Helminth species richness in wild wood mice, *Apodemus sylvaticus*, is enhanced by the presence of the intestinal nematode *Heligmosomoides polygyrus*

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SUMMARY

We analysed 3 independently collected datasets of fully censused helminth burdens in wood mice, *Apodemus sylvaticus*, testing the *a priori* hypothesis of Behnke *et al.* (2005) that the presence of the intestinal nematode *Heligmosomoides polygyrus* predisposes wood mice to carrying other species of helminths. In Portugal, mice carrying *H. polygyrus* showed a higher prevalence of other helminths but the magnitude of the effect was seasonal. In Egham, mice with *H. polygyrus* showed a higher prevalence of other helminth species, not confounded by other factors. In Malham Tarn, mice carrying *H. polygyrus* were more likely to be infected with other species, but only among older mice. Allowing for other factors, heavy residual *H. polygyrus* infections carried more species of other helminths in both the Portugal and Egham data; species richness in Malham was too low to conduct a similar analysis, but as *H. polygyrus* worm burdens increased, so the prevalence of other helminths also increased. Our results support those of Behnke *et al.* (2005), providing firm evidence that at the level of species richness a highly predictable element of co-infections in wood mice has now been defined: infection with *H. polygyrus* has detectable consequences for the susceptibility of wood mice to other intestinal helminth species.

Key words: *Apodemus sylvaticus*, associations of helminths, co-occurrence of helminths, *Heligmosomoides polygyrus*, helminths, helminth species richness, interactions between helminths.

INTRODUCTION

Interactions between different species of animals, or functional guilds of animals with shared or very similar niches, are the cornerstone of community ecology (Pedersen and Fenton, 2006; Suttle *et al.* 2007; Graham, 2008), and indeed it has been argued that such interactions define communities, contrasting them with assemblages of animals where the presence or absence of species is considered to be a purely random event (Janovy, 2002).

Most species of free-living hosts are subject to infection by a range of parasitic organisms and in nature, it is rare to encounter completely uninfected hosts. Indeed most wild animals, and also humans, particularly those living in impoverished communities in developing regions of the world (Keusch and Migasena, 1982), harbour several species of parasites concurrently, 'polyparasitism' in the medical literature (Buck *et al.* 1978 *a, b*). Whether multiple species infections actually constitute communities of helminths within hosts, so called infracommunities, has been a subject of debate for several decades. In some hosts, notably birds that harbour species-rich helminth communities, the evidence is indisputable, but in others such as fish and wild rodents characterized by relatively depauperate helminth communities, evidence in support of a role for interactions in structuring communities has been elusive (reviewed Behnke *et al.* 2001). This contrasts markedly with laboratory studies where interactions between helminths have been clearly demonstrated and mostly assigned to interactions mediated through the immune response of the host (Behnke *et al.* 2001; Behnke, 2008). The search for evidence of interactions among wild-caught hosts, has been hampered

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by a number of difficulties; for example, the statistical analyses required to dissect out the evidence for interactions is complex (Haukisalmi and Henttonen, 1998; Howard *et al.* 2001; Bottomley *et al.* 2005), and there is a lack of support for any specific interaction between particular parasites being a consistent enough feature wherever both species of parasites co-occur with the host. In contrast, a good example of a positive relationship that has been recorded many times in the medical literature, is that between the human intestinal nematodes *Trichuris trichiura* and *Ascaris lumbricoides* which not only co-occur but also show a positive quantitative relationship wherever the two species are endemic in human communities (Holland *et al.* 1989; Booth and Bundy, 1992; Howard *et al.* 2002; Tchuem Tchuenté *et al.* 2003; Behnke, 2008).

In our earlier paper (Behnke *et al.* 2005) we demonstrated 2 marked interactions among helminths of wild wood mice in the UK in 3 ecologically contrasting habitats; the positive relationship (evident as both co-occurrence and as a positive quantitative relationship) between *Heligmosomoides polygyrus* and the tapeworm *Catenotaenia pusilla*; and the positive relationship between *H. polygyrus* and species richness of other helminths. Both relationships are consistent with the idea that the long-lived *H. polygyrus* (Gregory *et al.* 1990), like its close relative *H. bakeri* (which, being a parasite of house mice, *Mus musculus*, has been explored extensively in laboratory work; Behnke, 1987; Behnke *et al.* 1992; Monroy and Enriquez, 1992), is aided in its survival strategy in wood mice by suppressing its host's capacity to mount protective immune responses in the intestine, and consequently provides an opportunity for other species to benefit from the altered host environment and to establish in and survive for longer than they otherwise might (reviewed by Behnke *et al.* 2001).

Whilst evidence of interactions between helminths in wild systems is not rare in the parasitological/ecological literature (Kisielewska, 1970; Montgomery and Montgomery, 1990; Haukisalmi and Henttonen, 1993), to date no relationship has stood the test of re-examination and been substantiated in other ecosystems where both the same host and parasite exist. In this paper, building on our initial report that the presence of *H. polygyrus* increases the likelihood that wood mice will harbour other species of helminths, we explore 2 entirely new datasets together with an extended dataset already partially analysed, with the specific aim of testing the robustness of our earlier findings. The first concerns helminths in wood mice from Portugal collected over a single calendar year, but from 6 ecologically contrasting habitats across all 4 seasons; the other 2 are from uniform habitats collected at the same time of the year (late summer) for 9 (Egham) and 16 (Malham) years. The extrinsic factors (e.g. year, season, site) known to influence helminth burdens differed between these 3 datasets, as

did helminth species richness. Hence, if the relationship between *H. polygyrus* and the species richness of other helminth species can be demonstrated in these 3 independently collected datasets, then for the first time we will have demonstrated a robust element of the infracommunity structure of the helminths of *A. sylvaticus* in Europe.

MATERIALS AND METHODS

Datasets

The first dataset is derived from wood mice trapped in a coastal sand dune region (Dunas de Mira) in Portugal and localities in close proximity. In this 'Portuguese' dataset, wood mice were caught in 6 ecologically distinct sites represented by sand dunes, dune meadows, coastal pinewoods, pinewoods burned in 1993, lake margins and agricultural fields. The mice analysed were caught between autumn 2004 and the summer of 2005, structured into 4 seasons corresponding to autumn, winter, spring and summer in the region. For full details of the ecology of each of the sites and the methods used for trapping and necropsy of animals, see Eira *et al.* (2006), where the methods used in the field and in the laboratory are comprehensively detailed. The range of helminth species identified in these mice and the number of factors recorded, together with the levels of each factor, are summarized in Table 1.

The second dataset is derived from wood mice trapped in the grounds of the Royal Holloway, University of London, at Egham in Surrey. This 'Egham' dataset is based on mice trapped only during late summer, so it is not affected by seasonal variation. We (Behnke *et al.* 1999) analysed the epidemiology of helminths in mice caught in the period 1994 to 1997 and data from these 4 years were also utilized in our earlier analysis of interactions between helminths (Behnke *et al.* 2005), although that paper was largely based on another study originally conducted by Abu-Madi *et al.* (1998, 2000) and the data from Egham played only a minor role. The current analysis is based on the addition of a further 5 years of data to the Egham dataset, now spanning the period from 1994 to 2002. The range of helminth species identified in these mice and the number of factors recorded, together with the levels of each factor, are summarized in Table 1.

The third dataset concerns wood mice trapped in the woodlands north of Malham Tarn in Yorkshire, UK. This 'Malham' dataset is based also on mice trapped only during late summer, but across a 16-year period from 1993 to 2008. The range of helminth species identified in these mice and the number of factors recorded, together with the levels of each factor, are summarized in Table 1. Details of the methods used to catch, necropsy mice and allocate them into age classes can be found in Rogan *et al.* (2007).

Table 1. *Prevalence of helminths from the Portuguese, Egham and Malham sites*

	Portugal*			Egham†			Malham§		
	Preva-lence (%)	Confidence limit		Preva-lence (%)	Confidence limit		Preva-lence (%)	Confidence limit	
Species		Lower	Upper		Lower	Upper		Lower	Upper
Nematodes									
Heligmosomoides polygyrus	54·9	48·3	61·2	77·7	73·5	81·4	73·0	68·5	77·4
Syphacia stroma	30·7	25·1	36·9	50·6	46·0	55·3	6·1	3·8	9·2
Syphacia frederici	24·4	19·2	30·4	0	—		0	—	
Rictularia proni	7·0	4·3	11·1	0	—		0	—	
Trichuris muris	0·2	0	2·6	14·2	11·1	17·8	0·3	0	2·1
Gongylonema neoplasticum	1·1	0	4	0	—		0	—	
Aspiculuris tetraptera	0·2	0	2·6	1·7	0·7	3·5	0	—	
Angiostrongylus dujardini	28·2	22·6	34·5	0	—		0	—	
Aoncotheca murissylvatici	0	—		6·0	3·9	8·8	6·4	4·1	9·6
Pelodera strongyloides	0	—		21·5	17·8	25·6	—	—	
Adult cestodes									
Rodentolepis straminea	1·8	0·6	4·7	0	—		0	—	
Skrajbinotaenia lobata	3·2	1·2	6·5	0	—		0	—	
Microsomacanthus crenata	0	—		28·3	24·2	32·8	0	—	
Larval cestodes									
Cladotaenia globifera	0·2	0	2·6	0	—		1·0	0	3·1
Taenia parva	14·9	10·8	20·1	0	—		0	—	
Taenia taeniaformis	0	—		4·3	2·6	6·7	0·7	0	2·4
Trematoda									
Brachylaemus recurvum	0	—		1·7	0·7	3·5	1·4	0·3	3·5
Corrigia vitta	0	—		19·3	15·8	23·4	0	—	
Plagiorchis muris	0	—		0	—		17·6	13·8	22·0
Factors measured	No. of levels			No. of levels			No. of levels		
Extrinsic									
Site	6			1			1		
Year	1			9			16		
Season	4			1			1		
Intrinsic									
Age	3			3			3		
Sex	2			2			2		
Hosts									
Males	235			153			179		
Females	208			80			117		
Combined	443			233			296		

* Eira *et al.* (2006).
† For the first 4 years (1994–1997) see Behnke *et al.* (1999). Data from 1998 to 2002 are in preparation for submission.
§ The full analysis of this dataset has not been published.

Hypotheses

In contrast to our earlier paper where we looked for evidence of interactions between species employing a range of different analytical procedures, in this paper we set out specifically to test the *a priori* hypothesis that mice infected with *H. polygyrus* would be predisposed to infection with other helminths, and should therefore show a higher prevalence and a higher species richness of other helminths. We used 2 response variables. Firstly, each animal was recorded as either infected or not infected with any of the other species of helminths (i.e. excluding *H. polygyrus*). This binary factor is referred to hereafter as 'presence/absence of other helminth species'. Secondly

we recorded the number of other helminth species in each animal; this factor is referred to as 'species richness of other helminths'.

Statistical analysis

Binary prevalence data were analysed by maximum likelihood techniques based on log-linear analysis of contingency tables, using the software package SPSS (Version 12.0.1.). Full factorial models incorporated the intrinsic factors age (3 age classes, all data-sets) and sex (2 levels, males and females), and the extrinsic factors of site (6 levels, Portuguese dataset), year of survey (9 levels [1994 to 2002] in the Egham

data-set or 16 [1993 to 2008] in the Malham dataset) and season (4 levels, corresponding to 4 seasons in the Portuguese data-set). Infection with *H. polygyrus* was considered as a binary factor (present/absent), as was also the presence/absence of other helminth species. One of the species of helminths recorded in Portugal, *Angiostrongylus dujardini* does not inhabit the intestine or peritoneal cavity of the host, and in a final phase of this analysis we also examined the effect of the presence/absence of *H. polygyrus* on other helminth species but this time excluding *A. dujardini*. At Egham *Pelodera strongyloides* was recorded, another species that does not inhabit the intestine, and the same approach was used here.

In each case, beginning with the most complex model, involving all possible main effects and interactions, elements that did not contribute significantly to explaining variation in the data were eliminated in a stepwise fashion, beginning with the highest-level interaction. A minimum-sufficient model was then obtained, for which the likelihood ratio (L.R.) of χ^2 was not significant, indicating that the model was sufficient in explaining the data. The importance of each term in the final model was assessed by the probability that its exclusion would alter the model significantly: these values are given in the text, but only for those terms that included both the presence/absence of *H. polygyrus* and the presence/absence of other helminth species. The number of other terms is also stated, but for brevity we do not give the details of each (these can be obtained from J.M.B. by request). Prevalence data (percentage of animals infected with various combinations) are shown with 95% confidence limits (CL$_{95}$), calculated with bespoke software following Rohlf and Sokal (1995).

Analysis of quantitative data was conducted in 2 phases. Firstly we fitted generalized linear models (GLM) with negative binomial errors, normal errors on Box-Cox transformed data, Poisson errors or quasibinomial errors, as relevant, using R version 2.2.1 (R Core Development Team). Models were fitted using the appropriate GLM routine in the MASS library of R (Venables and Ripley, 1997). We first fitted full factorial models and if these converged, we employed the STEP procedure in R to simplify the models. We then tested the significance of the highest-order interaction by comparing models with or without that interaction. Next, the significance of each 3-way interaction was tested by comparing models with and without that interaction, but incorporating all the other 3-way interactions, 2-way interactions and main effects. This was repeated for 2-way interactions and then main effects. Minimum-sufficient models were then fitted (all significant interactions and main effects + any non-significant main effects that featured in interactions) and the process was repeated to obtain values for changes in deviance, test statistics and probabilities. The residuals of these models were saved.

Secondly, following Lotz and Font (1994) and Behnke *et al.* (2005), we confined further analysis to animals that harboured *H. polygyrus* and at least 1 worm of another species. This approach avoids false conclusions generated by excessive zero values in correlational tests. We assessed the correlation (Pearson's coefficient of correlation) between the residuals of the minimum-sufficient GLM of *H. polygyrus* and the residuals of GLM of species richness of other helminths. For these we used one-tailed tests, since we were testing *a priori* hypotheses with clearly predicted positive relationships. In the case of the final analysis of the Malham Tarn dataset, we confined the analysis to mice infected with *H. polygyrus* and calculated the prevalence of other species, for reasons given in the Results section.

RESULTS

Prevalence of species

The prevalence of species of helminths in all 3 studies is summarized in Table 1. Analysis of the factors influencing the prevalence and abundance of helminths in the Portuguese dataset has been published (Eira *et al.* 2006). Behnke *et al.* (1999) provided the analysis of prevalence and abundance of helminths in the period 1994–1997 from Egham, but the analysis of the full dataset to 2002 is still in preparation. Only the data on *Plagiorchis muris* in the period 1993–2005 have been published for the Malham dataset (Rogan *et al.* 2007).

The Portuguese dataset was the most species-rich with 12 species of helminths recovered, 5 of which showed prevalence exceeding 10%. The prevalence of *H. polygyrus* was 54·9%, and the second most common species was *Syphacia stroma*. One of the listed species, *A. dujardini* was not associated with the intestine, but rather with the blood vessels connecting the lungs with the heart.

The dataset from Egham was based on fewer animals, and 10 species of helminths were identified. *H. polygyrus* was again the most common showing a higher prevalence than in the Portuguese dataset (77·7%). *S. stroma* was again the second most-common species. Six species showed prevalence exceeding 10%. One species, *P. strongyloides*, was not associated with the intestine, but rather with the skin, nasal passages and the eye sockets (Hominick and Aston, 1981).

Wood mice from Malham were infected with 8 species of helminths and, as elsewhere, *H. polygyrus* was the most common (73·0%). Here, *S. stroma* was rarer than in the first 2 datasets (6·1%) and instead the second most-common species encountered was the digenean *P. muris* (17·6%). *H. polygyrus* and *P. muris* were the only 2 species to occur in more than 10% of the animals. *P. strongyloides* was present in the population, but infections with this species were

only quantified in some years, and therefore the species was excluded from the analysis.

Only 1 species of nematode was common (*H. polygyrus*) but 3 others (*S. stroma*, *Trichuris muris* and *Aspiculuris tetraptera*), were present in all 3 studies. The last of these was recorded in 1 mouse from Malham but the identity was not confirmed and it was removed from the analysis. *S. stroma* was common in the Portuguese and Egham datasets but far less so in the Malham wood mice. *T. muris* was more common in the Egham dataset than in the Portuguese or Malham datasets. None of the adult cestodes overlapped among these studies, but larval *T. taeniaeformis* was encountered in both Egham and Malham datasets.

Multiple species infections

The species density distributions are illustrated in Fig. 1 (Janovy *et al.* 1995). In the first 2 datasets (Portuguese and Egham) the maximum number of helminth species harboured was 6, whereas at Malham no mice harboured more than 3 species concurrently. In the Portuguese dataset (Fig. 1A), 31·4% (25·5–37·8) were infected with just a single species of helminth, but 50·8% (44·3–57·3) harboured more than a single species. At Malham (Fig. 1C) most wood mice harboured just 1 species, (53·4%, 48·1–58·6) and the percentage harbouring more than 1 species concurrently was correspondingly lower (25·3%, 21·0–30·2). In the Egham dataset (Fig. 1B) 17·2% (13·8–21·1) of mice were infected with 1 species and 72·5% (68·2–76·5) harboured more than just 1 species.

Effect of Heligmosomoides polygyrus *on prevalence of other helminths*

Portuguese sites. In this dataset 52·5% (46·0–60·0) of mice without *H. polygyrus* were infected with other helminths, but the prevalence was almost 8% higher among mice with *H. polygyrus* (60·5%, 55·7–65·0). There was no significant difference between the sexes in this respect (otherwise sex would have been retained as a factor in the minimum sufficient model; see below) and not surprisingly the difference was evident in summary data in both males (57·0% of mice without *H. polygyrus* versus 67·4% of mice with *H. polygyrus*) and in females (48·0% versus 51·9%, respectively), with the discrepancy greater, but not significantly so among male mice (10·4% *vs* 3·9%).

Log-linear analysis revealed just 1 relevant significant interaction term (season∗presence/absence of *H. polygyrus*∗presence/absence of other helminth species: L.R. $\chi^2_3 = 9.43$, $P = 0.024$) in the minimum-sufficient model (goodness of fit of model L.R. $\chi^2_{114} = 111.55$, $P = 0.55$; note that this was a model in which
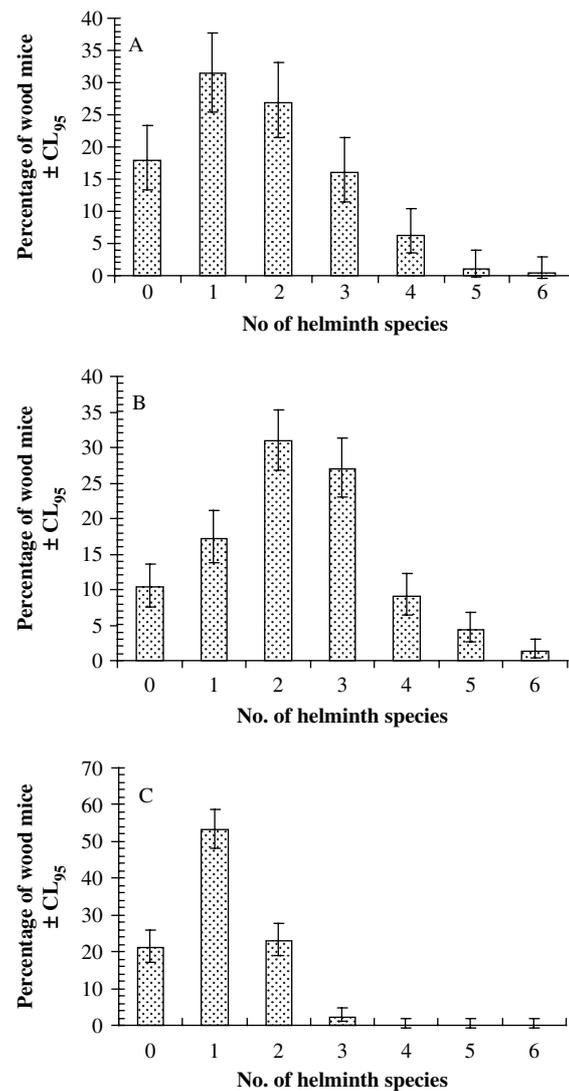

Fig. 1. Species density distributions for the Portuguese (A), Egham (B) and Malham (C) datasets.

age was not fitted). This interaction is illustrated in Fig. 2A, where it can be seen that in each of the 4 seasons, mice carrying *H. polygyrus* also showed a higher prevalence of other species of helminths.

Exclusion of *A. dujardini* (because it is not an intestinal species) from the category 'other helminth species' yielded a more complex model with an interaction between site, season and presence/absence of *H. polygyrus* (L.R. $\chi^2_{15} = 30.26$, $P = 0.011$), but it was not possible to probe this further because with 48 possible combinations (season = 4, site = 6, presence/absence of *H. polygyrus* = 2, therefore $4 \times 6 \times 2 = 48$) some cells had no data and others were based on very small sample sizes. However, examining the relationship with just season (excluding site) gave much the same outcome as earlier, with mice infected with *H. polygyrus* generally showing higher prevalence of other helminth species (excluding *A. dujardini*) in all months except the winter (prevalence of other helminths was 69·2% *vs* 56·3% in spring, 57·1% *vs* 42·2% in the summer, 67·5% *vs* 59·0% in the autumn for
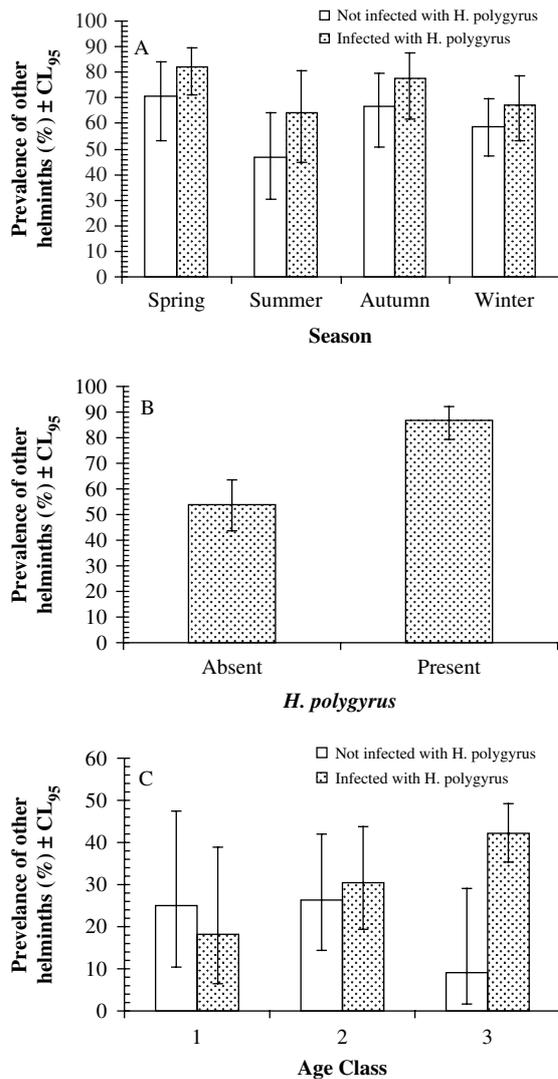
Fig. 2. Prevalence of other species of helminths in wood mice with or without *Heligmosomoides polygyrus*. (A) Interaction with season in the Portuguese dataset. The sample sizes were 48, 45, 39 and 68 for mice without *H. polygyrus*, and 78, 28, 40 and 97 for those with *H. polygyrus* in spring, summer, autumn and winter, respectively. (B) Unaffected by other factors in wood mice at Egham. The sample sizes were 52 for mice without *H. polygyrus* and 181 for those with *H. polygyrus*. (C) Interaction with age in the Malham dataset. The sample sizes were 20, 38 and 22 for mice without *H. polygyrus*, and 22, 92 and 102 for those with *H. polygyrus* in age classes 1, 2 and 3 respectively. For statistical analysis see text.

mice with and without *H. polygyrus* respectively: in the winter, prevalence was almost identical at 51·5% in mice with *H. polygyrus* compared with 52·9% in those without *H. polygyrus*).

Egham site. The Egham dataset showed a larger divergence in prevalence with other helminth species between wood mice infected (86·7%, 79·3–92·1) and not infected (53·9%, 43·7–63·5) with *H. polygyrus* (Fig. 2B). Analysis by log-linear procedures

generated a minimum-sufficient model with 4 terms (L.R.$\chi^2_{125} = 82\cdot36$, $P = 0\cdot99$), one of which was presence/absence of *H. polygyrus* * presence/absence of other helminth species: this was highly significant and not confounded by other factors (L.R.$\chi^2_1 = 23\cdot7$, $P < 0\cdot0001$).

The difference in prevalence was also clearly apparent in both sexes. In male wood mice not infected with *H. polygyrus* the prevalence of other helminth species was 55·3% (39·4–69·9) compared to 88·7% (83·1–92·7) among those with *H. polygyrus*. In females the values were very similar (50·0% [23·8–76·2] versus 83·3% [73·5–90·2]).

Here again, 1 species was not intestinal. *P. strongyloides* has a phoretic relationship with mice, exploiting them only for temporary refuge when environmental conditions are detrimental to survival (Hominick and Aston, 1981). It occurs in the lachrymal ducts, eye sockets and skin during its residence in mice, before emerging and completing its life cycle externally when the mice have died. Therefore, we repeated the analysis excluding *P. strongyloides* from the category 'other helminth species'. The outcome was much the same as earlier. The most-parsimonious model (L.R. $\chi^2_{151} = 125\cdot8$, $P = 0\cdot933$), included 4 terms, of which 1 was presence/absence of *H. polygyrus* * presence/absence other helminth species (excluding *P. strongyloides*), not confounded by other factors, and an essential component of the final model (L.R.$\chi^2_1 = 22\cdot95$, $P < 0\cdot0001$).

Malham Tarn site. As in the other datasets, prevalence of other helminths was higher among mice carrying *H. polygyrus* (34·7%, 30·49–39·16) compared with those without *H. polygyrus* (21·3%, 12·62–33·35), and this difference was evident in both males (34·5% [26·95–42·75] versus 24·3% [12·83–39·76]) and females (35·1% [24·58–47·05] versus 18·6% [8·38–35·09]).

Log-linear analysis generated a minimum-sufficient model (L.R. $\chi^2_{350} = 269\cdot2$, $P = 1\cdot0$) with just 2 terms 1 of which was relevant (presence/absence of *H. polygyrus* * presence/absence of other helminths * age class; L.R.$\chi^2_2 = 7\cdot0$, $P = 0\cdot029$), indicating that the prevalence of other helminth species depended on both the prevalence of *H. polygyrus* and host age (Fig. 2C). Although there was no difference in prevalence of other helminths among wood mice in age classes 1 and 2, among the oldest mice (age class 3) prevalence of other helminths was more than 4-fold higher in mice with, compared to those without, *H. polygyrus*.

Effect of Heligmosomoides polygyrus *on species richness of other helminths*

Abundance of infections and species richness. The mean abundance of each of the species recovered from each study site is summarized in Table 2. This

Table 2. *Abundance of helminths from the Portuguese, Egham and Malham sites*

(The table shows the pooled abundance across all subsets of data.)

Species	Portugal		Egham		Malham	
	Mean±	S.E.M.	Mean±	S.E.M.	Mean±	S.E.M.
Nematodes						
Heligmosomoides polygyrus	15·7	2·40	11·4	1·13	14·4	1·63
Syphacia stroma	16·7	2·65	23·6	5·53	0·8	0·39
Syphacia frederici	6·7	1·40	0	—	0	—
Rictularia proni	0·18	0·038	0	—	0	—
Trichuris muris	0·002	0·002	0·55	0·126	0·05	0·054
Gongylonema neoplasticum	0·02	0·013	0	—	0	—
Aspiculuris tetraptera	0·05	0·05	0·011	0·011	0	—
Angiostrongylus dujardini	1·1	0·18	0	—	0	—
Aoncotheca murissylvatici	0	—	0·23	0·095	0·4	0·19
Pelodera spp.	0	—	1·7	0·65	0	—
Adult cestodes						
Rodentolepis straminea	0·32	0·270	0	—	0	—
Skrajbinotaenia lobata	0·08	0·029	0	—	0	—
Microsomacanthus crenata	0	—	3·7	0·92	0	—
Larval cestodes						
Cladotaenia globifera	0·002	0·002	0	—	0·01	0·006
Taenia parva	0·22	0·032	0	—	0	—
Taenia taeniaeformis	0	—	0·24	0·146	0·007	0·005
Trematodes						
Corrigia vitta	0	—	1·2	0·24	0	—
Brachylaemus recurvum	0	—	0·047	0·035	0·13	0·12
Plagiorchis muris	0	—	0	—	2·0	0·50

shows quite clearly the remarkably similar abundance of *H. polygyrus* in these 3 study sites, despite their geographical separation and climatic differences. It also shows the degree to which this species dominated the infracommunities of the wood mice. Only *S. stroma* showed comparable abundances in 2 of the study sites, and the other species were far less abundant.

Mean species richness was highest at Egham ($2·25 \pm 0·087$), lower in Portugal ($1·67 \pm 0·058$) and lowest at Malham ($1·06 \pm 0·043$). When *H. polygyrus* was excluded these values became $1·48 \pm 0·074$, $1·12 \pm 0·050$ and $0·33 \pm 0·030$, respectively. Clearly at Malham very few animals were infected with helminths other than *H. polygyrus* (31·1%, 26·34–36·20), compared with 67·5% (61·11–73·35) in the Portuguese dataset and 79·4% (75·35–82·91) in Egham.

Portuguese sites. The species richness of other helminths was analysed by a GLM model with Poisson errors, initially fitting all 4 factors (site, season, host age and sex) in a full factorial model. However, the data were best accounted for by a minimum-sufficient model that comprised only the main effects of season ($\chi^2_3 = 10·3$, $P = 0·02$), site ($\chi^2_5 = 27·1$, $P < 0·00001$) and host age ($\chi^2_2 = 56·1$, $P < 0·00001$). The precise effect of each of these factors on species richness is described fully by Eira *et al.* (2006). The residuals from this analysis were saved.

Attempts to fit a model with negative binomial errors for the abundance of *H. polygyrus* failed to converge, so the data were Box-Cox transformed (Box-Cox lambda $= -1·018$, $Cl_{95} = -1·011$ and $-1·027$; Likelihood $= -754·635$) and analysed by a model with normal errors. The minimum-sufficient model was season ($F_{3,431} = 15·9$, $P < 0·00001$) + site ($F_{5,431} = 28·9$, $P < 0·00001$) + sex (P = NS) + age ($F_{2,431} = 15·7$, $P < 0·00001$) + season * site ($F_{13,429} = 4·47$, $P < 0·00001$) + age * sex ($F_{2,418} = 3·59$, $P = 0·028$). The residuals from this model were saved.

Finally the relationship between the *H. polygyrus* residuals and the species richness residuals was examined by correlation using data from mice with at least 1 individual of *H. polygyrus* and 1 worm of another species. These were significantly correlated ($r_p = 0·161$, one-tailed test, $n = 178$, $P = 0·016$), and are illustrated as raw values in Fig. 3A and arranged in convenient range classes in Fig. 4A.

Egham site. The species richness of other helminths was analysed by a GLM model with Poisson errors, initially fitting all 3 factors (year, host age and sex). Only age played a significant role in explaining the species richness of other helminth species ($\chi^2_2 = 28·4$, $P = 6·9 \times 10^{-7}$) with the effect of year just outside significance ($\chi^2_8 = 14·5$, $P = 0·069$): neither host sex nor any interactions were significant predictors. Accordingly, to err on the side of caution, we saved the residuals of the Poisson model with just host age and
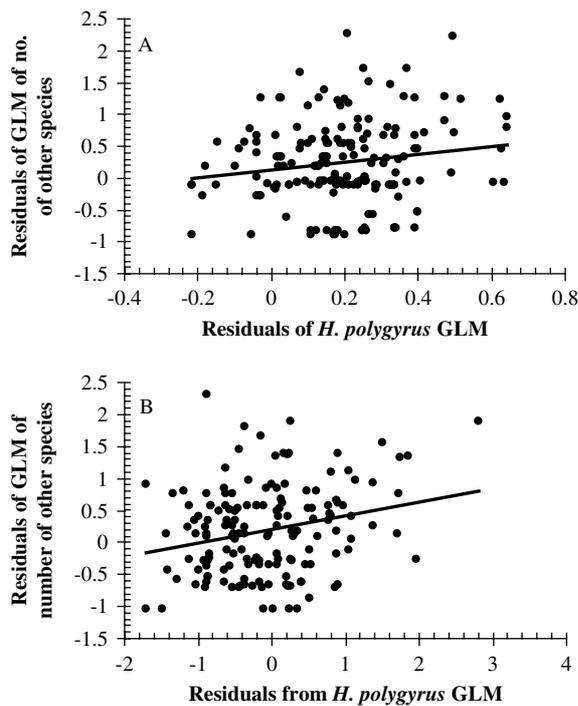
Fig. 3. Correlation between the intensity of
Heligmosomoides polygyrus infection and number of
other species of helminths harboured by wood mice.
(A) The relationship in the Portuguese dataset between
the residuals of a minimum-sufficient GLM of the
species richness of other helminths (Poisson errors, with
season, site and age as explanatory factors) and the
residuals of a minimum-sufficient GLM of the
abundance of *H. polygyrus* (Box-Cox transformed data
and model with normal errors, and season, site, sex, age,
season ∗ site and age ∗ sex as explanatory factors and
interactions). (B) The relationship at Egham between the
residuals of a minimum-sufficient GLM of the species
richness of other helminths (Poisson errors with year and
host age as explanatory factors) and the residuals of a
minimum-sufficient GLM of the abundance of
H. polygyrus (model with negative binomial errors and
year and host age as explanatory factors). Both analyses
were confined to mice which harboured at least 1
H. polygyrus and 1 worm of another species and the full
statistical analysis is given in the text. Lines of best fit are
provided to guide the eye, and these were fitted by a least
squares routine in Microsoft Excel.

year as the explanatory factors, and preferentially
conducted further analyses using this model.

Analysis of the abundance of *H. polygyrus* was
carried out by GLM with negative binomial errors.
The minimum-sufficient model was year ($LR\chi^2_8 =
26\cdot4$, $P = 0\cdot00088$) plus age ($LR\chi^2_2 = 39\cdot1$, $P = 3\cdot3 \times
10^{-9}$). The main effect of host sex and all possible
interactions were not significant. The residuals of
this model were saved.

The species-richness residuals and the *H. poly-
gyrus* abundance residuals were then correlated using
mice with at least 1 individual of *H. polygyrus* and
1 worm of another species. They were significantly

correlated ($r_p = 0\cdot24$, $n = 157$, $P = 0\cdot0015$ one-tailed
test) and are illustrated as raw values in Fig. 3B and
arranged in convenient range classes in Fig. 4B.

Additionally we also examined the relationship
between *H. polygyrus* abundance residuals and the
species-richness residuals of a model with just age as
the explanatory factor. This gave a lower value for
r_p but still significant ($r_p = 0\cdot191$, one-tailed test, $n =
157$, $P = 0\cdot008$).

Malham Tarn site. The Malham dataset could not
be examined in exactly the same manner because
species richness was low, and as explained earlier
only $31\cdot1\%$ of mice harboured helminths other than
H. polygyrus. Moreover, the maximum number of
other helminth species was 2, and only 7 mice fell into
this category. Therefore, we fitted a full factorial
GLM model with negative binomial errors to the
abundance of *H. polygyrus*. This model could not
be simplified, comprising year ($LR\chi^2_{15} = 27\cdot3$, $P =
0\cdot026$) + age ($LR\chi^2_2 = 35\cdot2$, $P = 2\cdot299 \times 10^{-8}$) + year ∗
sex ($LR\chi^2_{15} = 27\cdot8$, $P = 0\cdot023$) + year ∗ age ($LR\chi^2_{29} =
62\cdot1$, $P = 0\cdot0003$) + sex ∗ age ($LR\chi^2_2 = 7\cdot2$, $P =
0\cdot028$) + sex ∗ age ∗ year ($LR\chi^2_{17} = 28\cdot6$, $P = 0\cdot038$).
The main effect of sex was not significant but this was
retained because sex featured in significant interac-
tions. The residuals from this model were saved.

We then sorted the residuals of the *H. polygyrus*
GLM into 3 convenient abundance classes, and for
animals that were infected with *H. polygyrus* we
calculated the prevalence of other helminth species in
each of the 3 classes (Fig. 4C). These indicate that
after controlling for age, sex and year and their in-
teractions, the prevalence of other species increased
as *H. polygyrus* abundance increased. In order to test
the significance of this relationship, we fitted the fixed
factors year, sex and age, and the residuals of *H. poly-
gyrus* as a covariate in a GLM with quasibinomial
errors, with the dependent variable being the binary
presence/absence of other helminths species: the data
were confined to mice infected with *H. polygyrus*.
Full factorial models did not converge but fitting just
the main effects simplified to a model with just age
and *H. polygyrus* residuals (age, $\chi^2_2 = 8\cdot1$, $P = 0\cdot018$;
H. polygyrus residuals, $\chi^2_1 = 9\cdot8$, $P = 0\cdot002$).

In Fig. 4D we have reproduced the results of our
earlier published analysis (Behnke *et al.* 2005) based
on 3 study sites in the south of the UK, assessed in a
single calendar year, to emphasize the similarity of
the findings of all 4 studies.

DISCUSSION

In this study we tested the specific *a priori* hypothesis
that the presence of the dominant intestinal nema-
tode of wood mice, *H. polygyrus*, predisposes wood
mice to infection with other helminths. We predicted
that the prevalence of other helminth species would
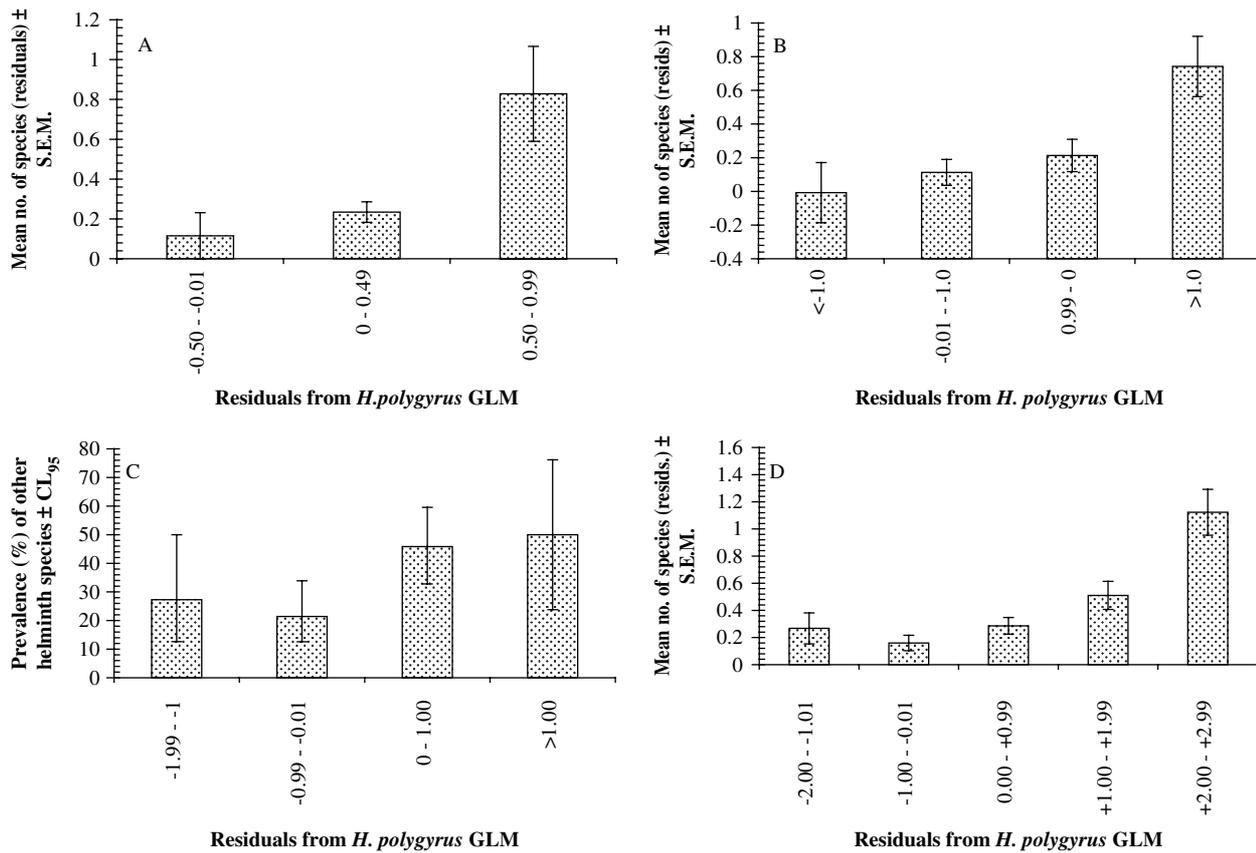be higher in the presence of *H. polygyrus*, and that the

Fig. 4. Relationship between the intensity of *Heligmosomoides polygyrus* infection and number or prevalence of other species of helminths harboured by wood mice expressed in the form of histograms, with the residuals of the *H. polygyrus* GLMs in class ranges as shown on the abscissa. (A) Portuguese dataset (no. of mice in columns from left to right = 21, 148 and 9). (B) Egham dataset (no. of mice in columns from left to right = 14, 73, 56, and 14). (C) Malham dataset (prevalence of other helminth species; no. of mice in columns from left to right = 22, 84, 96 and 14). (D) For comparison with the above, data reproduced from Behnke *et al.* (2005) showing the same relationship in the dataset of Abu-Madi *et al.* (1998, 2000) collected from wood mice in 3 different habitats in the south of the UK (no. of mice in columns from left to right = 16, 59, 76, 22 and 6).

species richness of other helminths would also increase with increasing worm burdens of *H. polygyrus*. These two predictions were based on the findings of Behnke *et al.* (2005) from 3 ecologically contrasting sites in the UK. Here we tested the robustness of this finding in 3 additional datasets unrelated to one another. Since the current analysis was focused on just 2 predictions, each analysed by a well-defined statistical approach, it was free of the confounding problems of multiple comparisons (including Type I errors) that invalidate many other studies of interactions between species. It is clearly evident from our results that our predictions were both borne out. Both relationships (the presence/absence of other helminth species and the species richness of other helminths) held up in 2 datasets, and there was strong support for a similar relationship in the third dataset (Malham) where overall species richness was poor in comparison to the first 2 study sites. The data were collected in geographically different extremes of Europe and in ecologically quite distinct habitats with different combinations of helminths. On the basis of these results we are confident

in concluding that indeed *H. polygyrus* has an effect on other co-infecting helminths in wood mice, and therefore that quantitative interactions between helminths infecting wild wood mice exist and contribute to infracommunity structure at the individual host level, and to component community structure at the population level.

Perhaps counter-intuitively, the current analysis does not indicate that the mice that are most heavily infected with *H. polygyrus* in any population are those most likely to carry also a wide range of other helminths. Rather, our data show that mice more heavily infected with *H. polygyrus* for their own particular subset (i.e. relative to the average burden for a particular year, site, season, age and sex) are the individuals that are most predisposed to being concurrently infected with other helminth species, and the more heavily they are infected for their subset, the more likely they are to carry a greater range of other species than their uninfected or less heavily infected conspecifics.

Whilst correlational analyses such as the current work can never prove cause and effect, there is

already a substantial body of information in the experimental field indicating that the very closely related congener *H. bakeri* has a marked immuno-depressive effect on its host, particularly at the local intestinal level, and that as a consequence other helminths reside longer than they normally would in concurrently infected mice (reviewed by Behnke, 1987). Our data are consistent with the idea that *H. polygyrus* in wild populations of wood mice behaves similarly, not only facilitating its own survival but equally providing an opportunity for other species of helminths to exploit the host's weakened intestinal defences to benefit their own survival and reproductive effort. We are unaware of any experimental assessments of the immunocompetence of wild wood mice carrying heavy burdens of *H. polygyrus*, but Jackson *et al*. (manuscript in preparation) have recently reported that the abundance of *H. polygyrus* in wild wood mice correlates negatively with innate immune responsiveness, implying that this parasite like *H. bakeri* does have immunomodulatory properties.

In recent years, the question of polyparasitism has attracted considerable attention (Graham, 2002; Lello *et al*. 2004; Graham *et al*. 2007), although in reality the subject has a long history of experimental investigation under laboratory conditions (Christensen *et al*. 1987; Behnke *et al*. 2001). The underlying mechanisms that lead to multiple-species infections have come under both theoretical (Dobson, 1985) and experimental scrutiny (reviewed by Behnke *et al*. 2001; Behnke, 2008), driven partly by the ideas that limited intrinsic resources leave hosts with life-history choices relating to their defences against infection, and hence resistance to some species of parasites may be traded off against susceptibility to others (Jolles *et al*. 2008). In part this may be mediated by the compartmentalization of the immune system into apparently mutually antagonistic Th1 and Th2 driven arms (the former of which is optimal in protection against intracellular organisms, the latter more appropriate for metazoan parasites such as helminths) with resultant consequences for the epidemiology of important human infections controlled by these two arms of the immune system e.g. exacerbation of malaria by concurrent helminth infections (Druilhe *et al*. 2005; Graham *et al*. 2007). Motivation for the study of polyparasitism is also driven by the urgency to understand the epidemiological consequence of immunodepression inducing HIV infection, and other emerging diseases, in the context of an array of already established pathogens and symbionts which the host may harbour simultaneously and/or be exposed to throughout life (Pedersen and Fenton, 2006). In the context of parasites of wildlife, a particular threat may be posed by introduced host species, which once established generally have impoverished helminth communities in foreign ecosystems because some of their parasites are unsuccessful in surviving in a novel environment (Vignon *et al*. 2009), but others may spread to indigenous hosts and farmed livestock with resultant consequences for the balance of parasite communities and their influence on host fitness and survival (Hudson *et al*. 1998; Gortázar *et al*. 2007). For these reasons models of co-infection, such as those provided by wild rodent systems, provide an important avenue for testing relevant hypotheses; in this case the consequences of a potentially immunodepression inducing intestinal helminth. Field studies involving intervention, for example by treatment to selectively remove *H. polygyrus* (Ferrari *et al*. 2004), are logically the next step in assessing the overall importance of this nematode in facilitating infection with other helminths in wood mice (Pedersen and Greives, 2008).

The history of studies of interactions between helminths in wild rodents is long (Elton *et al*. 1931; Kisielewska, 1970), but until now no relationship has been discovered that is robust enough to be repeatedly demonstrated in different host populations, and under different ecological circumstances. Our study is therefore the first to do so, and given the prevailing view that quantitative interactions between intestinal helminths play a minor if any role in structuring helminth communities (Haukisalmi and Henttonen, 1993; Poulin, 2001; Behnke *et al*. 2005), our results, together with those of Behnke *et al*. (2005), provide firm evidence that at the level of species richness, if not at the level of individual species, a highly predictable element of co-infections in wood mice has now been defined: infection with *H. polygyrus* has detectable consequences for the susceptibility of wood mice to other helminth species, particularly those associated with the intestine.

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