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Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: the influence of phylogeny and local environmental conditions

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The evolution of host specificity remains a central issue in the study of host-parasite relationships. Here we tackle three basic questions about host specificity using data on host use by fleas (Siphonaptera) from 21 geographical regions. First, are the host species exploited by a flea species no more than a random draw from the locally available host species, or do they form a taxonomically distinct subset? Using randomization tests, we showed that in the majority of cases, the taxonomic distinctness (measured as the average taxonomic distances among host species) of the hosts exploited by a flea is no different from that of random subsets of hosts taken from the regional pool. In the several cases where a difference was found, the taxonomic distinctness of the hosts used by a flea was almost always lower than that of the random subsets, suggesting that the parasites use hosts within a narrower taxonomic spectrum than what is available to them. Second, given the variation in host specificity among populations of the same flea species, is host specificity truly a species character? We found that host specificity measures are repeatable among different populations of the same flea species: host specificity varies significantly more among flea species than within flea species. This was true for both measures of host specificity used in the analyses: the number of host species exploited, and the index measuring the average taxonomic distinctness of the host species and its variance. Third, what causes geographical variation in host specificity among populations of the same flea species? In the vast majority of flea species, neither of our two measures of host specificity correlated with either the regional number of potential host species or their taxonomic distinctness, or the distance between the sampled region and the center of the flea's geographical range. However, in most flea species host specificity correlated with measures of the deviation in climatic conditions (precipitation and temperature) between the sampled region and the average conditions computed across the flea's entire range. Overall, these results suggest that host specificity in fleas is to a large extent phylogenetically constrained, while still strongly influenced by local environmental conditions.

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Evolution of ecological specialization is one of the most challenging issues in modern ecology. Although a large number of studies, both empirical and theoretical (Futuyma and Moreno 1988, Thompson 1994, Fry 1996, Hughes 2000, Desdevises et al. 2002), deal with the evolutionary ecology of specialization, it is still unclear why natural selection sometimes favours specialists over generalists whereas in other situations it favours generalists over specialists.

The crucial problem in understanding the evolution of specialization is to understand the role played by natural selection in this evolution. This, in turn, leads to another important question of whether a given level of specialization ("niche breadth") is a species attribute that can be subjected to natural selection, or whether it merely reflects the local restrictions caused by a variety of ecological, morphological, chemical and/or genetic factors (Fox and Morrow 1981). The latter possibility is supported by the substantial variation in the degree of specialization observed among populations within a species (Fox and Morrow 1981, Thompson 1994). Moreover, the degree of resource specialization of a species may differ depending on the scale of observation because of two interrelated factors, namely local resource specialization and the substitutability of those resources across locations (Hughes 2000). For example, a species that is considered to be a generalist on a local or within-habitat scale may appear as a specialist on a regional or among-habitat scale (Gaston et al. 1997, Hughes 2000). This suggests that availability of resources can profoundly affect the degree of resource specialization in a local population of a species.

Parasitic animals are useful models for studying the evolution and ecology of specialization. The ecological niche of a parasite is believed to be easier to define than that of free-living organisms (Timms and Read 1999) because the main living environment or habitat of a parasite and its food are represented by its host. Therefore, the degree of specialization of a parasite is usually considered in terms of its host specificity, which is traditionally defined as the number of host species exploited by a given parasite species. A parasite species using a single host species is usually considered as a specialist, whereas if it exploits multiple host species it is thought to be a generalist. However, the classification of a parasite species as either specialist or generalist can also be affected by the scale of observation, especially in the case of endoparasites. For example, a parasite can exploit multiple hosts but be highly specific in relation to its physical location within a host (Sukhdeo and Sukhdeo 1994). The study of hematophagous arthropod ectoparasites avoids this difficulty. Indeed, hematophagous arthropods are not usually highly restricted to any specific location on the host body (but see Hsu et al. 2002) and, therefore, their host specificity may be considered as a good estimator of resource specialization.

If the level of specialization expressed as host specificity is a true species character, it should be relatively constant across different populations of the same parasite species. In addition, from an evolutionary perspective, the host specificity of a parasite is not merely a function of how many host species it can exploit, but also of how closely related these host species are to each other (Poulin and Mouillot 2003). For example, consider two parasite species each exploiting the same number of host species; if one of these parasite species exploits only host species belonging to the same genus whereas the other exploits hosts belonging to different families or orders, then the host specificity of the former should be considered higher than that of the latter. Therefore, the study of host specificity should take into account phylogenetic (taxonomic) relationships among all host species of a parasite species.

Here we addressed fundamental questions about the evolution of specificity by studying spatial variation of host specificity in fleas (Siphonaptera) in 21 geographic regions. Fleas are parasites of higher vertebrates, being most abundant and diverse on small mammals. They usually alternate between periods when they occur on the body of their hosts and periods when they occur in their hosts' burrows or nests. In most cases, pre-imaginal development is entirely off-host. The larvae are usually not parasitic and feed on organic debris as well as on dried excreta of adult fleas and cannibalistically (e.g. feeding on flea eggs and larvae of younger age) in the burrow and/or nest of the host. The degree of association between a particular flea species and a particular host species varies, with flea species ranging from highly host-specific to host-opportunistic (Marshall 1981).

Arthropod ectoparasites, especially periodic (e.g. fleas and mesostigmatid mites) and temporary (e.g. mosquitos and tabanids) ones, are often influenced by their off-host environment (e.g. microclimate of host's nest/burrow, Krasnov et al. 2002). This dependence on the off-host environmental conditions can mask the true spatial pattern of host specificity in these parasites. However, this masking is likely to be unidirectional, decreasing rather than increasing the true level of host specificity. For example, some hosts that are exploited by a flea species in one locality may be dropped out of the host spectrum of this flea in another locality due to the unsuitability of the microclimate and/or substrate of the host's burrow in this locality for pre-imaginal flea development. In addition, the true level of host specificity in a parasite species can be distorted in its peripheral populations because the latter often live under conditions sharply different from those of core populations. Peripheral areas, on the edges of a flea's geographic range, are often characterized by variable and suboptimal conditions (in terms of both host populations and off-host environment), relative to core areas. Peripheral populations are thus expected to be more variable, since the variable conditions induce fluctuating selection, which itself maintains high genetic diversity (Fisher 1930, Volis et al. 1998). Alternatively, due to marginal ecological conditions at the periphery, populations there may be small and isolated and adapted to a narrower range of ecological conditions (Carson 1959). In any case, the host specificity of individual parasites from marginal populations may differ from that of individuals from the center of a parasite's geographic range.

The aims of this study were threefold. First, we asked whether host species used by a flea species are a random draw of locally available host species. In other words, does host taxonomy/phylogeny matter? This was tested by comparison of the host spectrum of a flea species with random selections from the entire pool of potential host species available in the region, using randomization tests and an index of host specificity that takes host taxonomy into account (Poulin and Mouillot 2003). Second, we studied whether host specificity is a true species character of fleas, i.e. is it repeatable across populations of the same flea species? Third, we asked what causes geographical variation in host specificity (if any) in the same flea species, i.e. whether host specificity relates to distance from the centre of the geographic range or to geographic variation in the off-host environmental conditions.

Materials and methods

Data set

Data were obtained from 21 published surveys that reported flea distribution and abundance on small mammals (Insectivora, Rodentia and Lagomorpha) in 21 different regions (Table 1). The detailed surveys that include all the relevant information are available mainly for the Palaearctic. These sources provided data on a number of individuals of a particular flea species found on a number of individuals of a particular host species. We cross-checked the species lists with the catalogue of Lewis and Lewis (1990a) to resolve cases of synonymy. Flea species with cosmopolitan distributions (e.g. *Xenopsylla cheopis*, *Nosopsyllus fasciatus*) that were likely introduced to many regions with humans, domestic animals and synanthropous rodents, were omitted from the analysis. In addition, single findings of a flea species on a host species or in a region were considered accidental and were not included in the analysis. We included in the analysis only those flea species that were recorded in at least two regions. In total, we used data on 401 flea samples, representing 662 856 flea individuals of 118 flea species collected from 351 926 individual mammals of 152 species. Repeatability analysis (see below) was carried out for all these flea species, whereas randomization tests and analyses of geographic variation of host specificity (see below) were conducted for the 23 flea species that were recorded in at least 5 regions.

All analyses were carried out on log-transformed data. The number of hosts examined often covaries with the number of parasite individuals and species found in a survey (Walther et al. 1995, Morand and Poulin 1998), and sampling effort (number of host individuals examined per host species, see below) was therefore included as a potential confounding variable, where relevant.

Table 1. Number of flea species from each of the 21 regions used in the analyses.

Region	Number of species	Source
North New Mexico	16	Morlan 1955
Idaho	27	Allred 1968
California	10	Davis et al. 2002
North Kyrgyzstan	29	Shwartz et al. 1958
Turkmenistan	25	Zagniborodova 1960
Kustanai region, northwestern Kazakhstan	18	Reshetnikova 1959
Tarbagatai region, eastern Kazakhstan	30	Mikulin 1958
Pavlodar region, eastern Kazakhstan	13	Sineltschikov 1956
Akmolinsk region, northern Kazakhstan	24	Mikulin 1959a
Moyynkum desert, Kazakhstan	29	Popova 1967
East Balkhash desert, Kazakhstan	33	Mikulin 1959b
Dzhungarskyi Alatau, Kazakhstan	23	Burdelova 1996
Selenga region, central Siberia	12	Pauller et al. 1966
Altai mountains	10	Sapegina et al. 1981
Tuva	27	Letov et al. 1966
Mongolia	22	Vasiliev 1966
Slovakia	11	Stanko et al. 2002
Volga-Kama region	28	Nazarova 1981
Khabarovsk region, southern Russian Far East	13	Koshkin 1966
North Asian Far East	13	Yudin et al. 1976
Negev desert, Israel	2	Krasnov et al. 1997 and unpubl.

Measures of host specificity

The two measures of host specificity we used were a) the number of mammalian species on which the flea species was found, corrected for sampling effort (residuals of the regression against number of hosts examined) and b) the specificity index, S_{TD}, and its variance $VarS_{TD}$ (Poulin and Mouillot 2003). The index S_{TD} measures the average taxonomic distinctness of all host species used by a parasite species. When these host species are placed within a taxonomic hierarchy, the average taxonomic distinctness is simply the mean number of steps up the hierarchy that must be taken to reach a taxon common to two host species, computed across all possible pairs of host species (see Poulin and Mouillot 2003 for details). For any given host species pair, the number of steps corresponds to half the path length connecting two species in the taxonomic tree, with equal step lengths of one being postulated between each level in the taxonomic hierarchy. The greater the taxonomic distinctness between host species, the higher the number of steps needed, and the higher the value of the index S_{TD}: thus it is actually inversely proportional to specificity. A high index value means that on average the hosts of a flea species are not closely related. Taxonomic distinctness is used as a surrogate for genetic or phylogenetic distances among hosts; although the latter measures would provide a better representation of the overall differences between hosts, data are not yet available for all mammalian host taxa included in the present analyses. Using the taxonomic classification of Wilson and Reeder (1993), all mammal species included here were fitted into a taxonomic structure with 5 hierarchical levels above species, i.e. genus, subfamily, family, order, and class (Mammalia). The maximum value that the index S_{TD} can take (when all host species belong to different orders) is thus 5, and its lowest value (when all host species are congeners) is 1. However, since the index cannot be computed for parasites exploiting a single host species, we assigned a S_{TD} value of 0 to these flea species, to reflect their strict host specificity. The variance in S_{TD}, $VarS_{TD}$, provides information on any asymmetries in the taxonomic distribution of host species (Poulin and Mouillot 2003); it can only be computed when a parasite exploits 3 or more host species (it always equals zero with 2 host species). To calculate S_{TD} and $VarS_{TD}$ and run the simulations, we developed a computer program using borland C++ Builder 5.0 (available at <http://www.otago.ac.nz/zoology/downloads/poulin/TaxoBiodiv1.2>).

The number of host species exploited by a flea species correlated positively with both S_{TD} and $VarS_{TD}$ ($r = 0.74$, $N = 401$ and $r = 0.41$, $N = 210$; respectively, $p < 0.05$ for both), indicating that these measures were influenced by the number of species in a flea's host spectrum. In addition, S_{TD} and $VarS_{TD}$ covaried positively with each other ($r = 0.45$, $p < 0.05$). The same relationships held when we used only flea species recorded in at least 5 regions ($r = 0.72$, $N = 154$; $r = 0.60$, $N = 89$ and $r = 0.59$, $N = 89$; respectively, $p < 0.05$ for all). Therefore, in the subsequent analyses S_{TD} and $VarS_{TD}$ were corrected for the number of host species in a flea's host spectrum.

Randomization tests

Using randomization tests, we tested the null hypothesis that the S_{TD} and $VarS_{TD}$ values for host species used by a flea in a region are no different from those of random subsets of the regional pool of available host species (i.e. all rodent, lagomorph and insectivore species caught by traps in each of the regional surveys). To do this, for each of the 23 flea species occurring in at least five regions, and for each region where they occurred, we generated 10 000 random selections of host species from the regional list, each selection having the same number of species as that used by the flea in that region. We only performed the test when a flea species occurred on at least 3 host species in a given region, because $VarS_{TD}$ cannot be computed on fewer host species. Values of S_{TD} and $VarS_{TD}$ were computed for each random subset. Observed values of S_{TD} and $VarS_{TD}$ were then compared with the null distributions of these random subsets; the probability that the observed values depart from the null distribution is equal to the proportion of simulated values that are greater (or lower) than the observed ones.

Repeatability analysis

To determine whether flea host specificity expressed either as the number of host species exploited, average taxonomic distinctness among hosts, S_{TD}, and its variance, $VarS_{TD}$, is a true flea species attribute, i.e. a parameter that varies less among populations of the same flea species than among host species, we performed a repeatability analysis following that of Arneberg et al. (1997). Using flea species for which at least two samples were available, we analyzed the variation in host number, taxonomic distinctness among hosts, S_{TD}, and its variance, $VarS_{TD}$ by a one-way ANOVA in which flea species was the independent factor. A significant effect of flea species would indicate that the measures are repeatable within flea species, i.e. that they are more similar to each other than to values from other flea species. We estimated the proportion of the total variance originating from differences among flea species, as opposed to within species, following Sokal and Rohlf (1995). First, we carried out the repeatability analysis using all flea species in the data set. Then, the analysis was repeated using only fleas which were recorded in at least 5 regions. Finally, we carried out the repeatability analysis across flea genera (48 genera), to see if host specificity is a generic character also.

Analyses of determinants of geographic variation in host specificity

To understand the possible causes of geographic variation in host specificity within a flea species, we calculated for each region and for each of 23 species with at least 5 across-region records the parameters that characterized host availability and abiotic environment (climate). Parameters of host availability were the total number of available host species, and their taxonomic distinctness, S_{TD}, and its variance, $VarS_{TD}$, of the entire regional pool of host species. Climatic factors were annual precipitation, mean surface air temperature of January and mean surface air temperature of July. These variables where calculated for each region using 30′ grid data (Kineman et al. 2000). In addition, we calculated the distance of a region where a flea species was found from the geometric center of the geographic range of this species. To obtain the values of these variables we composed distribution maps for each of the 23 flea species. The sources for distribution range maps were the taxonomic monographs and reviews of Hopkins and Rothschild (1962, 1966, 1971), Ioff et al. (1965), Lewis (1973), Traub et al. (1983), Kiefer et al. (1984) and Liu et al. (1986). Distribution range maps for each species were composed as polygon maps using the ArcView 3.2 software. For each of the 23 flea species we calculated the mean values of each of the three climatic parameters across the entire geographic range of this species and then calculated for each region an absolute deviation of each of these parameters from its across-geographic range mean value.

For each flea species, we regressed measures of host specificity in a region (number of host species, S_{TD} and $VarS_{TD}$) against a) the values of host availability (the number of host species, their taxonomic distinctness, S_{TD}, and its variance, $VarS_{TD}$, of the entire host pool of a region), b) distance from the geometric center of the geographic range and c) deviations of the climatic parameters from their mean across-geographic range values. We avoided an inflated Type I error by performing sequential Bonferroni corrections of the significance level.

Results

Of 118 flea species in the data set, 23 species where found in at least 5 regions. These consisted of 7 hystrichopsyllids, 9 leptopsyllids and 7 ceratophyllids (Table 2). Mean across-region numbers of host species exploited by these fleas ranged from 1.5 to 10.0, whereas the mean taxonomic distinctness of the host spectrum and its asymmetry ranged from 0.57 and 0.32 to 3.37 and 2.72, respectively (Table 2).

Using the 23 common flea species and the 21 regions, there were 86 cases where a flea species occurred on at least three host species in a region. In 58 of these 86 cases, the observed S_{TD} value for the flea species did not differ significantly from those of the 10 000 random selections of host species from the regional pool (see Fig. 1 for an example). However, in 26 of the 28 cases where there was a significant difference, the observed S_{TD} was lower than the values of the random subsets. Lower values than expected were significantly more frequent than higher values (26 versus 2: $\chi^2 = 20.6$, $DF = 1$, $p < 0.001$). This means that when host use departs from random, the parasite utilizes host species that are more closely related to each other than on average across the regional pool.

The 26 significantly lower S_{TD} values were scattered among the various flea species and regions, so that no particular flea species was responsible for this pattern. In many cases, however, significant departures from randomness appeared easier to detect when a flea exploited several host species (see Fig. 1). This is certainly due to the low power of the test when few host species are considered, i.e. the limited ability of the randomization test to reject the null hypothesis when few host species are involved. For $VarS_{TD}$, again most of the time, i.e. 61 out of 86 cases, the observed value did not differ from those of the random selections. In the 25 cases where it did, it was as likely to be lower as it was to be higher (13 vs 12, respectively). This suggests that the taxonomic affinities of host species chosen by a parasite do not tend to be more or less symmetrical than those of random selections from the regional pool of available host species.

The repeatability analysis for 118 flea species recorded in least two regions demonstrated that host specificity measured as the number of host species exploited, S_{TD} or $VarS_{TD}$ can be considered as a flea species character (Fig. 2). Estimates of number of hosts, host taxonomic distinctness (S_{TD}) and asymmetry of this distinctness ($VarS_{TD}$) from the same flea species are more similar to each other than expected by chance, and vary significantly among flea species ($F_{117,283} = 2.48$, $F_{117,283} = 1.54$, $F_{71,138} = 1.61$, respectively, $p < 0.002$ for all), with 30.4, 14.4 and 17.4%, respectively, of the variation among samples accounted by differences between flea species. Thus, estimates of host specificity are repeatable within the same flea species. This remained true when we included only flea species that were recorded in at least 5 regions ($F_{22,131} = 4.52$, $F_{22,131} = 1.66$, $F_{18,70} = 2.79$, respectively, $p < 0.04$ for all), with 34.5, 9.0 and 26.8%, respectively, of the variation among samples accounted by between-species differences. The number of host species exploited and the

Table 2. Mean(±SE) across-region values of host specificity measures for the 23 flea species used in the analyses. $VarS_{TD}$ can be calculated only for fleas that use >2 host species.

Species	Number of hosts	S_{TD}	$VarS_{TD}$
Neopsylla mana	8.83±1.47	2.80±0.55	1.07±0.26
Neopsylla pleskei	3.57±1.36	2.34±0.52	0.69±0.29
Neopsylla setosa	2.87±1.27	2.54±0.48	0.32±0.29
Doratopsylla birulai	2.60±1.61	2.87±0.61	2.72±0.41
Hystrichopsylla talpae	8.60±1.61	2.25±0.61	1.08±0.26
Palaeopsylla soricis	7.50±1.47	3.20±0.55	2.04±0.26
Ctenophthalmus assimilis	10.00±1.20	3.29±0.46	1.20±0.19
Amphipsylla dumalis	1.20±1.61	0.60±0.60	–
Amphipsylla primaris	3.33±1.47	2.39±0.55	1.01±0.33
Amphipsylla schelkovnikovi	1.40±1.61	2.00±0.61	–
Amphipsylla rossica	4.00±1.14	2.61±0.43	0.75±0.22
Amphipsylla sibirica	2.40±1.61	2.40±0.61	0.39±0.39
Frontopsylla elata	5.11±1.20	2.47±0.47	0.96±0.22
Mesopsylla hebes	1.75±1.27	0.57±0.48	0.42±0.41
Ophthalmopsylla volgensis	3.00±1.61	1.83±0.61	0.64±0.33
Pectinoctenus nemorosus	2.40±1.61	2.06±0.61	0.78
Amalaraeus penicilliger	5.41±1.04	2.97±0.39	1.14±0.18
Citellophilus tesquorum	4.62±1.28	2.72±0.48	0.86±0.26
Megabothris rectangulatus	9.60±1.61	3.09±0.61	1.15±0.26
Nosopsyllus consimilis	6.20±1.61	3.37±0.61	0.73±0.26
Nosopsyllus fidus	3.17±1.47	2.76±0.55	0.58±0.29
Oropsylla ilovaiskii	1.57±1.36	0.71±0.51	0.55±0.41
Oropsylla silantiewi	1.57±1.36	1.02±0.51	–

taxonomic asymmetry ($VarS_{TD}$) of host assemblages were repeatable also across flea genera ($F_{47,353} = 3.09$ and $F_{31,178} = 2.16$, respectively, $p < 0.001$ for both), with 20.8 and 15.4%, respectively, of the variation among samples accounted by between-genus differences. In contrast, the taxonomic distinctness (S_{TD}) of host assemblages was not more repeatable within flea genera compared to that across genera ($F_{47,353} = 1.12$, $p = 0.27$).

In most flea species, the degree of host specificity did not depend on either total number of available hosts in a region or on their taxonomic distinctness. The number of host species exploited by a flea species was not affected by the number of host species in the entire regional host pool ($r^2 = 0.0009$–0.47, $p > 0.1$ for all), except for *A. penicilliger* and *F. elata* where the number of hosts exploited increased significantly in regions with more host species ($r^2 = 0.49$ and 0.52, respectively, $p < 0.02$ for both). Similarly, the taxonomic distinctness (S_{TD}) of hosts exploited by a flea species did not change as a function of the taxonomic distinctness of the regional host pool ($r^2 = 0.01$–0.44, $p > 0.1$ for all) except for *A. penicilliger* and *N. fidus* which used more taxonomically distinct hosts in regions with taxonomically more diverse host assemblages ($r^2 = 0.49$ and 0.52, respectively, $p < 0.03$ for both). There was no correlation between the asymmetry in taxonomic distinctness of the host spectrum measured as $VarS_{TD}$ and the asymmetry in taxonomic distinctness of the entire regional host pool, for all flea species ($r^2 = 0.001$–0.59, $p > 0.1$ for all) except *N. consimilis*. In this species, the value of $VarS_{TD}$ for the host species exploited was higher in regions where the host assemblage also had a high value of $VarS_{TD}$ ($r^2 = 0.81$, $p < 0.03$).

No correlation between any of the host specificity measures and the relative distance of a location from the center of the geographic range was found in any of the 23 flea species ($r = 0.003$–0.39, $p > 0.3$ for all). In 17 of 23 flea species examined, at least one measure of host specificity correlated positively or negatively with at least one of the parameters that described the deviation of environmental conditions in a location from the mean value of the respective environmental factor calculated across the entire geographic range of a flea species (Table 3). Furthermore, as can be seen by contrasting Tables 2 and 3, significant correlations between host specificity measures and parameters reflecting environ-

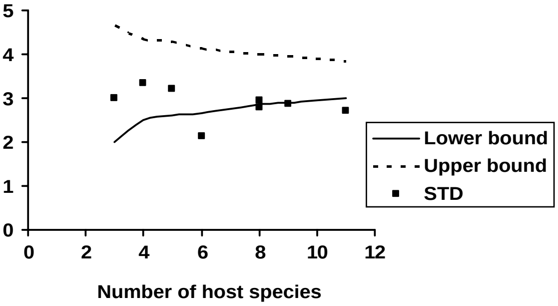

Fig. 1. Example of the comparison between the observed S_{TD} values of various flea species and the randomized selections of host species from the regional pool of host species. Shown here are results for 8 flea species from the Tarbagatai region. Each point represents the observed value for a flea species. The upper and lower bounds encompass 95% of the simulated random values, forming a funnel that gets narrower as the number of host species exploited by a flea increases. Points above the funnel indicate flea species with higher S_{TD} values than expected from random selections of host species, and points below indicate flea species with lower S_{TD} values than expected.

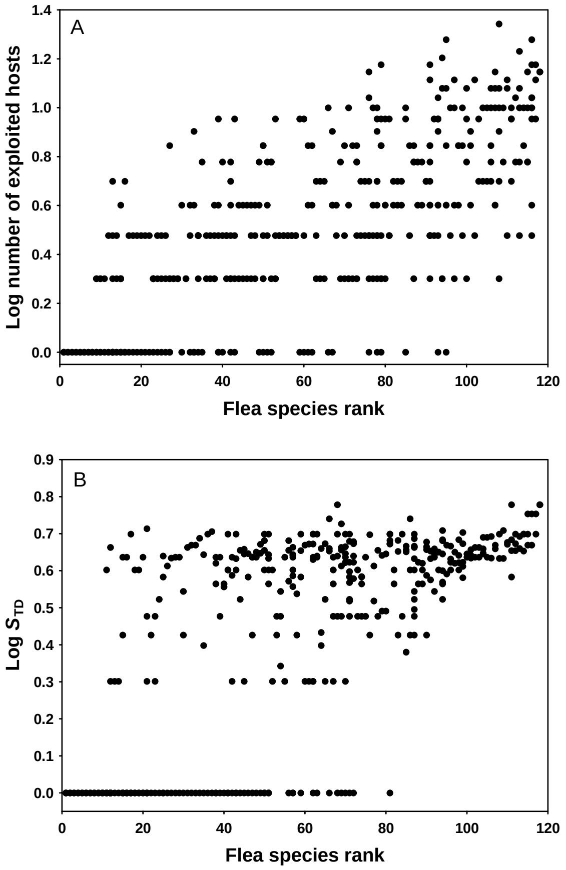

Fig. 2. Rank plots of number of host species (A) and average taxonomic distinctness (S_{TD}) between hosts (B). The 118 flea species recorded in at least two regions are ranked according to their mean log-transformed values of either number of host species (controlled for sampling effort) or S_{TD} (controlled for number of host species), with rank 1 given to the species with the lowest mean value; all sample estimates are plotted for each species. If variation is small within compared to between flea species, we expect the points to fall in a region of the plot stretching from the lower left to the upper right corner, with few or no points in either the upper left or lower right corner.

mental variation were found mainly in flea species that tend not to be very host specific.

Discussion

The most common explanation for the patterns of host affiliation or habitat preferences of a species is that traits leading to increased fitness in one host/habitat can be detrimental in others (Jaenike 1990). Consequently, trade-offs in fitness across hosts or habitats represent a main assumption of most hypotheses regarding the evolution of specialization (Futuyma and Moreno 1988, but see Mackenzie 1996). Moreover, Fry (1996) and Kawecki (1998) demonstrated that even when trade-offs are absent, selection can favour specialization. However, various factors may oppose the evolution toward higher specialization, such as the unpredictability of host quality or the energetic cost of host search (see Fry 1996 and references therein). The degree of specialization of a species may thus be the result of two opposing selection forces that are, in turn, associated with both historical and ecological factors.

Here we demonstrate that a) the host specificity of flea species is a true species character; b) in many cases, the set of host species used by a flea tends to be more taxonomically clustered, i.e. the host species are more closely related to each other than in random subsets taken from the regional pool of host species; however, the most common scenario is that there are no differences between the taxonomic properties of the set of host species used by a flea and random subsets of the regional pool; c) in the majority of cases, the characteristics of the regional pool of host species have no effect on the characteristics of the set of host species used by a flea; and d) in general, environmental conditions, measured as departures from average conditions across the entire geographical range, influence the measures of host specificity.

The level of host specificity of a parasite species is determined by the range of conditions to which this species is adapted. These conditions are related to ecological, behavioral, physiological and biochemical traits of a particular host species or group of species (Ward 1992, Poulin 1998). For fleas, these traits can be the structure of host skin, the physical and chemical properties of host blood, the parameters of the host's immune response, and the environmental conditions of the host burrow/nest. If we adopt a Hutchinsonian representation of the ecological niche of a parasite species as an n-dimensional hypervolume, the axes of which are host traits, then a parasite species would demonstrate either broad or narrow tolerance along each of these axes. This also corresponds to the more contemporary definition of the functional niche (Tokeshi 1999, Rosenfeld 2002). The repeatability of the degree of host specificity among populations of the same flea species found in this study suggests that host specificity is a true attribute of a flea species and can be envisaged as the entire set of flea's responses along all axes representing host traits. Natural selection can, thus, act on this set of responses as a whole. On the other hand, we did not find within-genus repeatability in S_{TD}, suggesting that congeneric fleas can vary in their host specificity in terms of the level of taxonomic distinctness of their host assemblage. This supports, albeit indirectly, the hypothesis that specificity, at least for the taxonomic diversity of the hosts, is not an evolutionary blind alley and that the evolution of specialization has no intrinsic direction (Thompson 1994, Desdevises et al. 2002). In

Table 3. Summary of significant ($p < 0.05$) correlations between measures of host specificity and environmental factors across regions for 17 flea species. Factors are deviations of mean annual temperature of January (TJA) and July (TJU) and mean annual precipitation (P) in a region from their mean values calculated across the entire geographic range of a flea species.

Species	Measure of host specificity	Environmental factor	r
Neopsylla mana	Host number	TJA	0.79
	$VarS_{TD}$	TJU	−0.81
Neopsylla pleskei	Host number	TJU	−0.76
Neopsylla setosa	Host number	P	−0.78
Hystrichopsylla talpae	$VarS_{TD}$	TJU	−0.79
Palaeopsylla soricis	Host number	TJU	−0.70
	$VarS_{TD}$	P	0.75
Ctenophthalmus assimilis	Host number	TJU	0.70
Amphipsylla primaris	Host number	TJU	−0.79
	S_{TD}	P	0.80
Amphipsylla schelkovnikovi	Host number	P	0.73
Amphipsylla rossica	$VarS_{TD}$	TJA	−0.70
Frontopsylla elata	S_{TD}	TJA	0.69
Ophthalmopsylla volgensis	S_{TD}	TJA	−0.74
Pectinoctenus nemorosus	Host number	TJU	0.75
Amalaraeus penicilliger	Host number	P	−0.55
Citellophilus tesquorum	$VarS_{TD}$	TJA	0.87
	S_{TD}	TJU	−0.76
Megabothris rectangulatus	S_{TD}	TJU	0.90
	$VarS_{TD}$	P	−0.89
Nosopsyllus consimilis	$VarS_{TD}$	TJA	−0.96
	$VarS_{TD}$	P	0.89
Nosopsyllus fidus	Host number	P	0.75
	$VarS_{TD}$	TJA	−0.88

addition, the difference between the two measures of host specificity in terms of repeatability (the number of host species was repeatable both within species and within genus, whereas the index S_{TD} was repeatable within species but not within genus) suggests that these two measures capture different aspects of host specificity and are complementary to each other (Poulin and Mouillot 2003).

In 28 out of 86 cases, the randomization tests indicated that fleas were using host species with a taxonomic distinctness different from that of the regional host species pool. In the many cases where the null hypothesis was not rejected, the power of the test may have been the reason, i.e. the randomization cannot easily discern between random and non-random host choice when a flea only uses 3, 4 or 5 host species. If we focus only on the cases where we found departures from the null model, then the main pattern (26 out of 28 cases) is that the hosts used by a flea are more closely related than those in the pool as a whole. The explanation may have something to do with host compatibility. The appropriateness of a host species for a parasite species is determined by a) the ability of the parasite to acquire the resources provided by this host and b) the ability of the parasite to use these resources successfully. The successful acquisition of resources is related to host defense mechanisms (e.g. grooming behaviour and immune responses) and the capability of a parasite to cope with these mechanisms, whereas the successful use of resources is related to certain properties of these resources (e.g. physical and chemical parameters of host blood). The tendency for the set of host species used by many fleas to be taxonomically clustered can stem from similarity of both the "defense" and "resource" parameters of closely related host species. Indeed, the major constituents of blood that are important for hematophagous arthropods (e.g. water, lipid, carbohydrate and protein content, see Lehane 1996) are likely similar in closely-related host species (Vatschenok 1988). Consequently, the efficiency of feeding on closely related hosts is expected to be similar and to differ from that on distant hosts. Indeed, the flea *Xenopsylla skrjabini* digested blood of *Mus musculus* and *Rhombomys opimus* (both belonging to family Muridae) during 15–18 h, whereas it took this flea 24–30 h to digest blood from guinea pig *Cavia porcellus* (Vatschenok 1988). Behavioural anti-parasite defence mechanisms also tend to be similar in closely-related species (Mooring et al. 2004). Although the similarity of the immune responses of closely related hosts to the same parasite species has never been tested specifically, some findings indirectly suggest that this is the case. For example, continuous infestation of house mouse *Mus musculus*, cotton mouse *Peromyscus gossipinus* and guinea pig by larvae of the tick *Ixodes scapularis* resulted in no resistance of both murids (*M. musculus* and *P. gossipinus*) and high resistance of the guinea pig measured as the parasite's feeding success (Galbe and Oliver 1992). Consequently, when a flea species adds a host species to its repertoire, this new host species is, as a rule, taxonomically related to one or more host species from the existing host spectrum.

We did not find any effect of the characteristics of the regional pool of host species on the characteristics of the host assemblage used by a flea. This means that, in general, local host availability (in terms of host number and taxonomic diversity of the host pool) does not influence flea host specificity. This finding supports the idea that the ability of a flea species to use a certain set of hosts is genetically constrained. The absence of coupling between the number of potential host species and host specificity was found in the monogenean genus *Lamellodiscus* parasitic on Mediterranean fish (Desdevises et al. 2002). However, the local availability of taxonomically-related species in a region can affect to some extent the level of host specificity of a given flea species in this region. Indeed, among parasites of Canadian freshwater fish, parasites exploiting hosts belonging to species-rich taxa have been found to parasitize other potential hosts closely related to the original host (Poulin 1992). A similar pattern was reported for digeneans parasitic on coral reef fish of the Great Barrier Reef (Barker et al. 1994) and for parasites of fish from Canada and the Mediterranean (Sasal et al. 1998).

In spite of the degree of host specificity of a flea species being a true species character, this character varied across the geographic range in many fleas, indicating that host specificity is also influenced by local factors. Geographic variation in the host specificity of a flea species was not related to differences between core and peripheral flea populations but rather was associated with environmental variation. Across-region variation in host specificity linked with environmental conditions was found in 17 of 23 studied flea species. This suggests the occurrence of a causal association between specificity and local ecological factors. A relationship between specificity and ecological factors has been reported for other victim-exploiter associations and, sometimes, has been seen as the primary determinant of host specialization (Smiley 1978). Our results suggest that environmental factors may play an important role in the geographic variation of flea host specificity, although they are not the only factors involved. The effect of environmental variables on the degree of local host specificity of a flea is likely related to the microclimatic preferences of the off-host stages of fleas (eggs, larvae and/or pupa). For example, the flea *Xenopsylla ramesis* parasitizes several gerbilline species throughout the Middle East (Lewis and Lewis 1990b). However, in some areas *Meriones crassus* is dropped out of the host spectrum of this flea (Krasnov et al. 1997). The reason for this is the unsuitability of microclimatic and substrate conditions in *M. crassus* burrows for the successful survival of eggs, larvae and newly-emerged imago of *X. ramesis* (Krasnov et al. 2001, 2002).

It should be noted, however, that flea taxonomy is based mainly on morphological characters. Therefore, in some cases, the existence of cryptic flea species can mask the true patterns of host specificity. For example, geographic variation in the degree of host specificity can arise when dealing with several geographically vicariate flea species erroneously considered as a single valid species. However, for the vast majority of flea species, molecular data are not yet available.

In conclusion, our results suggest that the ability of a flea species to exploit a certain set of host species is a) phylogenetically constrained, b) to a lesser extent, also constrained by the availability of taxonomically-related host species in the region, and c) to an important extent modulated by local environmental conditions.

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