$-transformed measures of flea species richness and specialization on $\log_{10}$-transformed study intensity did not change the conclusions (see Appendix 4).

We investigated the relationships between host coloniality and host T-cell response, respectively, and number of host species of fleas using path analysis (Wright, 1968; Li, 1975). This was done by creating four different models to provide likely scenarios for causal relationships: (1) coloniality and T-cell response mutually influence each other and both affect number of host species of fleas, (2) coloniality and T-cell response affect number of host species of fleas independent of each other, (3) coloniality affects T-cell response which affects number of host species of fleas and (4) T-cell response affects coloniality which affects number of host species of fleas.

Results

In the first series of analyses we investigated the relationship between T-cell mediated immune response of hosts and flea specialization using the phylogenetic relationship among bird hosts as the framework for our comparative analyses. As the T-cell mediated immune response of hosts increased, the number of main host species and the number of accidental host species parasitized by their flea species decreased (Fig. 3a, b; Table 1). The distribution of data points was clearly triangular with host species with weak immune responses supporting flea species with both few and many hosts, whereas hosts with strong immune responses were only

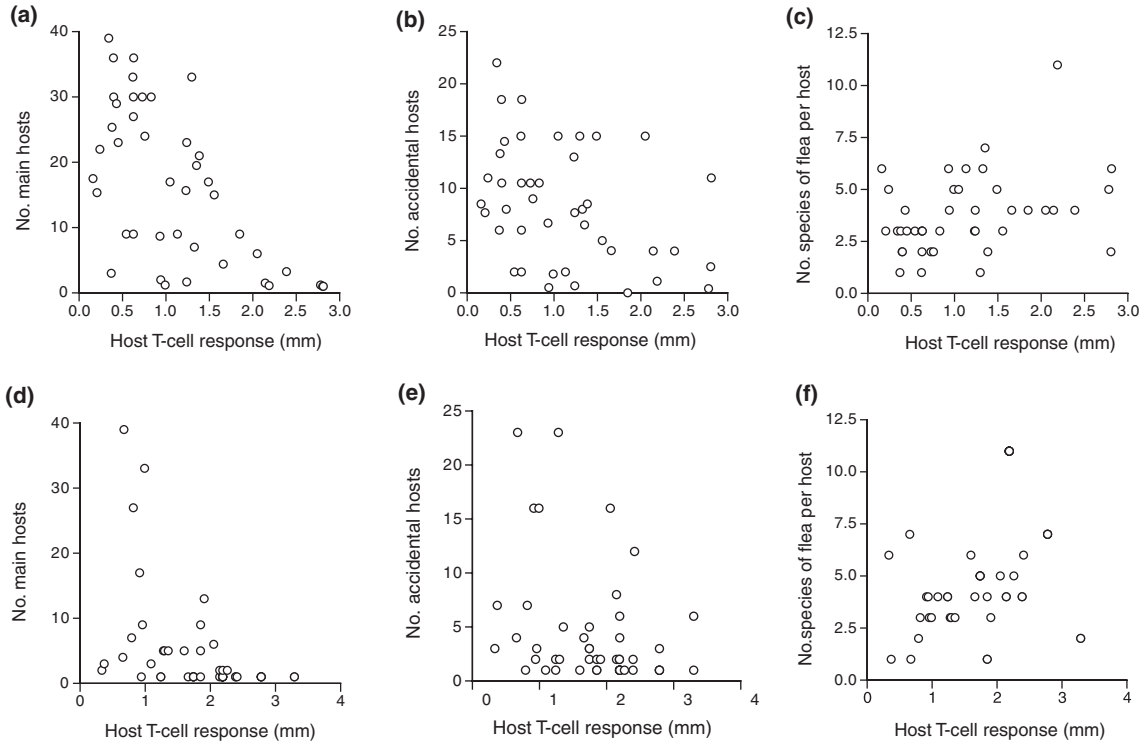

Fig. 3 Specialization by fleas in relation to T-cell mediated immune response (mm) of nestlings of their avian hosts, using host species (a–c) and parasite species (d–f) as the units of analysis. (a) No. main host species by their flea species in relation to mean T-cell response of hosts. (b) No. accidental flea species by their flea species in relation to mean host T-cell response of hosts. (c) No. species of flea per host in relation to mean T-cell response of hosts. (d) Mean no. main host species per host in relation to mean T-cell response of hosts. (e) Mean no. accidental flea species per host in relation to mean host T-cell response of hosts. (f) Mean no. species of flea per host in relation to mean T-cell response of hosts. The untransformed values are presented for clarity, but log-transformed values were used in the calculations (see Materials and methods).

exploited by fleas with few hosts. The number of species of flea per host tended to increase with host immune response although not significantly so (Fig. 3c, Table 1). The extreme data point in Fig. 3c was not an outlier when the data were log-transformed, as done in all analyses.

In a second series of analyses we investigated the relationship between T-cell mediated immune response of hosts and flea specialization using the phylogenetic relationship among fleas as the framework for our comparative analyses. The evolution of a stronger immune response of hosts correlated with a decrease in the number of hosts exploited by fleas, but also a decrease in the number of accidental hosts (Fig. 3d, e; Table 1). Again, the distribution of data points was triangular with hosts with weak immune responses supporting fleas with both many and few host species, whereas hosts with strong immune responses were only exploited by fleas with few host species. The number of species of flea per host increased significantly with host immune response (Fig. 3f; Table 1). These results are similar whether the host or the parasite side of the coevolutionary relationship is used for the analyses, with the exception of the number of species of flea per host which only increased significantly with host T-cell response when calculations were based on the flea phylogeny (Table 1). However, the two correlation coefficients from the analysis based on the bird phylogeny and the analysis based on the flea phylogeny are not significantly different from each other ($t = 0.54$, d.f. = 88, n.s.).

We investigated the relative importance of host sociality and host immune response for number of host species, using path analysis on the phylogenetic correlations based on the bird phylogeny (the corresponding phylogenetic model for correlated trait evolution: $\kappa = 0.284$, $\lambda = 0.750$, LR = 12.827, d.f. = 3, $P < 0.0001$). We started with four different models linking the three factors (Fig. 4), and this set of models was reduced to the best model 2, based on its greater explanatory power (amount of variance explained by the model $r^2 = 49.0\%$). The alternative models assumed that (a) coloniality and T-cell response mutually influence each other and both affect number of host species (model 1;

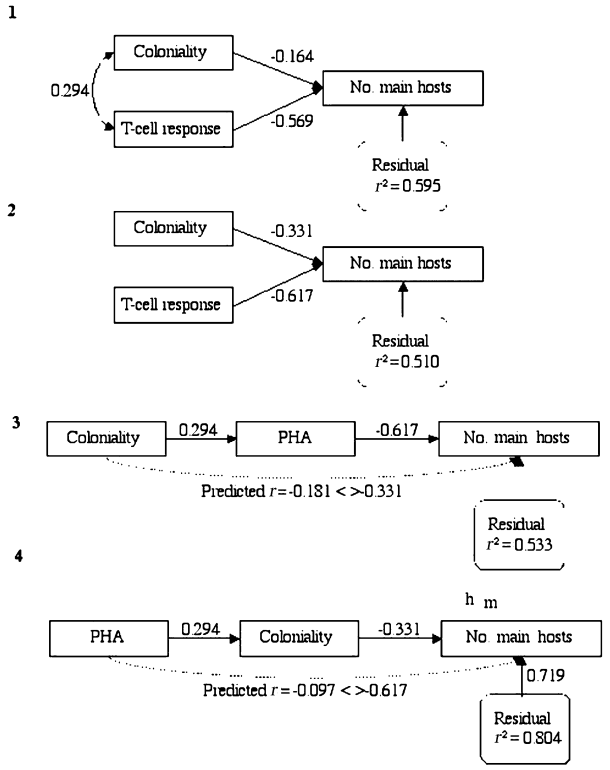

Fig. 4 Path analysis of the relationship between host coloniality, host immune response and number of host species. Values are the path coefficients.

$r^2 = 40.5\%$), (b) host coloniality directly affects host T-cell response which in turn affects the number of host species (model 3; $r^2 = 46.7\%$) and (c) host T-cell response affects host coloniality which in turn affects the number of hosts (model 4; $r^2 = 19.6\%$). The retained model 2 only explained 2.3% more of the variance than the second best model 3, but 8.5% more than the third best model 1 and 29.4% more than the worst model 4. Models 2 and 3 differed significantly from models 1 and 2. The best fit model 1 assumes independent effects of coloniality and T-cell response of hosts on the number of host species of fleas, and that coloniality and T-cell response do not affect each other mutually. This model 2 showed that the effect of T-cell response on the number of host species was considerably larger than the effect of coloniality. An increase in species richness of fleas occurred with the evolution of greater immune response of hosts (Fig. 4). The second best model 3, which explained a similar amount of variance, assumed that host coloniality directly affected host T-cell response, which in turn affected the number of host species used by fleas (Fig. 4).

Discussion

This study is the first to investigate the coevolutionary dynamics of parasite specialization and host immunity, using a comparative approach. Although many studies have suggested that the defenses raised by hosts against their parasites may contribute to the extraordinary species richness of parasites (e.g. Price, 1980; Combes, 2001), we are only aware of a couple of studies that have quantified host defenses and related these to species richness of parasites [Berenbaum (1981, 1983) for herbivorous insects]. We investigated the coevolutionary relationship between fleas and their avian hosts by determining the extent to which host immunity and parasite specialization (a reduction in the number of host species) covaried while simultaneously taking the phylogenetic relationships between hosts and parasites into account. The comparative analyses based on the avian phylogeny revealed that when the immune response of hosts became stronger, the number of host species exploited by their flea species decreased. In addition, the number of species of flea per host tended to increase with host immune response. When analysing the coevolutionary relationships between host defense and flea specialization, using the flea phylogeny as the basis for the comparative analysis, we found very similar results, with the additional result that the number of species of flea per host increased significantly with host immunity. Although the number of species of flea per host increased significantly with host immunity when calculations were based on the flea phylogeny, but not when calculations were based on the bird phylogeny, this apparent contradiction is not real since the two correlation coefficients are not significantly different from each other. These findings concerning the number of main and accidental hosts suggest that we are dealing with coevolution since phylogenetic analyses of the two sides of the coevolutionary relationship provided similar results. Thus, our results suggested that specialization in fleas occurred in response to T-cell mediated immune response of hosts, and conversely T-cell mediated immunity evolved in response to specialization by fleas. These findings parallel those of Berenbaum (1981, 1983) on herbivorous insects of plants in the parsnip family, where strong chemical defenses are associated with host specialization by herbivores. Our study goes beyond those by Berenbaum by statistically controlling for similarity in phenotype among taxa because of common descent, by analysing both sides of the coevolutionary equation (hosts and parasites) in a phylogenetic perspective, and by making inferences about the order of events in the coevolutionary scenario (see below).

Before discussing the evolutionary implications of our study, we briefly discuss the quality of the data. First, we used information on specialization and species richness of fleas using information as reported directly in Traub *et al.* (1983). We realize that this source defined specialization by assigning hosts as main or accidental hosts based on the number of records of a host being exploited by a given species (or subspecies) of flea. Since the foremost specialists in fleas at that time made these

categorizations, and since the categorizations were made independent of our subsequent hypotheses and predictions, this cannot possibly have caused any bias in the analyses. In addition, we note that Tripet *et al.* (2002b) found very strong correlations between specialization of fleas as defined by Traub *et al.* (1983) and a measure of jumping ability of fleas as determined from morphological measurements of leg structures. As dispersal ability of fleas is closely linked to specialization (Tripet *et al.*, 2002a), these correlations provide evidence of flea specialization as defined by Traub *et al.* (1983) has a strong biological basis. Any level of arbitrariness in the list published by Traub *et al.* (1983) would only cause noise in our analyses and hence render any detected relationships conservative. We used a reliable estimate of T-cell mediated immunity as our measure of immune response. While this measure was developed by poultry immunologists more than 25 years ago, and therefore may be considered to be antiquated, we note that no other techniques exist to measure T-cell mediated immune response under field conditions in a large number of species. We also note that this measure is a reliable indicator of viability of hosts (review in Møller & Saino, 2004), and that it reliably reflects the impact of parasite-mediated natural selection on different species of avian hosts (Martin *et al.*, 2001). Hence, we consider both our measure of parasite specialization and host T-cell mediated immune response to provide biologically relevant and reliable information about parasites and hosts in this study.

The relationships between number of hosts and host immune response were negative, but also had a clearly triangular distribution of data points. This implies that hosts with weak immune responses both harboured fleas with few and many hosts, whereas hosts with strong immune responses only harboured fleas with few hosts. We suggest that these patterns may arise for several different reasons. First, host species with weak immune responses may suffer from high rates of immigration by fleas with either few or many hosts, whereas host species with strong immune responses only are exposed to immigration by fleas with few host species. Tripet *et al.* (2002b) have shown that flea species that exploit colonial hosts, which tend to have strong immune responses (Møller & Erritzøe, 1996; Møller *et al.*, 2001), have weak dispersal abilities compared with flea species that exploit solitarily breeding hosts. If only dispersal ability of fleas accounted for the triangular distribution of the relationship between number of hosts and host immune response, we should expect colonial hosts to be colonized by fleas with weak and strong dispersal abilities, whereas solitary species should rarely or never be colonized by fleas with weak dispersal abilities. This would produce a triangular pattern that would be the mirror image of that shown in Fig. 3. Secondly, fleas with few and many host species may survive on hosts with weak immune responses, whereas only fleas with few host species may survive on hosts with strong immune responses. As hosts with weak immune responses do not produce immune substances that may limit population growth of fleas, or only do so to a limited extent, fleas with few host species may be inferior competitors on such hosts and therefore only or mainly occur on host individuals that are not currently exploited by generalist fleas. Such fleas would also be the only ones that would be able to cope with the strong immune responses of highly colonial hosts. These hypotheses are open to experimental test.

An important determinant of the level of host immune response is host coloniality, which is associated with increased immune defenses (Møller & Erritzøe, 1996; Møller *et al.*, 2001). Host coloniality has been shown to be associated with flea specialization and speciation (Tripet *et al.*, 2002a). Using path analysis relating host immune defense, host sociality and specialization by their flea parasites we obtained two models that explained the largest amount of variance. By doing so we assumed that the path analysis model that accounted for a larger amount of variance is the model that is most likely to accurately describe the relationship between host immune defense, host sociality and specialization by their flea parasites. The best model accounting for the largest amount of the variance suggests that host coloniality and T-cell response of hosts independently contribute to the number of main hosts exploited by fleas, but that the effect of T-cell response is considerably stronger than the effect of host coloniality. The observation that host defenses coevolve with parasite specialization is consistent with coevolutionary theory of parasite specialization (Price, 1980; Thompson, 1994). As parasites become specialists they tend to become more virulent, and, therefore, host offspring invest more in immune defense, at the cost of developmental rate (Møller *et al.*, 2001). The second best model, which explained almost the same amount of variance as the best model, assumed that host coloniality directly affected host T-cell response, which in turn affected the number of host species exploited by fleas. For example, this could be the case if host coloniality generally increased immunity of hosts, because coloniality promoted multiple infections or horizontal transmission and thereby parasite virulence in general. This evolutionary increase in immunity could subsequently affect host specialization by fleas. We hypothesize that such specialization by fleas may have important consequences for the evolution of parasite virulence. Furthermore, our results are consistent with the idea that intense selection from host populations via their immune system is an important factor promoting parasite specialization.

Acknowledgments

An anonymous reviewer kindly suggested the importance of the distribution of the data points. PC was supported by grants from the Swiss National Science

Foundation (No. 31-61450.00). J. Blount, S. Schjørring, K. McCoy and J. Fair kindly provided unpublished information on T-cell responses, while T. Martin, J. Shykoff and especially F. Tripet provided constructive criticism of previous versions. M. P. Whiting and B. Lewis provided novel information on the phylogenetic relationships of fleas.

References

Abbas, A.K., Lichtman, A.H. & Pober, J.S. 1994. *Cellular and Molecular Immunology*. Saunders, Philadelphia, PA, USA.

Becnel, J.J. & Andreadis, T.G. 2000. *Amblyospora salinaria* n. sp. (Microsporidia: Amblyosporidae), parasite of *Culex salinarius* (Diptera: Culicidae): its life stage in the intermediate host. *J. Invert. Pathol.* **71**: 258–262.

Bennett, P.M. & Owens, I.P.F. 2002. *Evolutionary Ecology of Birds*. Oxford University Press, Oxford, UK.

Berenbaum, M.R. 1981. Patterns of furanocoumarin distribution and insect herbivory in the Umbelliferae: plant chemistry and community structure. *Ecology* **62**: 1254–1266.

Berenbaum, M.R. 1983. Coumarins and caterpillars: a case for coevolution. *Evolution* **37**: 163–179.

Brinkhof, M.W.G., Heeb, P., Kölliker, M. & Richner, H. 1999. Immunocompetence of nestling great tits in relation to rearing environment and parentage. *Proc. R. Soc. Lond. B* **266**: 2315–2322.

Brown, C.R. & Brown, M.B. 1986. Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). *Ecology* **67**: 1206–1218.

Christe, P., Richner, H. & Oppliger, A. 1996. Begging, food provisioning, and nestling competition in great tits infested with ectoparasites. *Behav. Ecol.* **7**: 127–131.

Christe, P., Møller, A.P. & de Lope, F. 1998. Immunocompetence and nestling survival in the house martin: 'The tasty chick hypothesis'. *Oikos* **83**: 175–179.

Christe, P., Møller, A.P., Saino, N. & de Lope, F. 2000. Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, the house martin *Delichon urbica*. *Heredity* **85**: 75–85.

Cibois, A. & Pasquet, E. 1999. Molecular analysis of the phylogeny of 11 genera of the Corvidae. *Ibis* **141**: 297–306.

Combes, C. 2001. *Parasitism*. University of Chicago Press, Chicago, IL, USA.

Cramp, S., Simmons, K.E.L. & Perrins, C.M. (eds) 1982–1994. *Handbook of the Birds of Europe, the Middle East and North Africa*, Vol. I–IX. Oxford University Press, Oxford, UK.

Dunning, J.B. 1993. *CRC Handbook of Avian Body Masses*. CRC Press, Boca Raton, FL, USA.

Ehrlich, P.R. & Raven, P.H. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**: 586–608.

Freckleton, R.P., Harvey, P.H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *American Naturalist* **160**: 712–726.

Futuyma, D.J. & Moreno, G. 1988. The evolution of ecological specialization. *Ann. Rev. Ecol. Systemat.* **19**: 207–233.

Gandon, S., Capowiez, Y., Dubois, Y., Michalakis, Y. & Olivieri, I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc. R. Soc. Lond. B* **263**: 1003–1009.

Gilbert, L.E. & Raven, P.H. 1975. *Coevolution of Animals and Plants*. University of Texas Press, Austin, TX, USA.

Giorgi, M.S., Arlettaz, R., Guillaume, F., Nusslé, S., Ossola, C., Vogel, P. & Christe, P. 2004. Causal mechanisms underlying host specificity in bat ectoparasites. *Oecologia* **138**: 648–654.

González, G., Sorci, G. & de Lope, F. 1999. Seasonal variation in the relationship between cellular immune response and badge size in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **46**: 117–122.

Goto, N., Kodama, H., Okada, K. & Fujimoto, Y. 1978. Suppression of phytohaemagglutinin skin response in thymectomized chickens. *Poultry Science* **57**: 246–250.

Hoi-Leitner, M., Hoi, H., Romero-Pujante, M. & Valera, F. 1999. Female extra-pair behaviour and environmental quality in the serin (*Serinus serinus*): a test of the 'constrained female hypothesis'. *Proc. R. Soc. Lond. B* **266**: 1021–1026.

Hörak, P., Tegelmann, L., Ots, I. & Møller, A.P. 1999. Immune function and survival of great tit nestlings in relation to growth conditions. *Oecologia* **121**: 316–322.

Johnsen, A., Andersen, V., Sunding, C. & Lifjeld, J.T. 2000. Female blue throats enhance offspring immunocompetence through extra-pair copulations. *Nature* **406**: 296–299.

Kaltz, O. & Shykoff, J. 1998. Local adaptation in host–parasite systems. *Heredity* **81**: 361–370.

Kosoy, M.Y., Saito, E.K., Green, D., Marston, E.L., Jones, D.C. & Childs, J.E. 2000. Experimental evidence of host specificity of *Batonella* infection in rodents. *Comp. Immunol. Microbiol. Infect. Dis.* **23**: 221–238.

Lehane, M.J. 1991. *Biology of Blood Sucking Insects*. Harper Collins Academic, London, UK.

Li, C.C. 1975. *Path Analysis – A Primer*. Boxwood Press, Pacific Grove, CA, USA.

Møller, A.P. & Erritzøe, J. 1996. Parasite virulence and host immune defense: host immune response is related to nest re-use in birds. *Evolution* **50**: 2066–2072.

Møller, A.P. & Saino, N. 2004. Immune response and survival. *Oikos* **104**: 299–304.

Møller, A.P., Merino, S., Brown, C.R. & Robertson, R.J. 2001. Immune defense and host sociality: a comparative study of swallows and martins. *Am. Nat.* **158**: 136–145.

Marshall, A.G. 1981. *The Ecology of Ectoparasitic Insects*. Academic Press, London, UK.

Martin, T.E., Møller, A.P., Merino, S. & Clobert, J. 2001. Does clutch size evolve in response to parasites and immunocompetence? *Proc. Natl. Acad. Sci. USA* **98**: 2071–2076.

Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**: 646–667.

McCorkle, F. Jr, Olah, I. & Glick, B. 1980. The morphology of the phytohemagglutinin-induced cell response in the chi'ken's wattle. *Poult. Sci.* **59**: 616–623.

de Meeus, T., Renaud, F. & Gabrion, C. 1990. A model for studying isolation mechanisms in parasite populations – the genus *Lepeophtheirus* (Copepoda: Caligidae). *J. Exp. Zool.* **254**: 207–214.

de Meeus, T., Hochberg, M.E. & Renaud, F. 1995. Maintenance of two genetic entities by habitat selection. *Evol. Ecol.* **9**: 131–138.

Merino, S., Martínez, J., Møller A.P., Barbosa, A., de Lope, F. & Rodríguez-Caabiero, F. 2001. Physiological and hematological consequences of a novel parasite on the red-rumped swallow *Hirundo daurica*. *Int. J. Parasitol.* **31**: 1187–1193.

Merino, S., Møller, A.P. & de Lope, F. 2000. Seasonal changes in cell-mediated immunocompetence and mass gain in nestling barn swallows: a parasite-mediated effect? *Oikos* **90**: 327–332.

Moreno, J., Sanz, J.J. & Arriero, E. 1999. Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. *Proc. R. Soc. Lond. B* **266**: 1105–1109.

National Research Council 1992. *Biologic Markers in Immunotoxicology*. National Academy Press, Washington, DC, USA.

Pagel, M. 1997. Inferring evolutionary processes from phylogenies. *Zoologica Scripta* **26**: 331–348.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Poulin, R. 1997. Parasite faunas of freshwater fish: the relationship between richness and specificity of parasites. *Int. J. Parasitol.* **27**: 1091–1098.

Price, P.V. 1980. *Evolutionary Biology of Parasites*. Princeton University Press, Princeton, NJ, USA.

Richner, H. & Tripet, F. 1999. Ectoparasitism and the trade-off between current and future reproduction. *Oikos* **86**: 535–538.

Richner, H., Oppliger, A. & Christe, P. 1993. Effect of an ectoparasite on reproduction in great tits. *J. Anim. Ecol.* **62**: 703–710.

Saino, N., Calza, S. & Møller, A.P. 1997. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *J. Anim. Ecol.* **66**: 827–836.

Sheldon, F.H. & Winkler, D.W. 1993. Intergeneric phylogenetic relationships of swallows estimated by DNA-DNA hybridization. *Auk* **110**: 798–824.

Sibley, C.G. & Ahlquist, J.E. 1990. *Phylogeny and Classification of Birds: A Study in Molecular Evolution*. Yale University Press, New Haven, CT, and London, UK.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* **236**: 787–792.

Soler, J.J., Møller, A.P., Soler, M. & Martínez, J.G. 1999. Interactions between a brood parasite and its host in relation to parasitism and immune defence. *Evol. Ecol. Res.* **1**: 189–210.

Tella, J.L., Scheuerlein, A. & Ricklefs, R.E. 2002. Is cell-mediated immunity related to the evolution of life-history strategies in birds? *Proc. R. Soc. Lond. B* **269**: 1059–1066.

Thompson, J.N. 1994. *The Coevolutionary Process*. University of Chicago Press, Chicago, IL and London, UK.

Thresher, R.E., Werner, M., Hoeg, J.T., Svane, I., Glenner, H., Murphy, N.E. & Wittwer, C. 2000. Developing the options for managing marine pests: specificity trials on the parasitic castrator, *Sacculina carcini*, against the European crab, *Carcinus maenas*, and related species. *J. Exp. Marine Biol. Ecol.* **254**: 37–51.

Traub, R., Rothschild, M. & Haddow, J.F. 1983. *The Ceratophyllidae: Key to the Genera and Host Relationships*. Cambridge University Press, Cambridge, UK.

Tripet, F. & Richner, H. 1997. Host responses to ectoparasites: food compensation by parent blue tits. *Oikos* **78**: 557–561.

Tripet, F., Christe, P. & Møller, A.P. 2002a. The importance of host sociality for parasite specialization and speciation: a comparative study of avian hosts and their fleas (Siphonaptera: Ceratophyllidae). *J. Anim. Ecol.* **71**: 735–748.

Tripet, F., Glaser, M. & Richner, H. 2002b. Behavioural responses to ectoparasites: time-budget adjustments and what matters to blue tits *Parus caeruleus* infested by fleas. *Ibis* **144**: 461–469.

Walther, B.A., Cotgreave, P., Price, R.D., Gregory, R.D. & Clayton, D.H. 1995. Sampling effort and parasite species richness. *Parasitol. Today* **11**: 306–310.

Wikel, S.K. 1996. Host immunity to ticks. *Ann. Rev. Entomol.* **41**: 1–22.

Wright, S. 1968. *Evolution and the Genetics of Populations. Vol. 1. Genetic and Biometric Foundations*. University of Chicago Press, Chicago, IL, USA.

Wright, S. 1978. *Evolution and the Genetics of Populations. IV. Variability Within and Among Populations*. University of Chicago Press, Chicago, IL, USA.

Received 10 March 2004; revised 6 May 2004; accepted 6 May 2004

Appendix 1 Information on number of publications (studies published since 1945 on host genera as cited in the ISI Web of Science), mean nestling T-cell response (mm) of host taxa infested with a given flea species, no. main hosts per species of flea, no. species of flea per host, no. accidental hosts per species of flea and host taxa of species or subspecies of flea. The number of species of flea per host is the mean number of species of flea for all host species that are exploited by a given species (or subspecies) of flea. Nestling T-cell response of hosts in the mean value for all host species exploited by a given species (or subspecies) of flea. Sources are given in Materials and methods. Sample sizes and sources for the T-cell responses are given in Appendix 2.

Species or subspecies of flea	No. nestling publications	T-cell response (mm)	No. main hosts	No. species of flea per host	No. accidental hosts	Host taxa
Ceratophyllus a. affinis	689	1.24	1	4	2	*Hirundo rustica*
Ceratophyllus a. neglectus	689	1.24	1	4	1	*Hirundo rustica*
Ceratophyllus arcuegens	27	2.78	1	7	1	*Petrochelidon pyrrhonota*
Ceratophyllus avicitelli	278	0.33	2	6	3	*Oenanthe leucura*, *Saxicola rubetra*
Ceratophyllus breviprojectus	689	0.94	1	4	2	*Hirundo daurica*
Ceratophyllus c. celsus	425	2.26	2	5	1	*Riparia riparia*, *Petrochelidon pyrrhonota*
Ceratophyllus caliotes	86	2.19	1	11	2	*Delichon urbica*
Ceratophyllus delichoni	86	2.19	1	11	1	*Delichon urbica*
Ceratophyllus e. enefdea	118	1.30	5	3	2	*Pyrrhocorax graculus*, *Phoenicurus ochruros*
Ceratophyllus e. tjanschani	1253	1.60	5	6	1	*Pica pica*
Ceratophyllus f. chaoi	689	1.09	3	4	1	*Hirundo rustica*, *Hirundo daurica*
Ceratophyllus f. farreni	86	2.19	1	11	2	*Delichon urbica*
Ceratophyllus fringillae	1739	1.28	5	3	23	*Sturnus*, *Passer*
Ceratophyllus gallinae	11 431	0.99	33	3	16	*Lanius*, *Fringilla*, *Acrocephalus*, *Phylloscopus*, *Luscinia*, *Erithacus*, *Sturnus*, *Anthus*, *Ficedula*, *Pica*, *Emberiza*, *Turdus*, *Muscicapa*, *Alauda*, *Motacilla*, *Sitta*, *Passer*, *Troglodytes*, *Serinus*, *Carduelis*, *Sialia*, *Oenanthe*, *Sylvia*, *Phoenicurus*, *Parus*, *Corvus*
Ceratophyllus gilvus	27	2.78	1	7	1	*Petrochelidon pyrrhonota*
Ceratophyllus hirundinis	86	2.19	1	11	6	*Delichon urbica*
Ceratophyllus idius	169	0.37	3	1	7	*Tachycineta bicolor*
Ceratophyllus maculatus	86	2.19	1	11	1	*Delichon urbica*
Ceratophyllus niger	1993	0.79	7	2	1	*Passer*, *Pica*
Ceratophyllus orites	86	2.19	1	11	1	*Delichon urbica*
Ceratophyllus petrochelidoni	27	2.78	1	7	1	*Petrochelidon pyrrhonota*
Ceratophyllus pullatus	4607	0.96	9	3	3	*Erithacus*, *Sturnus*, *Parus*, *Passer*, *Sitta*, *Ficedula*, *Phoenicurus*
Ceratophyllus r. rossitensis	554	2.14	1	4	8	*Corvus corone*
Ceratophyllus r. swansoni	554	2.14	2	4	2	*Corvus corone*
Ceratophyllus rusticus	86	2.19	1	11	4	*Delichon urbica*
Ceratophyllus s. freyi	398	1.74	1	5	2	*Riparia riparia*
Ceratophyllus s. jordani	398	1.74	1	5	3	*Riparia riparia*
Ceratophyllus s. riparius	398	1.74	1	5	5	*Riparia riparia*
Ceratophyllus s. styx	398	1.74	1	5	3	*Riparia riparia*
Ceratophyllus scopulorum	27	2.78	1	7	3	*Petrochelidon pyrrhonota*
Ceratophyllus tribulis	740	2.41	1	6	12	*Passer montanus*
Ceratophyllus v. alpestris	58	1.66	1	4	4	*Pyrrhocorax graculus*
Ceratophyllus v. insularis	787	2.05	6	5	16	*Corvus*, *Rissa*
Ceratophyllus v. vagabundus	557	1.85	9	1	1	*Phalacrocorax carbo*
Dasypsyllus g. gallinulae	7390	0.82	27	3	7	*Phoenicurus*, *Parus*, *Passer*, *Carduelis*, *Phylloscopus*, *Luscinia*, *Sylvia*, *Erithacus*, *Ficedula*, *Troglodytes*, *Fringilla*, *Saxicola*, *Turdus*, *Muscicapa*, *Motacilla*
Dasypsyllus g. perpinnatus	502	1.91	13	3	2	*Turdus*
Emmareus borealis	378	0.91	17	4	16	*Saxicola*, *Phoenicurus*, *Motacilla*, *Luscinia*
Emmareus columbae	1767	3.29	1	2	6	*Columba livia*
Emmareus frigoris	149	0.66	4	7	4	*Phoenicurus*, *Alauda*
Emmareus garei	1152	0.67	39	1	23	*Luscinia*, *Anthus*, *Acrocephalus*, *Motacilla*, *Emberiza*, *Phylloscopus*, *Alauda*
Mioctenopsylla a. arctica	233	2.39	1	4	1	*Rissa tridactyla*
Mioctenopsylla a. hadweni	233	2.39	1	4	2	*Rissa tridactyla*

Appendix 1 Continued

Species or subspecies of flea	No. nestling publications	T-cell response (mm)	No. main hosts	No. species of flea per host	No. accidental hosts	Host taxa
Mioctenopsylla t. kurilensis	557	1.85	5	1	1	*Phalacrocorax carbo*
Mioctenopsylla t.traubi	557	1.85	1	4	2	*Phalacrocorax carbo*
Neornipsyllus stejnegeri	502	1.35	5	3	5	*Turdus*
Ornaceus gemina	1767	3.29	1	2	1	*Columba livia*
Ornaceus oreinus	86	2.19	1	11	1	*Delichon urbica*
Ornaceus waterstoni	86	2.19	2	11	1	*Delichon urbic*

Appendix 2 Information on number of publications (studies published since 1945 on each bird as cited in the ISI Web of Science), nestling T-cell response (mm)(S. E., sample size; number of broods; in species where the information on T-cell response was obtained from the literature or personal communication, sample size was not always available), no. main hosts per species of flea harboured by this host, no. species of flea per host, no. accidental hosts per species of flea harboured by this host, and coloniality, and mean body mass of hosts (g). The number of main hosts (and the number of accidental hosts) is the mean number of main hosts (accidental hosts) exploited by the species (or subspecies) of fleas that are exploiting a given host. Sources are given in Materials and methods.

Species	No. publications	Nestling T-cell response (mm) (S. E.) (*n*)	No. main hosts	No. species of flea per host	No. accidental hosts	Coloniality	Body mass (g)	Reference
Acrocephalus scirpaceus	320	0.40 (0.01) (2)	36.0	2	18.5	1	10.6	(1)
Alauda arvensis	89	0.38 (0.04) (4)	25.3	3	13.3	1	36.1	(1)
Carduelis chloris	214	0.83 (0.08) (4)	30.0	3	10.5	1	26.7	(1)
Columba livia	1767	3.29 (0.30) (5)	1.0	2	2.5	2	304.1	(1)
Corvus corone	554	2.14 (0.06) (5)	1.5	4	4.0	1	466.7	(1)
Corvus monedula	554	2.05 (0.16) (4)	6.0	4	15.0	2	154.9	(1)
Delichon urbica	86	2.19 (0.14) (21)	1.1	11	1.1	3	18.0	(1,2)
Emberiza citrinella	211	0.63 (0.03) (3)	36.0	3	18.5	1	25.0	(1)
Erithacus rubecula	272	1.24 (0.08) (2)	23.0	3	7.7	1	17.0	(1)
Ficedula hypoleuca	681	0.45 (-) (-)	23.0	3	8.0	1	14.0	(3)
Fringilla coelebs	188	0.73 (0.10) (3)	30.0	2	10.5	1	29.9	(1)
Hirundo daurica	689	0.94 (0.05) (8)	2.0	4	0.5	2	19.0	(2)
Hirundo rustica	689	1.24 (0.03) (24)	1.7	4	0.7	3	19.0	(2)
Lanius excubitor	151	1.30 (0.04) (1)	33.0	1	15.0	1	64.3	(1)
Larus fuscus	1345	0.24 (-) (-)	22.0	5	11.0	3	379.3	(4)
Luscinia svecica	116	0.43 (-) (-)	29.0	4	14.5	1	18.0	(5)
Motacilla alba	82	1.05 (0.10) (2)	17.0	5	15.0	1	19.3	(1)
Motacilla cinerea	82	1.49 (0.07) (2)	17.0	5	15.0	1	18.0	(1)
Muscicapa striata	29	0.40 (0.02) (3)	30.0	2	10.5	1	15.5	(1)
Oenanthe leucura	158	0.16 (0.02) (21)	17.5	6	8.5	1	35.0	(6)
Parus caeruleus	1724	0.54 (0.06) (4)	9.0	3	2.0	1	10.6	(1)
Parus major	1724	0.62 (0.09) (5)	9.0	3	2.0	1	17.0	(1)
Passer domesticus	740	1.33 (0.12) (4)	7.0	6	8.0	2	29.3	(1)
Passer montanus	740	2.81 (0.05) (3)	1.0	6	11.0	1	21.4	(1)
Petrochelidon pyrrhonota	27	2.78 (0.07) (48)	1.2	5	0.4	3	22.0	(2)
Phalacrocorax carbo	557	1.85 (-) (-)	9.0	4	0.0	3	2074.9	(7)
Phoenicurus ochruros	60	0.93 (0.05) (2)	8.7	6	6.7	1	14.5	(1)
Phoenicurus phoenicurus	60	1.13 (0.06) (2)	9.0	6	2.0	1	16.4	(1)
Phylloscopus trochilus	229	0.34 (0.17) (2)	39.0	3	22.0	1	8.5	(1)
Pica pica	1253	1.56 (-) (-)	15.0	3	5.0	1	205.1	(8)
Pyrrhocorax graculus	58	1.66 (0.15) (3)	4.4	4	4.0	2	244.9	(1)
Riparia riparia	398	1.74 (0.06) (21)	1.2	5	1.8	3	14.0	(1,2)
Rissa tridactyla	233	2.39 (-) (-)	3.3	4	4.0	3	299.9	(9)
Saxicola rubetra	120	0.21 (0.03) (4)	15.3	3	7.7	1	18.2	(1)
Serinus serinus	211	0.62 (-) (-)	33.0	1	15.0	1	11.2	(10)
Sialia mexicana	137	0.76 (-) (-)	24.0	2	9.0	1	29.5	(11)
Sitta europea	131	1.38 (-) (1)	21.0	2	8.5	1	22.6	(1)
Sturnus vulgaris	999	1.23 (0.12) (2)	15.7	3	13.0	1	69.0	(1)
Sylvia communis	1060	0.62 (0.04) (3)	30.0	2	10.5	1	15.1	(1)

Appendix 2 Continued

Species	No. publications	Nestling T-cell response (mm) (S. E.) (*n*)	No. main hosts	No. species of flea per host	No. accidental hosts	Coloniality	Body mass (g)	Reference
Tachycineta bicolor	169	0.37 (0.03) (18)	3.0	1	6.0	1	20.0	(2)
Troglodytes troglodytes	1373	0.62 (-) (1)	27.0	3	6.0	1	10.4	(1)
Turdus merula	502	1.35 (0.04) (4)	19.5	7	6.5	1	91.6	(1)

(1) This study; (2) Møller *et al.* (2001); (3) Moreno *et al.* (1999); (4) J. Blount personal communication; (5) Johnsen *et al.* (2000); (6) M. Soler, M. Martín-Vivaldi, J.M. Marín and A.P. Møller personal communication; (7) S. Schjørring personal communication; (8) Soler *et al.* (1999); (9) K. McCoy personal communication; (10) Hoi-Leitner *et al.* (1999); (11) J. Fair personal communication.

Appendix 3 Generalized least squares analysis of the relationship between species richness of fleas and host T-cell response based on the bird host phylogeny when the effect of body size on T-cell immune response is controlled by calculating residuals from a linear regression. See Table 1 for abbreviations.

	κ	λ	Phylogenetic correlation	LR	d.f.	*P*	*n*
Analyses based on the bird host phylogeny, host T-cell immune response							
No. main hosts	0.361	0.787	−0.568	8.518	1	<0.0001	42
No. accidental hosts	0.417	0.804	−0.346	2.677	1	0.021	42
No. species of flea per host	1.000	0.511	0.237	1.219	1	0.118	42

Appendix 4 Generalized least squares analysis of the relationship between species richness of fleas and host T-cell response based on the flea phylogeny when the effect of research intensity is controlled on species richness of fleas by residuals from the corresponding linear regressions. See Table 1 for abbreviations.

	κ	λ	Phylogenetic correlation	LR	d.f.	*P*	*n*
Analyses based on flea phylogeny, host T-cell immune response							
No. main hosts	1.000	0.000	−0.421	4.679	1	0.002	48
No. accidental hosts	1.000	0.000	−0.268	1.786	1	0.059	48
No. species of flea per host	1.000	0.000	0.204	1.024	1	0.152	48