

Host specificity under molecular and experimental scrutiny

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Current research on the patterns and processes underpinning host specificity in parasites goes well beyond field observations. Molecular studies are used increasingly on a range of parasite taxa to uncover levels of specificity not recognized previously. By contrast, the widespread use of experimental infections indicates that new host–parasite combinations are achieved easily in the laboratory, suggesting that parasites are less specific than they often appear. However, molecular and experimental studies of host specificity must be interpreted with caution: the usefulness of molecular studies is sometimes overstated, whereas experiments are often performed in an unnatural context. Here, the prospects offered by both approaches, as well as their limitations, are highlighted.

Host specificity: a defining feature of parasites

Host specificity is arguably one of the most fundamental properties of parasitic organisms. In simple terms, host specificity is inversely proportional to the number of host species that can be used by a parasite at a given stage in its life cycle. The range of hosts that can be used is constrained at a proximate level by both ecological and physiological factors and is determined ultimately by the evolutionary and biogeographical history of a parasite and its potential hosts [1]. Parasites that are highly host-specific occur in a single host species and their fate is linked closely to that of the host. By contrast, generalist parasites can persist following host extinction by continuing to exploit alternative hosts. Therefore, host specificity is a key determinant of the probability of parasite extinction [2]. Host specificity also reflects the ability of a parasite to colonize new host species when the opportunity arises. Not surprisingly, there is a tight association between the host specificity of parasites and their likelihood of ‘jumping’ to a novel host and initiating an emerging infectious disease [3–5]. Host specificity is thus clearly of central importance to parasites and of general interest to parasitologists. The many recent studies reviewed here challenge our understanding of host specificity: on the one hand, they reveal a degree of host specificity not detected previously in natural systems and, on the other hand, they demonstrate experimentally that specificity can break down easily under the right conditions. The prospects offered by molecular and experimental methods are highlighted here, and the inherent limitations of both approaches are considered with a critical eye.

Patterns of parasite specialization

Traditionally, host specificity has been quantified simply as the host range, that is, the list of host species actually used by a parasite in Nature. Although insufficient host sampling can create biases, this approach generally tells us that most parasites are highly host specific. Within many parasite taxa, the majority of species infect only one or sometimes a few host species, whereas only few parasite species are generalists that can exploit many host species. This pattern, although common (see illustrative examples in Figure 1), is far from universal. Different parasite taxa show different levels of host specificity, often apparently because of their mode of transmission. For instance, among parasites of primates, contact-transmitted parasites are restricted typically to fewer host species than are parasites that use intermediate hosts and that are acquired by ingestion [6]. Yet, even in parasite taxa with excellent mobility and dispersal, host specificity is often remarkably high [7]. Among parasites with the same mode of transmission, the extent to which several phylogenetically or ecologically similar host species are available within the same microhabitat can also cause variation in host specificity among parasite species [8]. Nevertheless, the general pattern remains: most parasite species co-evolve in association with only a few and often only one host species.

This generalization is based strictly on the number of host species used by parasites in Nature. The recent interest in host specificity has seen the development of many new, more sophisticated indices that measure specificity more accurately [9–12]. These indices take into account evolutionary and ecological differences among the host species used by a parasite. The phylogenetic relationships among hosts matter: obviously, a parasite infecting four host species belonging to the same genus can be said to be more specific than another parasite infecting four host species belonging to different families, even though they both exploit the same number of hosts. Similarly, the relative abundance of the parasite on its different hosts also matters: a parasite whose population is concentrated almost entirely on one of its four host species is more specific than another parasite spread among its four hosts more evenly. Only a few studies to date have used these new indices [13–15], enabling more subtle patterns to be detected. However, the most revealing new light cast on the study of host specificity comes from a series of recent molecular and experimental investigations.

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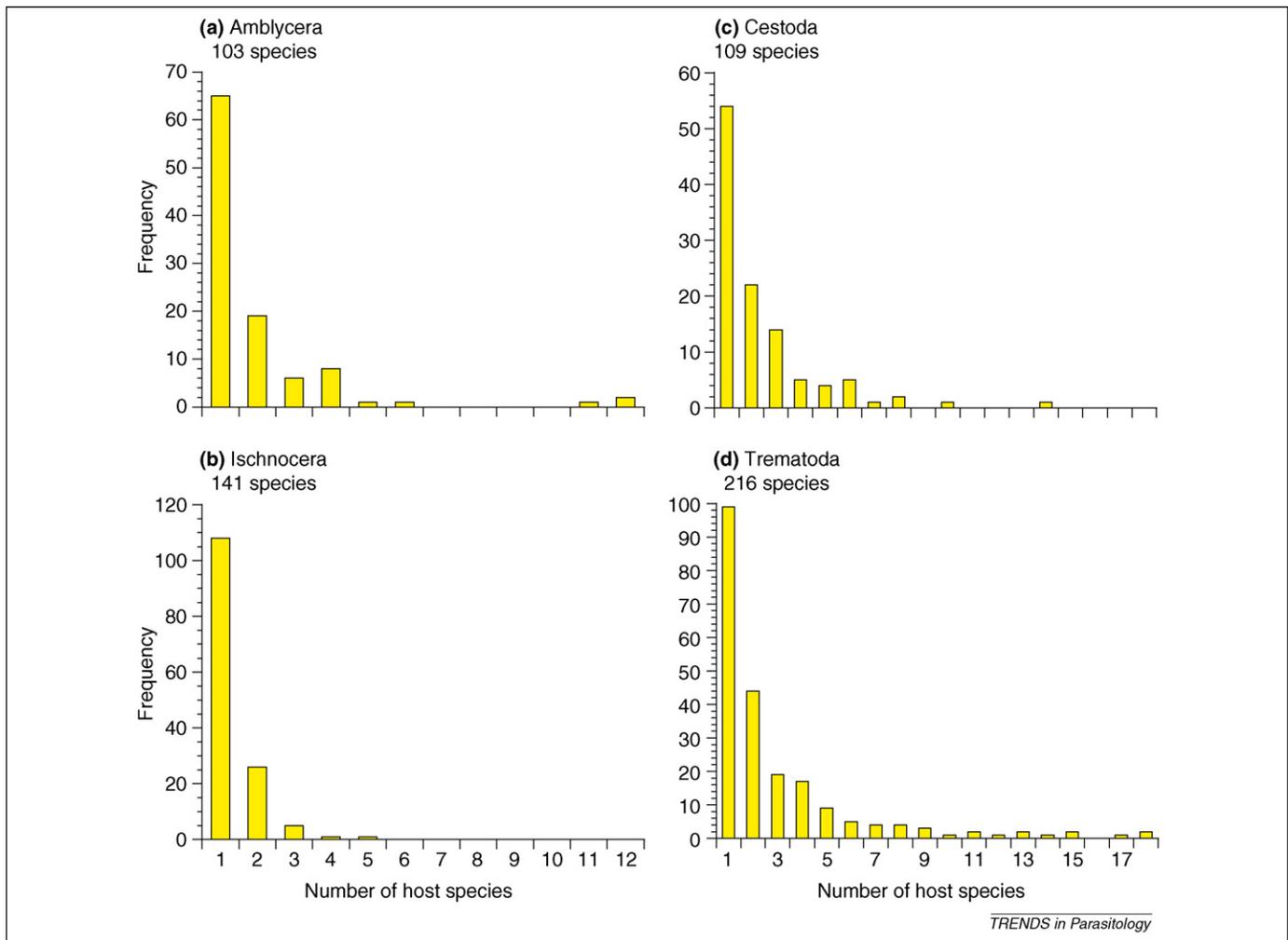


Figure 1. Frequency distribution of host specificity. The number of host species used by a parasite among all valid species of chewing lice worldwide that are parasitising rodents is shown separately for the suborders (a) Amblycera and (b) Ischnocera (data from Ref. [51]) and for (c) cestodes and (d) trematodes that are parasitising the birds of Azerbaïdzhān (data from Ref. [52]).

Host specificity under molecular scrutiny

In any field survey, parasite identification used to be based almost exclusively on morphological characters. Over the past decade, molecular tools have been applied widely in parasitological studies, and it is now routine to use DNA sequences to identify parasites and achieve a higher level of discrimination among morphologically similar species.

In the context of host specificity, genetic studies have generated new insights on several fronts. Numerous genetic investigations have revealed complexes of cryptic species in many taxa of parasites [16]. When morphology suggests the presence of one parasite species infecting several hosts, genetic data reveal multiple distinct parasite species showing high host specificity [17–22]. Thus, morphologically identical species designated under one species name formerly are turning out to consist of several different isolated gene pools (Figure 2a). The opposite is also observed frequently, that is, molecular data sometimes confirm that certain parasite taxa are true generalists [23–25]. One fascinating example is the identification of sexually dimorphic male and female strepsipteran insect parasites of the family Myrmecolacidae (each gender is specific to a distinct host species: males infect ants and females infect crickets) as conspecifics after almost

a century had elapsed without the identification of a female [26]. In addition, multiple cryptic parasite species have also been identified within single host species by molecular analyses [27]. Nevertheless, the general message from most recent molecular studies is that, in many cases, we have underestimated previously the levels of host specificity shown by parasites in Nature: many generalist species were in fact sets of highly host-specific species that we failed to distinguish [17–22].

An additional use of molecular tools to study host specificity focuses on the mechanisms responsible for enforcing specificity. For instance, genetic data can be used to pinpoint the moment at which specificity is manifested in the infection process (Figure 2b). Specificity can result from mechanisms acting before infection *per se*, by preventing contact between the parasite and unsuitable hosts, or it can result simply from the death of the parasite after infection of an unsuitable host [28]. For example, gene-sequence data have confirmed that adult specimens of the tetraphyllidean cestode *Echeneibothrium vernetae* occur in only one of the two species of skates in which immature worms were found [29]. In this case, specificity results from the failure of the parasite to establish and develop in alternative hosts and not from a failure to reach these

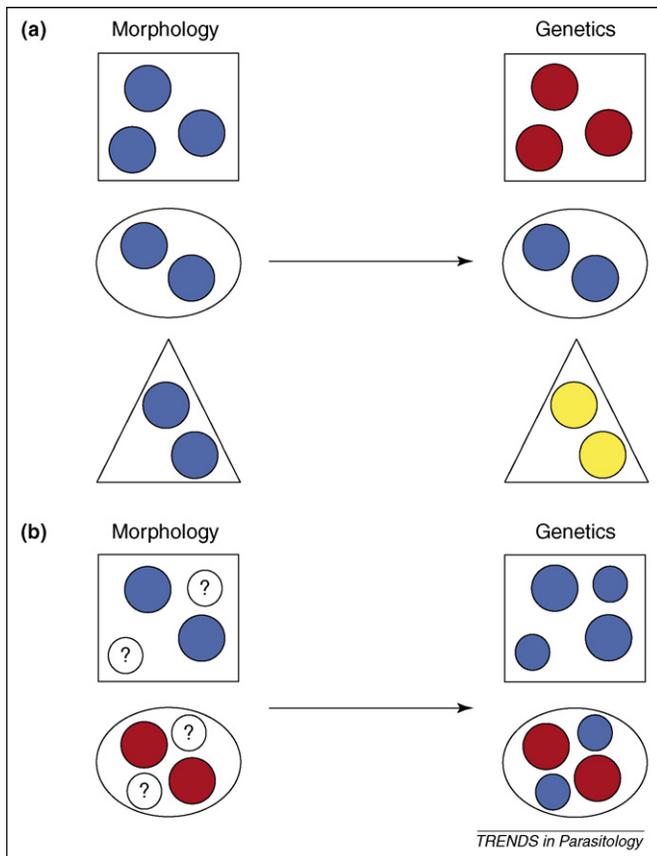


Figure 2. Host specificity revealed by genetic data. In (a), what appears based on morphological identification as a single parasite species (colored circles) found in different host species (different geometric shapes), often turns out to consist of different genetic entities, each specific to one or only a few host species, after the comparison of DNA sequences. The situation in (b) illustrates that genetic data can also be used to identify juvenile parasite stages arrived recently in a definitive host that often cannot be distinguished based on morphology (small circles enclosing a question mark). In the example shown, this approach demonstrates that a parasite can occur in a host species at the juvenile stage even if adult parasites are never found in that host (oval host); molecular data can thus pinpoint the time (i.e. post-infection) at which host specificity is manifested.

hosts. Because immature cestodes, as well as many other larval parasites, cannot often be identified to a particular species based on morphology, information of this kind is available only by using molecular tools.

Experimenting with host specificity

In Nature, certain host–parasite combinations seem possible, at least in principle, because both species co-occur in the same physical habitat; if they are not realized, it is assumed generally to result from ecological or physiological incompatibilities [28]. Other host–parasite combinations appear impossible because host and parasite never co-occur in the same habitat and thus never encounter each other. Species introductions can provide a kind of natural experiment, whereby an invading host or parasite is faced with novel interaction partners, sometimes leading to successful infection [30,31]. Only in the laboratory, however, is it possible to create novel combinations by bringing selected hosts and parasites together, to determine what exactly prevents infection from occurring in Nature.

In its simplest form, the experimental study of host specificity consists in exposing a host species to a parasite

found normally on a different (but related) host; if both host species harbor a specific parasite species, then a fully reciprocal transfer becomes possible experimentally [32–35]. A more comprehensive approach involves exposing a parasite to host species that are progressively more phylogenetically distant from the original host, that differ progressively more from the original host with respect to ecological features or that come from increasingly more distant geographical regions [36–39] (Figure 3). By comparing the performance of the parasite on these novel hosts, it is possible to assess what proximate mechanisms serve to maintain host specificity in Nature. Some studies indicate that host preferences by the parasite are important [40–43], whereas others point to the specific nature of host defenses [39,41]. Taken as a whole, these recent studies reveal the real potential of parasites to infect novel host species and they show that host specificity can break down when new hosts and parasites are brought together. These new findings [32–43] have important implications. Under experimental conditions, parasites chosen as biological control agents against a particular host species, in part because of their high host specificity, can infect non-target hosts [44]. Similarly, the monogenean *Gyrodactylus turnbulli*, assumed previously to be specific strictly to guppies, *Poecilia reticulata*, survives and reproduces on many other species in the aquarium fish trade, some not related closely to guppies [45]. No doubt these results are of concern to pest managers [46] and those in the ornamental fish industry. Experimental studies of host specificity can tell us which host switches will be possible, or even

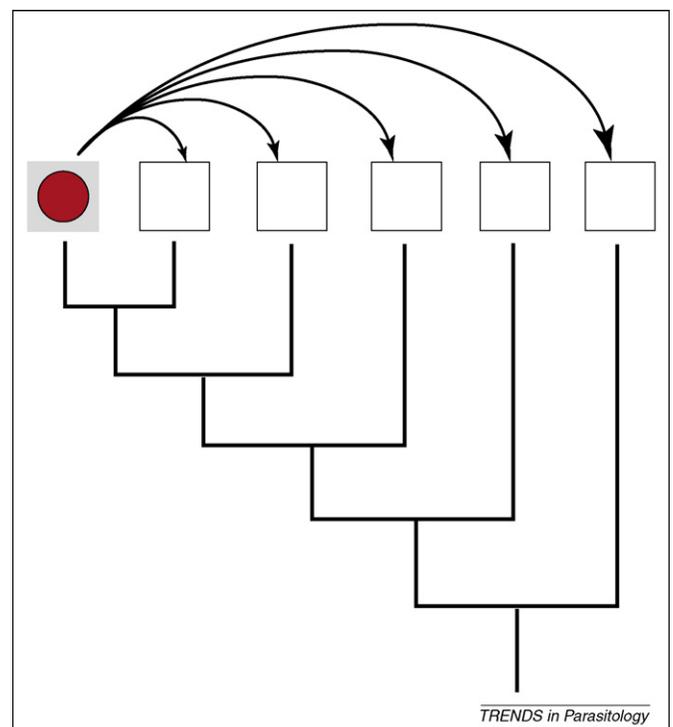


Figure 3. Experimental approach to the study of host specificity. Each square represents a host species, with their phylogenetic relationships illustrated by the tree joining them. An apparently host-specific parasite (red circle) that occurs naturally in a single host species is ‘transferred’ under controlled conditions to progressively more phylogenetically distant host species. The distance between the novel host species exposed to infection and the original host can be expressed in terms of ecology or geography, rather than phylogeny.

probable, in the aftermath of climate change, when modified geographical distributions will bring parasites into contact with new host species.

Experimental studies of host specificity can also shed light on evolutionary processes. Recent phylogenetic studies indicate that, on a macroevolutionary timescale (10^4 – 10^6 years), host specificity does not evolve irreversibly in one particular direction, that is, generalist ancestors do not produce more specialized descendants invariably [47,48]. The operation of natural selection on host specificity can be investigated on shorter timescales (<10 years) using cross-generational experiments. For example, by maintaining each of two or more parasite strains on different host species for several generations, one can determine whether the enforced single-host environment will select for specialization on that host, as well as measure the speed at which this happens [49]. The experimental approach has therefore much to offer to the study of host specificity.

Words of caution

Molecular studies indicate generally that host specificity was underestimated previously because of our inability to discriminate among closely related and morphologically similar species of parasites. By contrast, experimental studies have revealed that parasites can often infect many more hosts successfully than is seen in Nature. Entomologists have come to the same conclusion following insect-rearing experiments: many herbivorous insects, thought previously to be specific to a particular plant, can feed and develop successfully on other plants, prompting downward revisions of earlier estimates of insect biodiversity in tropical forests [50]. So, what do these recent results mean for parasitologists?

Although they are certainly powerful, we must be cautious not to overstate the usefulness of molecular methods versus morphological analyses. The term ‘cryptic species’ could become overused easily in the literature by geneticists who are not taxonomic experts. Thorough morphological studies carried out with care can often achieve similar levels of discrimination as genetic studies and are essential for the correct classification of species (which is necessary for investigations of host specificity above the parasite species level). Indeed, morphological differences among ‘cryptic’ species are often detected after the species are separated based on molecular evidence [22]. Gene sequences should not be seen as a panacea for imprecise morphological measurements or a lack of qualified taxonomists. Nevertheless, although morphological species descriptions remain essential, estimates of host specificity based solely on the morphological identification of parasites are fast losing their appeal and the specificity of most parasite taxa will need to be reassessed based on both morphological and genetic data.

The experimental approach promises much but it too must be used with caution. This is true in the literal sense because the accidental release of novel host–parasite combinations into the wild is always a risk in such studies. Caution is also necessary for interpreting experimental results: new host–parasite combinations created in a laboratory environment might mean little in Nature. They are created outside a real ecological context. In Nature, for

instance, other parasite species might exclude the parasite from exploiting the novel host; the possibility of inter-specific exclusion can also be studied in the laboratory but is too often overlooked. In addition, when parasites encounter and occasionally infect novel hosts in Nature, natural selection might intervene and curtail any new association. Given sufficient time and genetic variation, the host could evolve the ability to avoid or resist infection if it proved detrimental; otherwise, the parasite could evolve mechanisms to avoid contact with the host if development in this host yields lower fitness than in the original host. Most host–parasite combinations existing in Nature have passed through these co-evolutionary processes successfully. By contrast, novel combinations of hosts and parasites created in the laboratory have no prior evolutionary history and experimental studies rarely examine the long-term maintenance of a novel host–parasite combination across several generations. How should one interpret the failure of a parasite to infect a new host species under experimental conditions? There exists genetic variance within a parasite species; surely there must be pioneer genotypes capable of making the jump to infect a new host and many other genotypes not capable of this. Experimental studies cannot capture all the existing genetic variation and its importance for the capture of new host species. Similar to the use of molecular tools, the experimental approach is a promising avenue for the study of host specificity, as long as its users remain aware of its limitations.

Acknowledgements

We thank Dale Clayton, Haseeb Randhawa and members of the University of Otago’s Evolutionary and Ecological Parasitology Group for discussion and comments on an earlier draft. R.P. and D.B.K. are funded by a grant from the Marsden Fund of New Zealand.

References

- 1 Poulin, R. (2007) *Evolutionary Ecology of Parasites*, (2nd edn), Princeton University Press
- 2 Koh, L.P. *et al.* (2004) Species coextinctions and the biodiversity crisis. *Science* 305, 1632–1634
- 3 Daszak, P. *et al.* (2000) Emerging infectious diseases of wildlife: threats to biodiversity and human health. *Science* 287, 443–449
- 4 Cleaveland, S. *et al.* (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 356, 991–999
- 5 Dobson, A. and Foufopoulos, J. (2001) Emerging infectious pathogens of wildlife. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 356, 1001–1012
- 6 Pedersen, A.B. *et al.* (2005) Patterns of host specificity and transmission among parasites of wild primates. *Int. J. Parasitol.* 35, 647–657
- 7 Dick, C.W. and Patterson, B.D. (2007) Against all odds: explaining high host specificity in dispersal-prone parasites. *Int. J. Parasitol.* 37, 871–876
- 8 Jex, A.R. *et al.* (2006) The importance of host ecology in thelastomatoid (Nematoda: Oxyurida) host specificity. *Parasitol. Int.* 55, 169–174
- 9 Caira, J.N. *et al.* (2003) On a new index of host specificity. In *Taxonomy, Ecology and Evolution of Metazoan Parasites* (Vol. 1) (Combes, C. and Jourdane, J., eds), pp. 161–201, Presses Universitaires de Perpignan
- 10 Poulin, R. and Mouillot, D. (2003) Parasite specialization from a phylogenetic perspective: a new index of host specificity. *Parasitology* 126, 473–480
- 11 Poulin, R. and Mouillot, D. (2005) Combining phylogenetic and ecological information into a new index of host specificity. *J. Parasitol.* 91, 511–514

- 12 Rohde, K. and Rohde, P.P. (2005) The ecological niches of parasites. In *Marine Parasitology* (Rohde, K., ed.), pp. 286–293, CSIRO Publishing
- 13 Fallon, S.M. *et al.* (2005) Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. *Am. Nat.* 165, 466–480
- 14 Krasnov, B.R. *et al.* (2005) Host specificity and geographic range in haematophagous ectoparasites. *Oikos* 108, 449–456
- 15 Edwards, D.D. and Vidrine, M.F. (2006) Host specificity among *Unionicola* spp. (Acari: Unionicolidae) parasitising freshwater mussels. *J. Parasitol.* 92, 977–983
- 16 Poulin, R. and Morand, S. (2004) *Parasite Biodiversity*, Smithsonian Institution Press
- 17 Donald, K.M. *et al.* (2004) Host specificity and molecular phylogeny of larval *Digenea* isolated from New Zealand and Australian topshells (Gastropoda: Trochidae). *Int. J. Parasitol.* 34, 557–568
- 18 Sehgal, R.N.M. *et al.* (2005) Molecular evidence for host specificity of parasitic nematode microfilariae in some African rainforest birds. *Mol. Ecol.* 14, 3977–3988
- 19 Pouyaud, L. *et al.* (2006) Phylogenetic relationships among monogenean gill parasites (Dactylogyridea, Ancyrocephalidae) infesting tilapia hosts (Cichlidae): systematic and evolutionary implications. *Mol. Phylogenet. Evol.* 38, 241–249
- 20 Smith, M.A. *et al.* (2006) DNA barcodes reveal cryptic host specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proc. Natl. Acad. Sci. U. S. A.* 103, 3657–3662
- 21 Cepicka, I. *et al.* (2006) New evolutionary lineages, unexpected diversity, and host specificity in the parabasalid genus *Tetratrichomonas*. *Mol. Phylogenet. Evol.* 39, 542–551
- 22 Whiteman, N.K. *et al.* (2006) Cryptic host specificity of an avian skin mite (Epidermoptidae) vectored by louseflies (Hippoboscidae) associated with two endemic Galápagos bird species. *J. Parasitol.* 92, 1218–1228
- 23 Krizanauskienė, A. *et al.* (2006) Variation in host specificity between species of avian hemosporean parasites: evidence from parasite morphology and Cytochrome *b* gene sequences. *J. Parasitol.* 92, 1319–1324
- 24 Palm, H.W. *et al.* (2007) Genetic diversity in the trypanorhynch cestode *Tentacularia coryphaenae* Bosc, 1797: evidence for a cosmopolitan distribution and low host specificity in the teleost intermediate host. *Parasitol. Res.* 101, 153–159
- 25 Jones, C.M. *et al.* (2007) Host specificity of two species of *Gnathia* (Isopoda) determined by DNA sequencing blood meals. *Int. J. Parasitol.* 37, 927–935
- 26 Kathirithamby, J. and Johnston, J.S. (2004) The discovery after 94 years of the elusive female of a myrmecolacid (Strepsiptera), and the cryptic species of *Caenocholax fenyesi* Pierce sensu lato. *Proc. R. Soc. Lond. B Biol. Sci.* 271 (suppl.), S5–S8
- 27 Miura, O. *et al.* (2006) Introduced cryptic species of parasites exhibit different invasion pathways. *Proc. Natl. Acad. Sci. U. S. A.* 103, 19818–19823
- 28 Combes, C. (2001) *Parasitism: The Ecology and Evolution of Intimate Interactions*, University of Chicago Press
- 29 Randhawa, H.S. *et al.* (2007) Establishment of the onset of host specificity in four phyllobothriid tapeworm species (Cestoda: Tetraphyllidae) using a molecular approach. *Parasitology* 134, 1291–1300
- 30 Font, W.F. (2003) The global spread of parasites: what do Hawaiian streams tell us? *Bioscience* 53, 1061–1067
- 31 Poulin, R. and Mouillot, D. (2003) Host introductions and the geography of parasite taxonomic diversity. *J. Biogeogr.* 30, 837–845
- 32 Moen, D.S. and Stockwell, C.A. (2006) Specificity of the monogenean *Gyrodactylus tularosae* Kritsky and Stockwell, 2005, to its natural host, the White Sands pupfish (*Cyprinodon tularosa* Miller and Echelle 1975). *Comp. Parasitol.* 73, 278–281
- 33 Ruiz-González, M.X. and Brown, M.J.F. (2006) Honey bee and bumblebee trypanosomatids: specificity and potential for transmission. *Ecol. Entomol.* 31, 616–622
- 34 Olstad, K. *et al.* (2007) Variation in host preference within *Gyrodactylus salaris* (Monogenea): an experimental approach. *Parasitology* 134, 589–597
- 35 Kuris, A.M. *et al.* (2007) An experimental evaluation of host specificity: the role of encounter and compatibility filters for a rhizocephalan parasite of crabs. *Int. J. Parasitol.* 37, 539–545
- 36 Perlman, S.J. and Jaenike, J. (2003) Infection success in novel hosts: an experimental and phylogenetic study of *Drosophila*-parasitic nematodes. *Evolution* 57, 544–557
- 37 Munoz-Antoli, C. *et al.* (2006) Infectivity of *Echinostoma friedi* miracidia to different snail species under experimental conditions. *J. Helminthol.* 80, 323–325
- 38 Bush, S.E. *et al.* (2006) Ecomorphology of parasite attachment: experiments with feather lice. *J. Parasitol.* 92, 25–31
- 39 Bush, S.E. and Clayton, D.H. (2006) The role of body size in host specificity: reciprocal transfer experiments with feather lice. *Evolution* 60, 2158–2167
- 40 Esberard, C.E.L. *et al.* (2005) A method for testing the host specificity of ectoparasites: give them the opportunity to choose. *Mem. Inst. Oswaldo Cruz* 100, 761–764
- 41 Gerardo, N.M. *et al.* (2006) Ancient host–pathogen associations maintained by specificity of chemotaxis and antibiosis. *PLoS Biol.* 4, 1358–1363
- 42 Ohhashi, Y. *et al.* (2007) Involvement of host recognition by oncomiracidia and post-larval survivability in the host specificity of *Heterothrium okamotoi* (Monogenea: Dicliphoridae). *Int. J. Parasitol.* 37, 53–60
- 43 Glennon, V. *et al.* (2007) Experimental infections, using a fluorescent marker, of two elasmobranch species by unciliated larvae of *Branchotentes octohamatus* (Monogenea: Hexabothriidae): invasion route, host specificity and post-larval development. *Parasitology* 134, 1243–1252
- 44 Goddard, J.H.R. *et al.* (2005) Host specificity of *Sacculina carcini*, a potential biological control agent of introduced European green crab *Carcinus maenas* in California. *Biol. Invasions* 7, 895–912
- 45 King, T.A. and Cable, J. (2007) Experimental infections of the monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *Int. J. Parasitol.* 37, 663–672
- 46 Briese, D.T. (2005) Translating host-specificity test results into the real world: the need to harmonize the yin and yang of current testing procedure. *Biol. Control* 35, 208–214
- 47 Simkova, A. *et al.* (2006) Specificity and specialization of congeneric monogeneans parasitising cyprinid fish. *Evolution* 60, 1023–1037
- 48 Poulin, R. *et al.* (2006) Evolution of host specificity in fleas: is it directional and irreversible? *Int. J. Parasitol.* 36, 185–191
- 49 Little, T.J. *et al.* (2006) Parasite–host specificity: experimental studies on the basis of parasite adaptation. *Evolution* 60, 31–38
- 50 Novotny, V. and Basset, Y. (2005) Host specificity of insect herbivores in tropical forests. *Proc. R. Soc. Lond. B Biol. Sci.* 272, 1083–1090
- 51 Price, R.D. (2003) *The Chewing Lice: World Checklist and Biological Overview*, Illinois Natural History Survey Special Publication no. 24
- 52 Gregory, R.D. *et al.* (1991) Life history, ecology and parasite community structure in Soviet birds. *Biol. J. Linn. Soc.* 43, 249–262