Evolutionary trends in body size of parasitic flatworms

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Evolutionary trends in body size have been identified within several lineages, but not all have followed Cope's rule, which states that average body size within a taxon tends to increase over time. In organisms such as parasites, space constraints may have shaped the evolution of body sizes, favouring small-bodied taxa capable of exploiting new niches. Here, the average adult body sizes of families in three groups of parasitic flatworms, the Digenea and two clades of Monogenea (Monopisthocotylea and Polyopisthocotylea), are related to their clade rank. Clade rank reflects the number of branching events, and thus the total path length, between an extant family and the root of the phylogenetic tree. Among families of Digenea, all of which are endoparasites of vertebrates, there was no trend in body size evolution. In contrast, the Monopisthocotylea and Polyopisthocotylea, which are (with the exception of Polystomatidae and Sphyranuridae) ectoparasites of fish, revealed significant negative relationships between family body size and clade rank, suggesting an evolutionary trend of decreasing size. In addition, an analysis of body size distributions in monogenean families also provides support, albeit weak, for this trend. From an ancestor parasitic on the skin of fishes, monogeneans have diversified by colonizing other microhabitats on their hosts, including such space-limited sites as the gaps between secondary gill lamellae. Using a conservative likelihood ratio test, however, a random walk, or null model of evolution could not be discarded in favour of the directional trends mentioned above. Nevertheless, these results suggest that body size has taken different evolutionary paths in endo- and ectoparasitic flatworms.   © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, **85**, 181–189.

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INTRODUCTION

Body size is the single most important characteristic of an animal, as it relates strongly to life-history traits, metabolic rate, population density and geographical distribution (see Peters, 1983; Schmidt-Nielsen, 1984; Brown, 1995). Evolutionary trends in body size indicate that average sizes have increased over evolutionary time in several distinct animal taxa, a phenomenon known as Cope's rule. Fossil evidence from taxa as diverse as foraminiferans, molluscs and mammals generally supports Cope's rule (Stanley, 1973; Bonner, 1988; Arnold, Kelly & Parker, 1995; Alroy, 1998). The rule is not universal, however, and sufficient exceptions exist to suggest that body size may follow different evolutionary trajectories in different groups of organisms (Jablonski, 1997; Knouft & Page, 2003). Not only is the direction of evolutionary trends variable among taxa, but the nature of the

underlying factors can also vary from selection-driven to passive or random processes (McShea, 1994, 1998; Wagner, 1996; Alroy, 2000).

In certain groups of organisms, evolutionary decreases in body size are expected a priori. For instance, lineages making an evolutionary transition from a free-living to a parasitic mode of life are generally assumed to evolve smaller body sizes (Price, 1980; Hanken & Wake, 1993). The comparative evidence, however, is equivocal. Among extant members of groups ranging from nematodes to crustaceans, parasitic lineages are sometimes larger, sometimes smaller than their closest free-living relatives (Kirchner, Anderson & Ingham, 1980; Poulin & Hamilton, 1995; Poulin, 1995a, b).

What remains unclear is what happens after the transition to parasitism. Given that parasites living in or on other animals normally face more severe space constraints than most free-living animals, what have been the evolutionary trends in their body sizes following the adoption of a parasitic mode of life? If the

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first parasites in a lineage exploited the most accessible organs of their hosts (gastrointestinal tract for endoparasites, skin for ectoparasites), what happened to their body sizes when some groups colonized new microhabitats where space constraints may have been more intense (e.g. gill lamellae for fish ectoparasites)?

The frequency distribution of body sizes, even when the latter are log-transformed, tends to be right-skewed in most higher taxa (e.g. Van Valen, 1973; Dial & Marzluff, 1988; Blackburn & Gaston, 1994; Brown, 1995), and the same is true of all major parasitic taxa (Poulin & Morand, 1997). This could be taken as evidence that, in parasites and free-living animals in general, small-bodied taxa proliferate at higher rates than related but larger-bodied taxa. Proper comparative tests of this idea, however, indicate that body size is not a universal determinant of rates of diversification in free-living animals (Gardezi & da Silva, 1999; Orme *et al*., 2002b; Orme, Isaac & Purvis, 2002a) and in certain parasite groups (Poulin, 2002).

In higher taxa experiencing directional evolutionary trends in body size, one might expect that the skewness of body sizes would differ between species issued from basal phylogenetic branches and those from more derived lineages, as evolution would need time to shape the body size distributions of new lineages. To test for evolutionary trends in body size within higher taxa of parasites, one would need to determine whether the size of recent taxa is consistently different from that of ancestral taxa. Such a test can be problematic because there is no adequate fossil record of extinct parasite taxa; only extant taxa are available for analyses of evolutionary trends.

Using phylogenetic information, however, it is possible to identify basal and derived taxa (i.e. taxa that have originated at different distances from the base of the tree; see Pagel, 1997, 1999, 2002; Knouft & Page, 2003). Although this still does not provide information on the characteristics of extinct taxa, the body sizes of extant taxa give an indication of how ancient and recent branches in the same phylogenetic tree have evolved since branching out.

In this paper, I examine evolutionary trends in body size of basal and derived families in the Monogenea and Digenea, groups of flatworms belonging to the same phylum (Platyhelminthes). This also makes possible a contrast between monogeneans, which are, for the most part, ectoparasites of aquatic vertebrates, and digeneans, which are endoparasites of vertebrates. A priori, I expected a tendency for body size to decrease over evolutionary time as more derived groups invaded smaller microhabitats (e.g. gill lamellae for monogeneans, blood vessels for digeneans, etc.).

I also examine how body size distributions vary among families, expecting stronger right-skewed distributions (i.e. biased toward small body sizes) in the more basal families (i.e. lineages that have had a longer evolutionary history). The present analyses are the first to make use of large data sets, combined with recent and well-supported phylogenies, to uncover phylogenetically informative statistical trends in the evolution of body size in parasitic organisms.

METHODS

Data on monogenean body sizes used here are the same as those previously compiled (Poulin, 2002) from earlier monographs. Body size was taken as total body length, as most monogenean taxa have slender bodies that are much longer than they are wide. Average body lengths were first obtained for each genus by averaging species values, and family values by averaging generic values. The phylogeny used here was the complete tree of monogenean families proposed by Boeger & Kritsky (1997, 2001). Although based on morphological data, it is generally very well supported by molecular evidence (Olson & Littlewood, 2002). Several molecular analyses have recently cast doubt on the monophyly of the Monogenea (Mollaret *et al*., 1997; Justine, 1998; Littlewood, Rohde & Clough, 1999). Because of the strong possibility that they are paraphyletic, I here treat the two major branches of monogeneans, the Monopisthocotylea and the Polyopisthocotylea, as separate clades.

Data on digenean body sizes have been taken from the appendix in Poulin (1997); these were compiled from extensive searches through the literature. Body size is measured as the product of adult body length and width; given the wide range of shapes among digeneans, from long and thin to almost circular, this proved a better index of body size than simple length. Average family values were obtained as for monogeneans. The digenean phylogeny used here was the comprehensive molecular phylogeny proposed by Olson *et al*. (2003).

All data on body sizes were log-transformed prior to analysis. Evolutionary trends were examined separately within each of the three groups of parasites (Monopisthocotylea, Polyopisthocotylea and Digenea) using a generalized least squares regression (GLS). This allows one to determine whether body size correlates with clade rank among families within a group (Pagel, 1997, 1999, 2002; Knouft & Page, 2003). Clade rank is not equivalent to taxonomic rank; rather, it is the number of branching or speciation events between an extant family and the root of a phylogenetic tree, the root being the bifurcation from which all taxa in the group have descended (Norell & Novacek, 1992; Knouft & Page, 2003). It quantifies the evolutionary position of a family within a clade, or the total path length from the root of the tree to a given family. Basal

families are taxa with the lowest rank, while derived families have the highest rank.

Although data on body size were not available for all families in the monogenean and digenean phylogenies, all families were included in the computations of clade ranks and in the GLS model in order to obtain accurate phylogenetic information. Average body size for each family is predicted from the GLS regression of body size on clade rank (i.e. the distance from the root of the phylogeny to the family; Pagel, 1997, 1999, 2002; Knouft & Page, 2003). In this directional model of evolution, the slope defines the phylogenetically correct slope of the line relating clade rank to log-transformed family body size (Pagel, 1997, 1999, 2002; Knouft & Page, 2003). Information on branch length was not available, and all branches within a clade were set equal; this places a constraint on the evolutionary model, where most of the divergence in body size between sister taxa must be assumed to take place at, and not between, speciation events.

A likelihood-ratio (LR) test statistic was used to assess the probability that the GLS model was a better predictor of body size evolution within a clade than a null, or random walk, model in which the slope equals zero. In the test, $LR = -2\log (H_0/H_1)$, where H_0 is the likelihood associated with the null hypothesis and H_1 is the likelihood associated with the directional hypothesis (Pagel, 1997, 1999). The significance of the test is determined by comparing LR to a Chi-square statistic with d.f. = 1 (alpha = 0.05). The test was implemented using the program Continuous (available at http://sapc34.rdg.ac.uk/meade/Mark/).

The skewness of body size distributions was computed among log-transformed species values within the families of Monopisthocotylea and Polyopisthocotylea for which data were available on at least 20 species; insufficient families were available for a similar analysis in the Digenea. For each distribution, the standard coefficient of skewness, g_1, was computed (Sokal & Rohlf, 1995). This coefficient is scaled to equal zero in symmetrical, non-skewed distributions; negative values indicate a left-skew and positive values a right-skew (i.e. a distribution mode shifted toward larger and smaller body sizes within the range, respectively). Because values of g_1 co-vary with mean body size ($P = 0.004$ in this analysis), they were corrected for body size by taking the residuals of the regression of g_1 against mean body size across all family values; these residuals were then correlated with clade rank.

RESULTS

Data on body sizes were available for 13 families of Monopisthocotylea, 26 families of Polyopisthocotylea, and 53 families of Digenea. These numbers represent,

respectively, 65, 81 and 69% of the families in the comprehensive phylogenies of these higher taxa used in this study. There was no difference between the clade ranks of families included in the analysis and those of families for which no body size data were available, in any of the three higher taxa (Mann–Whitney U-tests, all $P > 0.21$).

Among monogeneans, the body sizes of Monopisthocotylea were smaller on average than those of Polyopisthocotylea (Fig. 1). At the level of mean family body sizes, a right-skew is not clearly evident in these two taxa, because the influence of a few species-rich families with small body sizes is negated. Among digeneans, however, the right-skew is apparent among family mean body sizes (Fig. 1).

In all three parasitic taxa, many families with relatively large average body sizes tend to be basal (i.e. they have a low clade rank), whereas many families with relatively small sizes tend to be more derived (Fig. 2). This resulted in negative slopes in the GLS regressions (Table 1). In the Monopisthocotylea, the regression is actually significant ($r^2 = 0.531$, $P = 0.0047$; see Fig. 2). However, using the more conservative LR test, the trend between body size and clade rank is not significant in any of the three higher taxa (Table 1).

If two families that do not exploit fish hosts (Polystomatidae and Sphyranuridae, two points in the bottom left corner of the figure; see Discussion) are excluded from the analysis of the Polyopisthocotylea, the regression becomes significantly negative ($\beta = -0.077$, $r^2 = 0.264$, $P = 0.0102$; see Fig. 2), and the LR test also comes close ($P = 0.066$) to indicating that there has been a significant evolutionary decrease in body size among the families that exploit fish hosts. Still, the random walk model cannot be rejected in any of the three groups, due to the fact that the directional model of evolution is not explaining significantly more of the variance in average family body sizes.
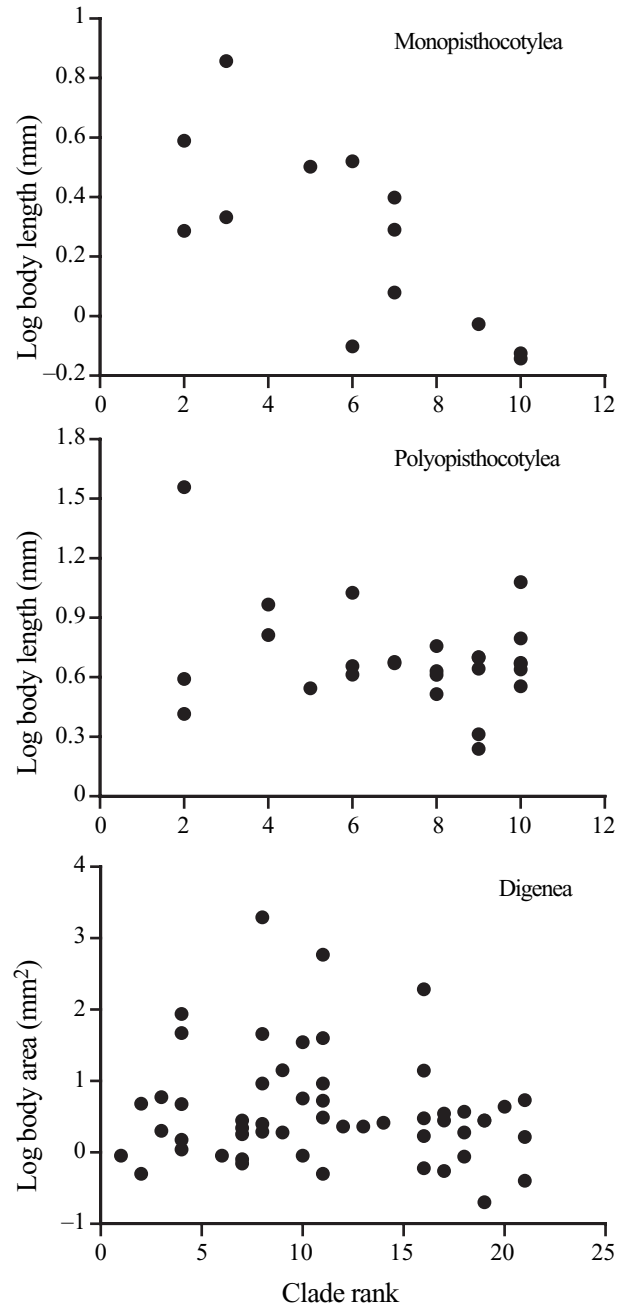
Except for significantly right-skewed body size distributions in the families Dactylogyridae and Gyrodactylidae (two-tailed t-tests, both $P < 0.001$), there were few strong skew values for other monogenean families (Table 2). There was a weak general tendency for skewness of body sizes within a family to go from right to left (i.e. from positive to negative values) with increasing clade rank (Fig. 3). This was true for both Monopisthocotylea ($r = -0.753$, $N = 5$, $P = 0.142$) and Polyopisthocotylea ($r = -0.613$, $N = 8$, $P = 0.106$), as well as for all monogenean families pooled together ($r = -0.677$, $N = 13$, $P = 0.011$), although the fact that few families could be included limited the power of the analyses. Still, the more derived families tend not to show the typical right-skewed body size distributions commonly found in most taxa.
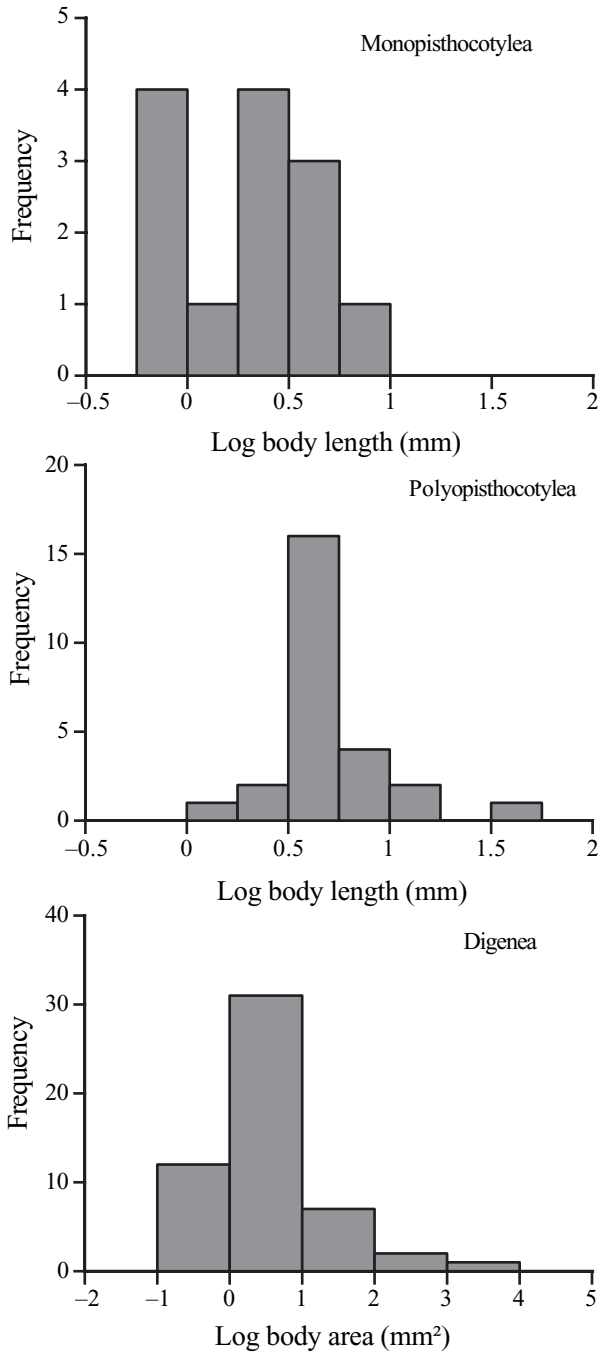
Figure 1. Frequency distribution of mean body sizes among 13 families of Monopisthocotylea, 26 families of Polyopisthocotylea, and 53 families of Digenea.

Figure 2. Mean family body size vs. clade rank for three lineages of parasitic flatworms: Monopisthocotylea, Polyopisthocotylea and Digenea. Each point represents an extant family.

DISCUSSION

Every lineage of parasites has diversified from a single ancestor, with different taxa within the same lineage colonizing and adapting to slightly different micro-habitats within the host. The hosts themselves have changed over evolutionary time, with extinction and speciation causing several turnovers of available host species. It is likely, however, that the internal and external structures that provide habitats for parasites have remain more or less constant within any higher host taxa.

Adult monogeneans and digeneans live in or on vertebrates. While host species come and go, their

Table 1. Results of the analyses of the relationship between mean family body size (log-transformed) and clade rank within three higher taxa of parasitic Platyhelminthes

Taxon	No. families	Mean no. species per family*	α† (SE)	β† (SE)	P†
Monogenea					
Monopisthocotylea	13	67.9	0.755 (0.273)	−0.101 (0.063)	0.1422
Polyopisthocotylea	26	16.2	0.980 (0.198)	−0.055 (0.048)	0.2460
Digenea	53	14.3	0.780 (0.235)	−0.017 (0.019)	0.3593

*Number of species used to measure body size, not actual species diversity.
†α is the Y-axis intercept and β the phylogenetically correct slope of the regression of log family body size on clade rank from the directional model of evolution; P is from the likelihood ratio test based on the difference between the likelihoods of the directional and random walk models.

Table 2. Skewness of species body size distributions in 13 monogenean families

| Family	No. species	Size range (mm)	Clade rank	Skewness (g_1)
Monopisthocotylea				
Capsalidae	51	0.90–22.50	3	−0.072
Dactylogyridae	462	0.13–4.97	9	0.792
Diplectanidae	51	0.32–1.66	10	0.187
Gyrodactylidae	132	0.20–3.30	6	1.110
Monocotylidae	34	0.48–12.00	2	0.385
Polyopisthocotylea				
Axinidae	29	1.60–8.10	8	−0.535
Diclidophoridae	20	0.99–10.00	8	−0.800
Discocotylidae	20	1.36–19.00	6	0.046
Gastrocotylidae	25	1.40–12.00	9	−0.084
Hexabothriidae	27	1.62–22.50	4	−0.440
Mazocraeidae	31	0.51–12.50	6	−0.023
Microcotylidae	75	1.15–15.00	9	−0.269
Polystomatidae	31	1.50–10.0	2	0.413

internal anatomy probably remains broadly the same, at least on the scale that matters to parasites. The same is probably true of the external structures of fish (skin, fins, gills) to which monogeneans attach themselves. The digestive system and external surfaces of extant fishes are probably not much different from those of fish that lived several million years ago. From the parasites' perspective, the host landscape has probably remained roughly constant during much of their evolution. It is the colonization of new microhabitats within the host paralleling the diversification of parasites that must have shaped the evolution of body sizes, since nutrient and space availability may be key determinants of optimal size.

The present analyses suggest that there has been no trend in the evolution of body sizes in digenean parasites. In the two clades of monogeneans, mean family body size covaried significantly and negatively with clade rank, but this trend could not be distinguished from a random walk process. The cautious conclusion is therefore that the most derived taxa are not necessarily smaller-bodied than the more basal ones.

Inferring evolutionary trends based solely on data from extant species is not without problems. Most phylogenetic methods used to reconstruct ancestral phenotypes make assumptions that are likely to be violated in many cases (see Cunningham, Omland & Oakley, 1998; Oakley & Cunningham, 2000). Only fossil evidence from a direct ancestor can allow unambiguous interpretation of evolutionary trends. The methods used here, based on the identification of basal and derived taxa in a phylogeny, provide one way of obtaining a rough idea of the evolutionary direction taken by these lineages since branching out (Pagel, 1997, 1999, 2002; Knouft & Page, 2003).
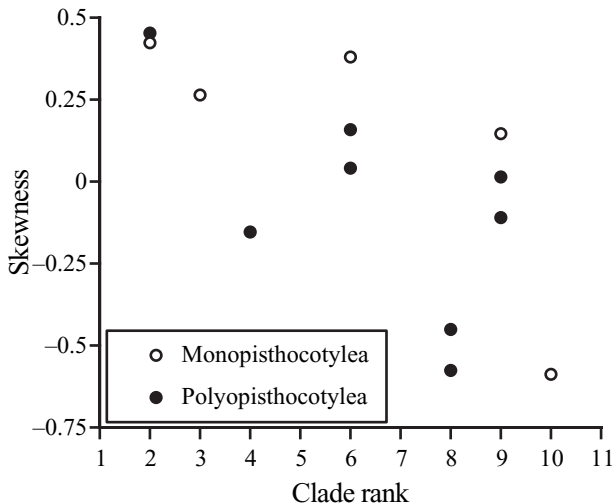
Figure 3. Skewness of body size distribution among species within a family vs. clade rank for five families of Monopisthocotylea and eight families of Polyopisthocotylea. Skewness values are corrected for mean body size. Each point represents an extant family.

Two potentially confounding factors can probably be eliminated as sources of artefacts in the analyses. First, taxon sampling was relatively good, with between 65 and 81% of families in each of the three higher taxa being included. Their positions were evenly distributed among the branches of the phylogenetic trees when compared to missing families, and there was no difference in clade rank (i.e. path length from the root to the family) between the included and excluded families. Thus the analyses covered representative samples of existing families.

Second, the lack of information on branch length meant that a speciational model of evolution was adopted instead of a model of gradual change. This probably did not matter. Using the imperfect information on branch lengths for some of the families derived from recent molecular analyses (Olson & Littlewood, 2002; Olson *et al.*, 2003) I carried out partial analyses that suggested that including estimates of branch lengths in the model has no effect on the results other than altering intercept or slope values (unpubl. data). Therefore, the speciational model used here is unlikely to have led to erroneous results. When the evolution of a trait is associated with niche shifts, as with body size being linked to the colonization of new microhabitats within hosts, a speciational model of evolutionary change often provides an accurate representation of evolutionary trends (e.g. Mooers, Vamosi & Schluter, 1999).

The first monogeneans, whether Monopisthocotylea or Polyopisthocotylea, were probably opportunistic browsers on the skin of early fishes (Llewellyn, 1965,

1970; Kearn, 1998). Most extant Monopisthocotylea still live on the skin of fish hosts, feeding on epidermal cells. These range from the basal family Capsalidae (largest-bodied Monopisthocotylea family in Fig. 2) to the more derived Gyrodactylidae (third smallest-bodied Monopisthocotylea family in Fig. 2). Physical factors and the risk of detachment, as well as potential predation by cleaner organisms feeding on fish external surfaces (Grutter, 2002), may have constrained any increases in body size and their associated increases in reproductive output.

Colonization of the buccal and branchial cavities has taken place more than once among the Monopisthocotylea (Kearn, 1998), perhaps favoured because of the protection this offered from cleaners. Some members of the Gyrodactylidae live in the gill cavity, but the most specialized gill inhabitants are some of the most derived Monopisthocotylea families, as well as some of the smallest-bodied, such as the Dactylogyridae, Tetraonchidae and Diplectanidae (the three points in the bottom right corner in Fig. 2). These monogeneans live between the secondary gill lamellae of fish, in spaces where other Monopisthocotylea would not fit. The pattern in Figure 2 suggests that invasion of these space-limited microhabitats by derived families may have been associated with a general decrease in body size. The fact that the trend is not supported by the statistical analysis may simply be due to the lack of sufficient families in this taxon and therefore to the low power of the test.

In contrast to Monopisthocotylea, most Polyopisthocotylea are blood feeders living in the gill cavity, or on the gills themselves, of their fish hosts (Kearn, 1998). The two exceptions are the Polystomatidae and Sphyranuridae (the two points in the bottom left corner of Fig. 2), which are endoparasites of non-fish aquatic vertebrates (i.e. amphibians and turtles), though one polystomatid species lives on the skin below the eyes of the hippopotamus (Kearn, 1998).

Excluding these two families, a trend appears, similar to that in the Monopisthocotylea. Among the fish parasites, the largest-bodied and most basal family, the Chimaericolidae, infect holocephalan fishes; the more recently derived families all tend toward smaller sizes, as smaller spaces within the gill cavity were colonized. Mode of attachment may also have played a role in body size evolution in these parasites. The Chimaericolidae penetrate the soft gill tissue and clamp into the cartilage, whereas more derived families attach to the surfaces of the gill lamellae; this difference may well have influenced the sizes they could attain.

Once again, a weak trend in body size evolution may exist, although there are not enough families in the analysis to reject the random walk model using the conservative LR test. The fact that the earliest mono-

geneans appear to have been large worms may also provide an ecological clue as to the identity of the sister-group of the Neodermata, the clade to which monogeneans and digeneans belong. Phylogenetic studies have yet to resolve which flatworms are the closest free-living relatives of the Neodermata (Little-wood *et al.*, 1999).

In both Monopisthocotylea and Polyopisthocotylea, the analyses of body size distributions within families also reveal a possible similar trend. The more derived families tend not to show the typical right-skewed body size distributions commonly found in most higher taxa (Van Valen, 1973; Dial & Marzluff, 1988; Blackburn & Gaston, 1994; Brown, 1995). If small-bodied species are favoured and diversify at a higher rate than larger species, it would take time for the body size distribution of species in a new lineage to adopt the characteristic right-skewed shape, with the mode shifting toward smaller sizes. Again, it may only be a lack of statistical power that kept the trend non-significant within each of the two taxa (it is significant when all 13 monogenean families are pooled).

Digeneans have complex life-cycles, in which larval development almost always begins in a mollusc, and adult worms almost always live in vertebrate hosts. One scenario postulates that the ancestor of all living digeneans was a ciliated flatworm that developed a symbiotic association with a mollusc (Llewellyn, 1965; Pearson, 1972; Kearn, 1998). As fish became domi-nant predators in aquatic habitats, frequent preda-tion on the free-living stages of these early digeneans may have led to the addition of fish hosts to the life-cycle.

In an alternative scenario, supported by recent phy-logenetic evidence, the vertebrate host came first, and the molluscan intermediate host was adopted subse-quently (Cribb *et al.*, 2003). In any event, the first digenean with a complex life-cycle matured and lived as an adult in the gastrointestinal tract of fish. From there, digeneans colonized other organs in their ver-tebrate definitive host. These include: the circulatory system, now used by three relatively basal and small-bodied families (Sanguinicolidae, Spirorchidae and Schistosomatidae); the liver, inhabited by the large Fasciolidae (second largest digenean family in Fig. 2) of intermediate clade rank; and the connective tissue and muscle, where the Didymozoidae, the largest digenean family in the analysis (and in Fig. 2) is found.

Several extant families still inhabit the gastro-intestinal tract of their vertebrate host, but these include both basal and derived families, as well as small-bodied and large-bodied ones (i.e. the Microphallidae and Gorgoderidae, which differ in size by more than two orders of magnitude). Some very basal digenean lineages may have never used the host gut as an infection site: adults of the Trans-versotrematidae, for instance, live beneath the scales of teleost fish (smallest-bodied family in the bottom-left corner of Fig. 2).

It appears that the evolutionary history of body size in digeneans has been less constrained by opportu-nities than that of monogeneans. A comparative anal-ysis has shown that digenean body size does not correlate with host body size (Poulin, 1997), and the site of infection itself seems to be a more important determinant. From an ancestral site of infection in the gut of the vertebrate host, digeneans have repeatedly invaded sites where space constraints were either more relaxed or more severe. This is unlikely to have led to a directional change in body size within the group.

Trends in body size evolution have been reported for a range of free-living taxa (Stanley, 1973; Bonner, 1988; Arnold *et al.*, 1995; Jablonski, 1997; Alroy, 1998; Knouft & Page, 2003). The present analysis is the first to investigate body size evolution in parasitic taxa. Based on the available evidence, it is impossible to reject the null, or random walk, model of evolution, and therefore there may have been no directional trend in the evolution of monogenean and digenean body sizes.

However, the appearance of similar tendencies for mean family body size to decrease as a function of dis-tance from the root of the phylogeny in both groups of monogeneans suggests that the power of the analyses may be solely responsible for the null model not being rejected. From an ancestor parasitic on the skin of fish, the only options open for monogeneans, in terms of unexploited microhabitats on the outside of the host, were sites requiring small body size. This may have placed constraints on body size evolution in monogeneans, and on the evolutionary success and diversification rates of new lineages.

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