Parasite burdens differ between sympatric three-spined stickleback species

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The ecological theory of adaptive radiation states that differences in ecological circumstances among local populations are the cause of divergence that leads to speciation. The role of parasites in contributing to divergence has seldom been considered, despite their ubiquity and known selective effects. The potential for parasites to contribute to divergence between closely related taxa was examined by quantifying the variation in parasite burdens between sympatric three-spined stickleback species (*Gasterosteus aculeatus* complex) in two lakes in coastal British Columbia, Canada. In doing so the relative importance of geographical differences between lakes and trophic or microhabitat differences between species within lakes were evaluated. The entire metazoan parasite burdens of a total of 255 limnetic and benthic sticklebacks in Paxton and Priest lakes were assayed over five time points between spring and autumn. Despite their sympatric distributions, there were large differences in parasite burdens between benthic and limnetic sticklebacks within lakes and these were consistent across both lakes. In particular, limnetics suffered greater burdens of the parasites *Schistocephalus solidus* and *Diplostomum scudderi* and benthics had much higher burdens of parasitic glochidia (mollusc larvae). Parasite burdens also differed quantitatively between lakes, but in general such differences were less pronounced than those between the stickleback species. The documented differences in parasite burdens between stickleback species have potential to contribute to divergent selection on life history, immunological and secondary sexual characters that could contribute to reproductive isolation between the species.

In recent years there has been a revival of interest in the ecological theory of adaptive radiation (Simpson 1953) which states that differences in ecological circumstances among local populations are the cause of divergence that leads to speciation (Schluter 2000). This has led to a concomitant interest in the ecological causes of divergence among populations of individual species. Traditionally the ecological theory has concentrated on the role of competition for food, and the associated evolution of trophic specialisation traits, as the predominant cause, and form, of divergence between populations (Schluter 2000). There is now good evidence in support of the role of competition as an agent of divergent selection (Schluter 2000). However there is a growing realisation that other aspects of ecology could contribute to divergence between populations. Interactions with other species are likely to be an important component of the ecology of any population. The evolutionary dynamism of such interactions has great potential to result in divergent coevolution (Thompson 2005). Recently the role of predators as agents of divergent natural selection has begun to be explored (Vamosi and Schluter 2002, Nosil and Crespi 2006) but the role of parasites in contributing to divergence between host populations has hardly been considered (although see Buckling and Rainey 2002, Thomas et al.

2003, Rauch et al. 2006). This is surprising given their ubiquity in natural populations and their acknowledged potential as selective agents (Summers et al. 2003).

Parasites could be important as primary agents of divergence, or secondarily by strengthening the divergence initiated by other ecological interactions. Parasites could make a primary contribution to divergence and speciation if the parasites to which hosts are exposed differ sufficiently between local populations that divergent selection results in reproductive isolation as a by-product. For example variation in parasite imposed selection could favour divergence of life history (Agnew et al. 2000), secondary sexual (Hamilton and Zuk 1982) or immunological characters (Kalbe and Kurtz 2006, Scharsack et al. 2007) that are associated with mate choice. Parasites could contribute secondarily to divergence if host populations experience different parasites following primary divergence for other reasons. For example, this could happen if populations living sympatrically diverge trophically because of competition for food. Such divergence could then be strengthened by the different parasites encountered as diets or microhabitats diverge. It could also be reinforced (sensu Blair 1955) by increased parasite susceptibility in hybrids between the diverging populations (Moulia 1999).

If parasites are to have the potential to drive adaptive radiation of hosts it is necessary to show: 1) that parasitism varies between host populations in ways that are of sufficient magnitude to result in divergent selection. 2) Closely related host species should exhibit consistent differences in parasitism across geographical locations. In order to show that parasites do contribute to adaptive radiation of their hosts it is necessary to show that 3) parasites cause selection on their hosts. Here, I address the potential for parasites to be involved in the divergence of populations of a host species, the three-spined stickleback, *Gasterosteus aculeatus* complex, that is a model for the study of adaptive radiation. I demonstrate satisfaction of criteria 1) and 2) for several parasites, including one species that has been repeatedly implicated as a cause of selection in the host. Numerous studies have found variation in parasite communities across geographical locations within a host species distribution (Kennedy and Hartvigsen 2000, Behnke et al. 2001). This includes three-spined sticklebacks (Kalbe et al. 2002) for which variation in parasite communities has been associated with the evolution of diversity of the major histocompatibility locus (Wegner et al. 2003). However there are very few studies that have described differences in parasitism between closely related taxa within geographical locations (Dorucu et al. 1995, Knudsen et al. 1997, 2003). Only one of these studies examined whether differences in parasitism between taxa were consistent across geographical locations (Knudsen et al. 2003). None of these studies have considered the potential evolutionary consequences of such variation within an ecological framework.

In this paper I exploit the unusual biogeography and ecology of sympatric three-spined sticklebacks in western Canada to explore differences in metazoan parasite communities between pairs of closely related species within and between two lakes. In coastal British Columbia there are seven lakes which contain, or have until recently contained, coexisting, reproductively isolated populations of two types of three-spined sticklebacks. The types are generally accepted to comprise good biological species given that they are substantially reproductively isolated with only low levels of hybridisation (McPhail 1992, Gow et al. 2006). These "species pairs" are thought to have resulted from a double invasion of the lakes from the sea, rather than from sympatric speciation (McPhail 1993). In each lake one species feeds predominantly on zooplankton in the pelagic zone and is termed "limnetic". It is slender, small and has more lateral boney plates and relatively larger spines than the other species which feeds predominantly on bottom dwelling invertebrates in the littoral zone and is termed "benthic". Benthics are deeper bodied, larger and have less "armour" (lateral plates and spines). The species pairs have been the subject of extensive ecological and evolutionary research in the last two decades. This has concentrated on divergence in feeding ecology (Schluter 1994, 1995), mate choice (Boughman 2001) and predator-prey interactions (Vamosi and Schluter 2002) between the species. Until recently almost nothing has been known about the parasite faunas of the two species. Here I describe variation in the metazoan parasite faunas, their compositions and abundances, of benthics and limnetics in two separate lakes. In doing so I demonstrate that there is important variation in parasitism in this system, both geographically and between the trophically divergent species. This supports the idea that variation in parasites could be an important agent of primary or secondary divergent selection.

Methods

In this study I sampled parasite faunas of sticklebacks in Paxton lake and Priest lake (hereafter Paxton and Priest) both on Texada island (49°40′N, 124°25′W) in the Georgia Strait, British Columbia, Canada. The lakes are ca 4 km apart in separate catchments that drain to opposite sides of the island. Both lakes contain stickleback species pairs. Available evidence suggests that these species pairs are evolutionarily independent (Taylor and McPhail 2000). Paxton and Priest were sampled on five separate occasions at approximately monthly intervals from April to September 2004 (22 April, 26 May, 28 June, 27 July, 14 September). Fish were caught in minnow traps ("Gee's Minnow Traps", Tackle Factory, Fillimore, NY). Twenty of these were set from the bank or a boat in ca 20–200 cm of water, to cover a range of microhabitats. Traps were set in approximately the same locations in each trapping session. Traps were lifted the day after setting and the contents emptied into a bucket of lake water. About 15 fish (mean 12.8, range 5–17) of each species were selected haphazardly from among those in the bucket and the rest were returned to the lake. Pure sticklebacks of the two species are easily separated in the field by experienced observers, using morphological criteria. A low level of hybridisation does occur between limnetics and benthics in the wild. Any fish with intermediate morphology were excluded from samples.

Fish selected for parasite screening were returned alive to the laboratory in Vancouver in sealed aerated polystyrene boxes within 24 h. Fish were then kept in 100 litre aquarium tanks for up to 48 h prior to having their external *Gyrodactylus alexanderi* burdens counted. During this period sticklebacks from different species, but not different lakes, were mixed. This could have facilitated transfer of *G. alexanderi*, which is directly transmitted, between the stickleback species and made estimates of their differences in burdens conservative. To count *G. alexanderi*, fish were anaesthetised, placed in 0.5 cm of water in a petri dish and their entire external surface and fins were examined under a dissecting microscope. Fish were then either spine clipped for identification and returned to a tank to be euthanised and dissected fresh within one week, or were euthanised and stored in 70% ethanol for dissection at a later date. The latter method was used only for the entire June sample.

To assess burdens of metazoan parasites, fish were systematically dissected under a microscope. We follow Bush et al. (1997) in defining "prevalence" as the proportion of hosts in a population that are infected by a particular parasite and "abundance" as the number of individuals of a parasite in or on a single host. Both terms are used here as measures of the parasite "burden" of individual hosts. The body surface was examined for parasites under the skin. The opercular cavity and gills on the right side were examined. The right eye was removed and completely dissected. Counts of parasites found in the

opercular cavity, on the gills and in the eye were doubled for the purposes of analysis, as this will have given a more accurate estimate of the total abundance of these species. The body cavity was opened and all internal organs were examined externally and internally. All metazoan parasites seen were recorded. *Glugea anomala* was also recorded.

Two hundred and fifty five fish were examined, dissected and had their macroparasite fauna recorded. These comprised 67 Paxton benthics, 62 Paxton limnetics, 70 Priest benthics and 56 Priest limnetics. Only data from these 255 fish with complete records were used in statistical analyses. An additional 18 fish that died in transit to the lab were dissected (ca 2–10 h post mortem) and had their macroparasites recorded apart from *G. alexanderi* and *Dactylogyrus* sp. A further 40 fish were externally examined and had only their *G. alexanderi* burden recorded. Data from the 58 fish with partial records were only used in the calculation of mean prevalence in Table 1. Only presence/absence, and not burden, of *Dactylogyrus* sp. was recorded for most fish because of difficulty making accurate counts of this species, especially in preserved material. Burdens were in any case low (generally <10 per fish). *Dactylogyrus* sp. was not recorded in the sample caught in June, and this parasite was excluded from the multivariate and univariate analyses.

Parasite identification

In most cases parasites were identified to genus, and sometimes to species, using keys for parasites of Canadian (Beverley-Burton 1984, Kabata 1988, Arai 1989, Gibson 1996) and North American (Hoffman 1967) freshwater fishes, and by reference to the work of Lester (1974). Thus far it has not been possible to identify encysted trematode metacercaria found in the body cavity and in the eyes. These metacercaria are referred to here as "other metacercaria" to distinguish them from the encysted metacercaria of *Apatemon* sp. ("blackspot"). It has also not been possible to identify a larval nematode found at low prevalence and

abundance in the intestines of both limnetics and benthics from Priest.

Multivariate analyses

Statistical analyses were performed in GenStat (release 8.1). In order to explore the overall differences in community composition between stickleback species and lakes, multivariate methods were used to analyse data on parasite abundances. Each individual fish can be represented as a point in a multidimensional space whose axes are the abundance of different parasite species. Canonical variates analysis (CVA) was used to identify the combinations of parasites that were best at differentiating between the stickleback populations. Only data for the 11 species of parasite that were common to both lakes were used in the CVA. Data on the abundances of each of these parasite species were transformed prior to multivariate analyses using the function natural logarithm (abundance + 1) and standardised by subtracting mean (transformed) abundance and dividing by the standard deviation of (transformed) abundance for that parasite across all populations. It is important to note that this method only used information on parasite abundances and population membership to differentiate between the stickleback populations. It did not include information about the known geographical (lake) and ecological (species) relationships between the populations. Hence the positions of the stickleback populations in the new space determined by the canonical variate (CV) axes is a function only of parasite abundances. Loadings of parasite species on the different CV axes indicate how the different parasite species contribute to the scores on that axis. They can be portrayed in a biplot that reveals the combinations of parasites that are most useful for discriminating between the different fish populations. CVA is a descriptive ordination technique which is useful for identifying the important differences between populations, but which cannot be used for making inferential tests about

Table 1. The overall prevalence (% infected ± 95% binomial confidence intervals) of metazoan parasite taxa (and *Glugea anomala*) in three-spined sticklebacks of Paxton and Priest lakes, British Columbia, averaged over five sampling points from April to September 2004. Samples sizes for all parasites except *Gyrodactylus alexanderi* (n =77, 68, 84, 66) and *Dactylogyrus* sp. (n =43, 37, 43, 32) were 68, 65, 71, 69 for Paxton benthic, Paxton limnetic, Priest benthic and Priest limnetic respectively.

Taxon	Species	Paxton		Priest	
		Benthic	Limnetic	Benthic	Limnetic
Mollusca	*Anodonta glochidia*	44.1 (33.7, 56.9)	20.0 (11.7, 31.6)	54.9 (43.3, 66.0)	17.4 (10.5, 28.7)
Crustacea	*Thersitina gasterostei*	0.0 (0.0, 6.9)	0.0 (0.0, 7.3)	76.1 (65.7, 85.6)	95.7 (88.4, 98.9)
Nematoda	unidentified	0.0 (0.0, 7.1)	0.0 (0.0, 7.3)	9.9 (4.8, 19.2)	2.9 (0.6, 10.1)
Cestoda	*Schistocephalus solidus*	0.0 (0.0, 7.0)	55.4 (43.8, 67.5)	0.0 (0.0, 6.9)	37.7 (27.3, 49.8)
	Proteocephalus sp.	10.3 (5.3, 20.7)	53.8 (41.8, 65.6)	2.8 (0.7, 9.9)	73.9 (62.5, 83.1)
	Paradilepis sp.	0.0 (0.0, 7.1)	0.0 (0.0, 7.3)	0.0 (0.0, 6.9)	4.3 (1.6, 13.1)
Trematoda	*Gyrodactylus alexanderi*	98.7 (93.6, 99.9)	75.0 (63.5, 83.9)	98.8 (94.0, 99.9)	90.9 (81.6, 96.0)
	Dactylogyrus sp.	31.0 (19.0, 46.9)	40.5 (27.0, 58.6)	24.4 (14.1, 40.9)	0.0 (0.0, 16.4)
	Crepidostomum cooperi	48.5 (37.0, 60.9)	35.4 (25.3, 48.1)	43.7 (33.0, 55.7)	18.8 (11.3, 29.9)
	Bunodera sp.	26.5 (17.6, 38.6)	50.8 (38.8, 63.2)	9.9 (4.8, 19.2)	44.9 (33.8, 56.8)
	Diplostomum scudderi	83.8 (73.5, 91.3)	95.4 (88.1, 99.0)	4.2 (1.7, 12.9)	52.4 (40.6, 65.0)
	Ichthyocotylurus sp.	7.4 (3.3, 17.0)	0.0 (0.0, 7.3)	1.4 (0.3, 8.7)	1.4 (0.2, 8.8)
	Apatemon sp. metacercaria	36.8 (26.4, 48.9)	6.2 (2.7, 15.9)	9.7 (4.8, 19.1)	0.0 (0.0, 7.0)
	other metacercaria	64.7 (53.1, 75.5)	21.5 (13.5, 33.8)	31.0 (21.2, 42.6)	8.7 (4.1, 18.1)
Acanthocephala	*Neoechinorhynchus rutili*	7.4 (3.3, 17.0)	9.2 (4.6, 19.7)	1.4 (0.3, 8.7)	14.5 (8.1, 25.4)
Microsporidia	*Glugea anomala*	4.5 (1.7, 13.2)	3.2 (0.7, 10.3)	1.4 (0.3, 8.7)	1.8 (0.3, 9.1)
Total species		12	12	14	14
Metazoan species		11	11	13	13

the importance of different factors in contributing to those differences.

Univariate analyses

To explore the relative magnitude of differences between the lakes and species in abundances of individual parasites and in overall parasite species "richness" (see below) and abundance, generalised linear models (GLMs) were used. These were all carried out on untransformed and unstandardised data. Overall richness and abundance were calculated for each fish as, respectively, the number of parasite species and the total number of individual parasites (summed over parasite species). Burdens of individual parasite species were only analysed if the parasite occurred in both lakes or if mean prevalence across populations and seasons exceeded 10%. Each response variables was modelled as a function of the factors "species" (benthic or limnetic), "lake" (Paxton or Priest) and "season" (month of collection) and the covariate "length" (standard length of individual fish in mm). As variation in parasite burdens between the species and the lakes is the primary focus of this paper, and for the sake of simplicity of interpretation, interactions were not included in these models. Season and length were included in the models to control for the most important sources of residual variation. The best-fitting models were constructed, from maximal models containing the main effects, by the sequential dropping and readdition of individual terms, until all terms remaining in the models were significant (Crawley 1993). Negative binomial error structures and logarithm link functions were normally used, following the inspection of residual plots. The significance of a term was then assessed by dropping the term from the best fitting model and comparing the change in deviance to χ^2. In one instance a normal error structure and identity link function were used, and then the significance of terms was assessed by comparing the change in fit to the F distribution.

Results

Parasite species richness and distribution across populations

Fifteen species of metazoans and *G. anomala* were recorded in total in the two lakes, including 13 species of helminths (Table 1). The remaining two metazoans were a crustacean (the ectoparasitic copepod *Thersitina gasterostei*) and one species of mollusc embedded in the gill tissue (larval "glochidia" of a freshwater mussel *Anodonta* sp.). Total parasite species richness (summed over all fish) was slightly higher in Priest than in Paxton (Table 1). There was no difference in total parasite species richness between benthics and limnetics within lakes, although this conceals the fact that the parasite communities of benthics and limnetics were comprised of slightly different species (Table 1). In particular *Schistocephalus solidus* was missing from benthics in both lakes.

Multivariate analyses

A biplot representing the CVA results is shown in Fig. 1. The main separation along the first CV axis was between species, and accounted for 54.8% of the variation. Low scores on this axis (predominantly limnetic fish) are particularly associated with high burdens of *Diplostomum scudderi*, *Proteocephalus* sp., *S. solidus* and *Bunodera* sp. High scores are associated with *G. alexanderi* and "other" metacercaria. Lakes separated along the second axis which accounted for 37.2% of the variation (Fig. 1). High scores on this axis (predominantly Paxton) are associated with high burdens of *D. scudderi*, *Apatemon* sp., other metacercaria and low burdens of *Proteocephalus* sp. Multivariate (Mahalanobis) distances between stickleback population means are not shown, but are closely correlated with the (Euclidean) distance between population means in Fig. 1. Mahalanobis distances were lowest for the same species in different lakes, highest for different species in different lakes and intermediate for different species in the same lake.

Univariate analyses

The number of metazoan species per fish and the total abundance of metazoan parasites per fish were both significantly higher in limnetics and higher in Paxton (Table 2). There was also important seasonal variation in these measures.

The abundance of most individual parasite species varied significantly between stickleback species and lakes (Table 2). Important exceptions were *G. alexanderi* and *Crepidostomum* sp. which did not show significant overall differences between stickleback species, and *G. alexanderi* and *Proteocephalus* sp. which did not vary significantly between lakes. There were important seasonal differences in abundance for many parasite species, and in some cases these disguise important difference between the stickleback species. For example, *G. alexanderi* abundances were lower but more constant on benthics, while peak abundances tended to be higher on limnetics (data not shown).

Most parasite species were more abundant on limnetics (Table 2) than on benthics. This included *S. solidus* which was found only in limnetics among the sampled fish (Table 1). Its overt, external symptoms (Arme and Owen 1967) were seen in only one benthic (in Priest) among several hundred that were individually measured between April and September 2004 (MacColl 2009). Two parasites which were significantly more common on benthics were glochidia (*Anodonta* sp.) and other metacercaria. Most parasite species were also more abundant in Paxton (Table 2). Exceptions were the ectoparasitic copepod *T. gasterostei*, which only occurred in Priest, and glochidia.

Discussion

In order to implicate parasitism in the adaptive radiation of hosts it is necessary to show: 1) that parasitism varies between host populations and 2) that closely related host species exhibit consistent differences in parasitism across geographical locations. It is also necessary 3) that the
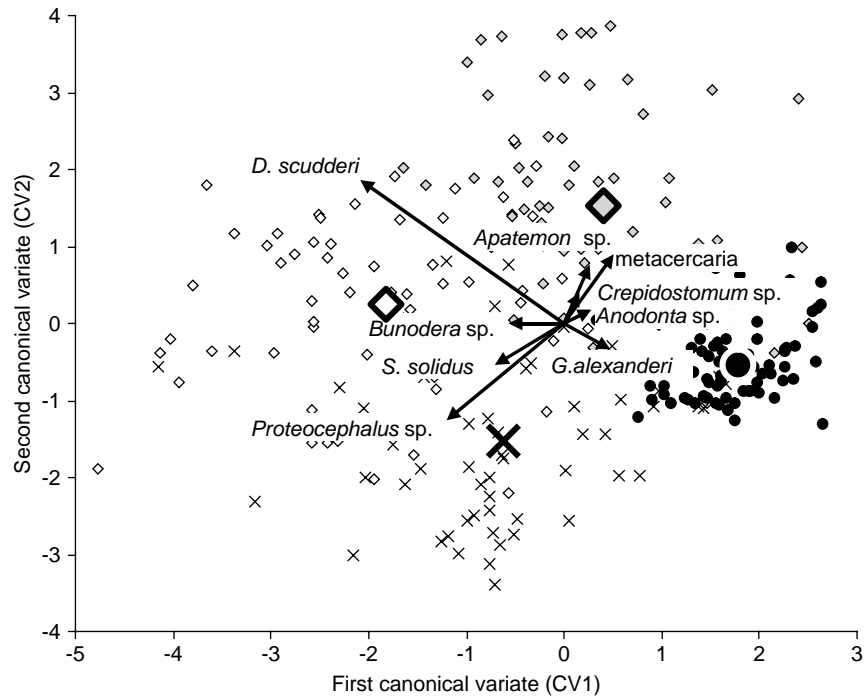
Figure 1. A biplot of the canonical variate (CV) scores for 255 individual fish from the four different populations, and vectors representing the loadings of individual parasite species on the first two CV axes. Mean CV scores of the four populations are also shown with enlarged symbols. Shaded diamonds are Paxton benthics, open diamonds are Paxton limnetics; filled circles are Priest benthics, crosses are Priest limnetics. Black arrows represent loading vectors. Loading vectors are only shown for those parasites with loadings greater than 0.1 on at least one of the axes. For clarity loading vectors in the figure have been multiplied by 2 in both directions. Note that the vector for *Crepidostomum* sp. is overlain by that for *Apatemon* sp.

parasites that differ between host populations cause selection on their hosts. These three criteria are first of all discussed in relation to the present results before highlighting specific mechanisms by which the differences in parasitism between host populations documented here could favour the evolution of reproductive isolation in these populations.

The analysis presented here suggests that parasites could contribute to the adaptive radiation of three-spined stickle-backs. It clearly demonstrates that there are differences in the prevalence and abundance of parasites between the stickleback species that are consistent across lakes. Hence the first two criteria (above) are satisfied in this system. Eight out of the twelve common parasites analysed in detail

Table 2. Differences in total parasite species richness and abundance, and in the abundance of individual parasite species, between stickleback species and lakes. "Difference" is the difference between mean abundances for the two stickleback species (limnetics–benthics) and lakes (Paxton–Priest). Results are for the best-fitting models derived from maximal models containing the main effects for species, lake, season and length only. The model for "Total number of metazoan species" used normal errors and an identity link function. For this model the value of F was used as the test statistic, with 247 denominator degrees of freedom (DF). For all other response variables, models used negative binomial errors and a logarithm link function. χ^2 was used as the test statistic. Species, lake and length are all associated with 1 DF. Season is associated with 4 DF. Probability values associated with the model terms are shown as: *** $=p < 0.001$, ** $=p < 0.01$, * $=p < 0.05$, - $=0.10 > p \geq 0.05$, -- $=p \geq 0.10$.

Response variable	Species			Lake			Season	Length
	Difference ($\pm$ SE)	F or χ^2	p	Difference ($\pm$ SE)	F or χ^2	p	p	p
Total number of metazoan species	0.9 ± 0.2	21.9	***	0.5 ± 0.2	6.7	*	***	***
Abundance of metazoans	32.8 ± 5.2	47.0	***	25.4 ± 4.4	36.7	***	***	***
Glochidia	-13.0 ± 2.2	79.8	***	-4.9 ± 1.8	8.5	**	***	*
Thersitina gasterostei	11.4 ± 2.4	34.6	***	-16.1 ± 2.1	441.2	***	*	***
Schistocephalus solidus	0.9 ± 0.1	125.9	***	0.4 ± 0.1	15.0	***	***	--
Proteocephalus sp.	1.9 ± 0.3	165.3	***	-0.3 ± 0.2	2.7	-	--	**
Gyrodactylus alexanderi	3.4 ± 2.3	2.2	--	0.4 ± 1.9	0.0	--	***	***
Crepidostomum sp.	-0.3 ± 0.2	1.4	--	1.3 ± 0.2	39.6	***	***	***
Bunodera sp.	1.3 ± 0.3	75.2	***	0.6 ± 0.2	11.0	**	***	--
Diplostomum scudderi	26.0 ± 4.9	66.8	***	34.4 ± 5.0	224.0	***	***	***
Ichthyocotylurus sp.	-0.03 ± 0.03	1.1	--	0.04 ± 0.02	6.8	**	--	*
other metacercaria	-1.2 ± 0.2	49.8	***	1.2 ± 0.2	52.8	***	--	--
Apatemon sp.	-0.1 ± 0.2	0.5	--	0.9 ± 0.2	32.0	***	***	*
Neoechinorhynchus rutili	0.1 ± 0.05	9.4	**	0.05 ± 0.05	0.9	--	**	--

differed significantly between benthics and limnetics over the two lakes. Limnetics generally had higher burdens. Several of the differences in parasites are associated with trophic specialisation of the hosts (e.g. *Bunodera* sp., *Proteocephalus* sp., *S. solidus*), a pattern that seems to be a common feature of closely related fish taxa in post-glacial lakes (Dorucu et al. 1995, Knudsen et al. 1997, 2003). *Bunodera* sp., *Proteocephalus* sp. and *S. solidus* use planktonic crustacea as intermediate hosts. The higher burdens of these parasites seen in limnetics are almost certainly at least partly the result of the pelagic, planktivorous niche of limnetics (Schluter 1993). Other differences in parasite abundances are associated with differences in habitat specialisation (glochidia, *Apatemon* sp.). Still others may be the result of differences in resistance or an interaction between resistance and exposure. Limnetics suffer higher burdens of at least one parasite, *D. scudderi*, to which they are probably less exposed. Sticklebacks are infected with *D. scudderi* after coming into contact with the free-swimming cercaria (larvae) that erupt from the intermediate host, a freshwater snail of the genus *Stagnicola*. The snails are found predominantly in the littoral zone that is occupied year round by the benthics, but only by breeding limnetics. Artificial infection experiments have shown that limnetics have lower resistance to infection by *D. scudderi* (MacColl et al. unpubl.). Limnetics are shorter-lived than benthics and trade-offs among life history traits may have favoured increased investment in reproduction during their single reproductive bout, at the expense of investment in immunity (Miller et al. 2007, MacColl 2009).

It is difficult to be certain that the differences in parasitism documented here result in divergent selection between host populations, although this seems likely. Differences in parasite burdens within and among local populations of sticklebacks (Kalbe et al. 2002) and other animals (Behnke et al. 2001) have previously been demonstrated. However the consequences of such differences for natural selection on the hosts have not been well explored. There exists a widespread theoretical and popular consensus that parasites play an important role in the evolution of their hosts (Anderson and May 1982, Summers et al. 2003, Thompson 2005) but it is sobering how little we actually know about the selective consequences of the kinds of differences in parasitism documented here, even for widely distributed and well known fish parasites like *Gyrodactylus*, *Schistocephalus* and *Diplostomum*. There are correspondingly few specific macroparasites for which the selective effect on wild hosts in any taxa has been unequivocally demonstrated (Hudson 1986, Gulland et al. 1993). It is clear that we need to know more about the impacts of macroparasites on host fitness and how virulence varies between parasite species and host populations.

Despite widespread recording of the parasite burdens of three-spined sticklebacks there has been little attempt to demonstrate that parasites can cause selection in this species (although see Wegner et al. 2003). *Schistocephalus solidus* is an exception, perhaps because it is such an impressive parasite. Individual worms can be almost the same weight as the host (unpubl.). Its impact on the survival and reproductive capabilities of its host are well documented (Arme and Owen 1967, Barber and Svensson 2003). For this reason, and because there is a qualitative difference in its occurrence in limnetics and benthics much of the discussion below of mechanisms by which parasites could contribute to divergent selection focusses on the role of *S. solidus*. However in all cases it is possible that other parasites could have similar effects.

Gyrodactylus is another parasite genus with well established effects on host fitness. *Gyrodactylus alexanderi* is known to reduce the survival of three-spined sticklebacks (Lester and Adams 1974) and thus it has the potential to be an agent of divergent selection in a similar way to *S. solidus*. For other species of parasites recorded here it is usually necessary to rely on what little is known about the effect of similar parasites on other host species. For example glochidia of some bivalve species are acknowledged as having a detrimental effect on their hosts (Karna and Millemann 1978), but the extent to which this is true of the glochidia of *Anodonta* is not known.

If all of the criteria discussed above are met, then there are several possible mechanisms by which adaptation to parasites could contribute to adaptive radiation of hosts. All require that the selection caused by parasites could contribute to the accumulation of host reproductive isolation. The most likely mechanisms include: 1) divergence in life history. If the greater parasite abundances of limnetics (especially *S. solidus*) causes significant adult mortality then this is likely to lead to selection for early reproduction (Agnew et al. 2000), a pressure that would be absent from benthics. Such differences in selection pressure could contribute to differences in size at maturity that are strongly associated with reproductive isolation in three-spined sticklebacks (Agnew et al. 2000, McKinnon et al. 2004). Differences in life history between benthics and limnetics are consistent with this possibility (MacColl 2009), although these could also arise from differences in predation pressure (Reznick et al. 1990, Vamosi and Schluter 2002).

2) Divergence in sexually selected characters. Sexually selected characters may evolve in response to variation in parasitism (Hamilton and Zuk 1982). Greater investment in signalling by males is expected where parasitism is more severe. Red carotenoid colouration is an important secondary sexual trait in sticklebacks which has been implicated in reproductive isolation (Boughman 2001). Male limnetics in Paxton and Priest exhibit much greater investment in carotenoid based nuptial colouration than benthics (Albert et al. 2007). This carotenoid colouration is associated with resistance to *S. solidus* in other stickleback populations (Barber et al. 2001). These facts suggest a role for parasites in the evolution of mate choice and reproductive isolation in sticklebacks. However the extent of carotenoid colouration is also associated with variation in the light regime in which the different stickleback populations breed (Boughman 2001). It may also be a simple result of dietary differences.

3) Breakdown of resistance in hybrids. Reduced fitness of hybrids is an important source of reproductive isolation in the species pairs (Gow et al. 2007). Hybrids between closely related taxa commonly show increased susceptibility to parasites (Moulia 1999). Artificial infection with *D. scudderi* of limnetics, benthics and F2 hybrids from Paxton lake show that limnetics and F2s have very similar resistance and that this is significantly less than that of benthics

(MacColl et al. unpubl.). This could result in reinforcement of mating preferences (Blair 1955).

4) Interactions between parasitism and competition. The currently favoured model for the origin of the stickleback species pairs in British Columbia is that they are the result of two separate waves of colonisation of the lakes (McPhail 1993, Taylor and McPhail 2000). It is thought that the first wave occupied the more profitable benthic niche and that the second wave was excluded from it by competition and became limnetic. Each species does have a competitive advantage in its own habitat (Schluter 1995), but this does not mean that competition was dominant in driving the patterns that are seen today. Selection may have favoured those individuals in the original wave (which became benthics) that occupied the niche that had the fewest parasites. Competition or apparent competition (Holt 1977) could then have driven the second wave (limnetics) into a niche that experienced more parasite exposure, with subsequent consequences for evolution such as those described above.

The results of this study show that, as well as the differences between the species, there were also important differences in parasite faunas between the lakes. These differences were mainly quantitative rather than qualitative, with most parasites occurring in both lakes. Total parasite abundance, and the abundance of many individual species of parasites were higher in Paxton than in Priest, giving rise to a general impression of "worminess" in Paxton fish. There are two obvious possible explanations for this: 1) differences in ecology that favour transmission in Paxton. For example Paxton has more extensive areas of littoral habitat which may equate to a higher density of intermediate and final hosts, such as snails and ducks. 2) Differences in population size. Paxton is a smaller lake, and mark-recapture indicates that stickleback populations are correspondingly smaller (D. Schluter pers. comm.). Genetic drift could have resulted in less variability for host resistance in Paxton and consequently higher burdens.

In conclusion, this study suggests that parasites have the potential to cause divergent selection even between sympatric, closely related species. Differences in parasite occurrence could therefore be a primary ecological cause of divergence between populations or parasites could be a secondary ecological contributor to divergence begun by other ecological mechanisms such as resource competition. These possibilities have been surprisingly little explored but the results presented here suggest that they warrant further study.

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