FACTORS INFLUENCING THE PINWORM COMMUNITY (OXYURIDA: NEMATODA) PARASITIC IN THE HINDGUT OF THE AMERICAN COCKROACH *PERIPLANETA AMERICANA*

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

Large cockroaches, such as *Periplaneta americana*, typically harbour in their hindgut two or more species of parasitic pinworm (*Nematoda: Oxyurida*). Our laboratory colony was infected with three, possibly four species. The mechanism(s) permitting the sympatry of these potentially competing species were investigated by: i) repeatedly sampling over time hosts of various size to determine the structure, if any, in the pinworm guild and ii) infecting uninfected hosts with known doses of infective eggs and monitoring population changes via daily host dissections. Results indicate that chemically-mediated intraspecific interference competition maintains pinworm populations at densities well below the apparent carrying capacity of the majority of hosts. The concomitant reduction of interspecific pressures thus permits the co-habitation of multiple pinworm species in what is essentially a single niche. This intraspecific population limitation is likely a response to pressures produced by the large size of the parasite in relation the hindgut of early instar hosts.
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INTRODUCTION

The american cockroach, *Periplaneta americana*, is found worldwide (Bell and Adiyodi 1981), and harbours a variety of parasites. At least 12 nematode species, representing 9 genera, are found worldwide in varying combinations within the hindgut of the american cockroach (Strand and Brooks 1977). Our colony contained *P. americana* infected with four species of "pinworm" (Nematoda, Oxyurida, Thelastomatidae) representing three genera: *Thelastoma periplaneticola* Leibersperger, 1960; *T. bulhoesi* (Magalhaes, 1900) Trayassos, 1929; *Hammerschmidtiella diesingi* (Hammerschmidt, 1838) Chitwood, 1932 and *Leidynema appendiculatum* (Leidy, 1850) Chitwood,1932. The occurrence of species with such apparently high degrees of niche overlap leads to suppositions as to the mechanism(s) permitting the co-existence. Previous workers have attributed this sympatry to niche diversification within the pinworm species (Hominick and Davey 1972a, 1973). However, before evidence for the mechanisms permitting stable co-existence can be proposed, repeated sampling of the pinworm fauna of *P. americana* over time and over a range of host sizes was necessary to determine the long term structure and stability of the pinworm guild.

The colony of *P. americana* at U.B.C. has been maintained for twenty five years under uniform conditions of heat, light, relative humidity and food supply. This afforded an opportunity to repeatedly sample the cockroach population over time to determine the long term pattern of the pinworm distribution. Also, infective-stage pinworm eggs were collected and fed to uninfected roaches and the subsequent development of the pinworms studied via daily dissections of these experimentally infected hosts. This provided an interpretation of the long term pinworm distribution pattern in terms of the biological interactions which occur during development within the host.

The co-occurrence of several pinworm species and therefore the possible effects of interspecies interactions on guild structure were also considered. To this end, a
colony of *P. americana* infected with one species of pinworm only (*L. appendiculatum*) was established, and the distribution of its pinworms compared to that found in the colony containing a mix of species.

There are four morphological types of male pinworm present in our colony: *T. periplaneticola, T. bulhoesi, H. diesingi* and *L. appendiculatum*. To date I have not been able to identify two morphological types of female *Thelastoma* spp. Data collected from the infected colony was combined with that from experimental infections to determine whether this represents a male polymorphism or a cryptic female species, but at this point the answer remains unclear. It will be explained later that this makes little difference to the analysis. However it is awkward to repeatedly use "three or perhaps four" species when describing the pinworm guild. Thus the guild will usually be referred to as if it contained three species, but the presence of a fourth male type must be considered when discussing the male distribution. Pinworms have a direct life cycle (Dobrovolny and Ackert 1934). Gravid females in the hindgut lay eggs which are passed to the outside environment in the fecal pellets of the host. Within the egg, pinworms develop into first stage larvae which moult to produce ellipsoid second stage larvae. After ingestion by a suitable host, the second stage larva hatches in the midgut and immediately mouls the third-stage which establishes in the hindgut. Here pinworms feed and develop, moultting twice to produce adults (fifth stage). Pinworms are haplodiploid (Adamson 1989, Van Luc and Spiridonov 1990, Zervos 1988b); males are haploid and develop from unfertilized eggs, while female are diploid and develop from fertilized eggs.
METHODS and MATERIALS

Maintenance of Cockroaches

Three colonies of *Periplaneta americana* were maintained in standard plastic garbage cans, each covered by a clear plastic lid with mesh-covered holes for ventilation. Two colonies (#1 and #2) were infected with *Thelastoma periplaneticola*, *T. bulhoesi*, *Hammerschmidtia diesingi* and *Leidynema appendiculatum* (Nematoda: Oxyurida: Thelastomoidea). A third colony (#3) was infected with *L. appendiculatum* only. Water and food were provided *ad libitum*. Food consisted of a ground mix of oat bran, brewers yeast, dry dog food and unsalted peanuts, with lettuce offered every two to four days. In this way, breeding colonies of 200 to 300 roaches were maintained over the study period.

Colonies #1 and #2 have been maintained at U.B.C. for 25 years or more. Although they are housed in two containers, they can be considered a single colony for the purposes of this study. The population of roaches in a colony episodically declines to the point where it must be supplemented from the other, or a new colony must be established from the other. Thus roaches and their parasites from the two containers have been periodically mixed. Comparison of data from the two colonies revealed no significant differences in mean pinworm burdens or prevalences for any of the pinworm species, and therefore data were combined for this study.

Longitudinal Distribution

To investigate longitudinal distribution of the pinworms, 44 hosts (late instar and adult) were dissected and their hindguts quickly transferred to a liquid nitrogen bath for about 10 seconds. The frozen hindguts were then sectioned into 5 approximately equal parts and the adult female pinworm burden of each part was
recorded. The location of the pinworm head was used to assign it to a particular fifth of the hindgut.

Guild structure in mid-instar hosts

To investigate the infraguild structure of a "typical" host, samples of five mid-instar roaches (4th-6th instar) were collected approximately weekly from the colonies infected with all three pinworm species, dissected, and the numbers of adult female pinworms of each species recorded. Host instar was approximated from rear femur length (mean femur length=5.1mm for mid instars). Samples were collected from September 1987 to February 1990 and data were later combined into semi-monthly samples to study changes in prevalence and intensity over time. A total of 328 mid-instar roaches were examined.

Effect of host size on guild structure

Repeated samples of four roaches each were collected to investigate the effect of host size and age on adult male and adult female pinworm burden. Each sample consisted of an adult female (mean femur length=10.0 mm), an adult male (mean femur length=9.9 mm), a late instar (8th-9th instar, mean femur length=8.2 mm) and an early instar (2nd-3rd instar, mean femur length=2.1 mm) cockroach. In all, 192 hosts (48 of each class) were examined and the structure of their guild compared. The egg burden was measured in pinworms from 90 hosts examined consecutively to avoid bias as to the selection of hosts for pinworm fecundity measurement. Mean fecundity for each species was calculated for each host.

Effect of Host Moult

To investigate the effect of the host moult on adult female pinworm burden, 38 pairs of roaches, each pair consisting of a newly moulted roach and a fully tanned
control roach, were removed from the colony and dissected. The burden of adult female pinworms of each species was recorded.

Cockroaches which have recently moulted are recognizable by their white colour. After approximately two days the cuticle tans and the roaches regain their typical brown colour. The cast hindgut lining of recently moulted roaches, although separated from the new underlying hindgut lining, is not passed out of the host until approximately two days after the molt. For this reason, recently molted hosts and their controls were held for three days before being dissected.

**Leidynema appendiculatum** in isolation

Sets of four roaches each, consisting of an adult female, an adult male, a late instar and an early instar, were collected from the colony infected with *L. appendiculatum* only and their burden of adult male and adult female pinworms recorded. In all, 76 (19 of each class) hosts were collected from this colony. Fecundity of the adult female pinworms present was measured for 48 roaches examined consecutively and the mean fecundity calculated for each.

**Experimental Infections**

Uninfected roaches were obtained by placing oothecae, which had been washed in water and rinsed briefly in 70% ethanol, in 1 gallon plastic containers covered with mesh lids. After hatching, the roaches were maintained in the same containers and fed as above.

Nematode eggs were obtained by dissecting roaches from the infected colonies and removing gravid female worms. Worms were put in distilled water and placed in an incubator at 29 C for three to four days, after which eggs containing infective third-stage juveniles were recovered. These were placed on a small piece of apple and
offered to roaches from which food had been withheld for three to six days. After the apple had been consumed, roaches were placed in an incubator at 29 C.

In the first set of infections, twenty cockroaches were given thirty eggs each. Roaches were then dissected two per day on days 1, 2, 3, 4, 5, 6, 9, 10, 11 and 12.

In the second set, sixteen roaches were each given twenty eggs. Roaches were dissected two per day on days 2, 6, 9, 13, 14, 16, 18, and 21.

In the third set, twelve roaches each received twenty eggs. Dissections were performed, two per day, eight hours after infection and on days 3, 6, 9, 12, and 19.

In the fourth set, fifteen roaches each received thirty eggs. Dissections were performed, three per day, on days 12, 15, 17, 19 and 20.

Statistical analysis

Data were analysed on an IBM Olivetti computer using the "Pipestat" data manipulation program (version 5.3, Gary Perlman, Wang Institute of graduate studies, Tyngsboro, MA.). Unless otherwise stated, all tests were evaluated at the 0.05 level of significance.

Terminology

Difficulties in terminology often arise when parasite distributions are described in the same terms as free living populations. Further confusion arises when the entire complement of pinworms in a host (the guild) is differentiated from the members of a specific species of pinworm present (the population). To avoid such confusion, the terminology of Margolis et al. (1982) has been used. Thus "infrapopulation" refers to the pinworms of a particular species within an individual host, and "suprapopulation" to the summation of all such infrapopulations in the host population under study. "Infraguild" refers to pinworms from all species present in an individual host, and "supraguild" to the summation of all infraguilds.
Prevalence defines the proportion of hosts that are infected with the specified parasite, and abundance the number of parasites in each host. Intensity is the number of parasites in each infected host.
RESULTS

Longitudinal Distribution in the Hindgut

Most adult female pinworms were found in the anterior portion of the host hindgut (Fig.1). Regardless of the number or combination of species present, the ileum always contained pinworms in infected hosts. Although proportionately more Thelastoma spp. females were found more posteriorly in the hindgut as compared to H. diesingi or L. appendiculatum (Fig.1), Thelastoma spp. females found in the more posterior region were always accompanied by pinworms located in the ileum and there was a significant correlation between the number of Thelastoma spp. females present and their tendency to be located in a more posterior position in the gut ($r^2=0.26$, P<0.001). There was no evidence that any of the species preferred a more posterior position whether in mixed or single-species infections.

The Guild Over Time-Mid Instar Hosts

Samples of five mid-instar roaches, collected approximately weekly, were combined into semi-monthly samples representing two months each. Changes in prevalence and intensity are shown in Figure 2.

Thelastoma spp. was the most prevalent adult female pinworm in 9 of 14 of these semi-monthly samples (Fig.2). H. diesingi was most prevalent in the remaining 5 samples. Four of the five samples in which H. diesingi was most prevalent occurred consecutively from March 1988 to Nov.1988 (Fig.2). L. appendiculatum was the least prevalent pinworm in 10 of the 14 samples.

Thelastoma spp. had the highest mean intensity in 13 of 14 semi-monthly samples, the only exception being Nov.1988 (Fig.2). In 10 of the 14 samples, the relative order of mean intensity was Thelastoma spp. > H. diesingi > L. appendiculatum (Fig.2).
Figure 1. Longitudinal distribution of adult female pinworms in the hindgut of 44 hosts dissected to investigate longitudinal distribution. Location refers to the percentage of the host hindgut which was anterior to the head of the pinworm.

A-Anterior hindgut (Ileum)

P-Posterior hindgut (Colon/Rectal constriction)
Figure 2. Changes in prevalence (top) and intensity (bottom) over time of adult female pinworms in mid instar hosts. Time is represented on the X-axis by the first letter of the first month in each sample, thus S=September/October, N=November/December, J=January/February etc. Prevalence refers to the percentage of hosts infected. Intensity refers to the mean number of adult female pinworms per infected host.

Dark circles- *Thelastoma* spp.
Open circles- *H. diesingi*
Open diamonds- *L. appendiculatum*
Characterization of Infraguilds-Mid Instar Hosts

96.1% of 328 hosts contained at least one gravid female pinworm (Table 1). 75% (247) of hosts contained *Thelastoma* spp., 62% (205) contained *H. diesingi* and 40% (120) contained *L. appendiculatum*.

Hosts were classified according to number of pinworm species present. Observed numbers of hosts harbouring 0, 1, 2 or 3 species did not differ significantly from expected values. Expected values were calculated by multiplying the proportion of hosts harbouring the stated combination of species, based on their independent distributions, by the total number of hosts (Table 1, $\chi^2=5.95$, $P>0.05$, df=3).

However, the 3 species were not distributed independently of one another. *H. diesingi* occurred more often than expected in combination with *Thelastoma* spp. or *L. appendiculatum* (Fig.3, $\chi^2=7.3$, $P<0.01$, df=3) and there were fewer hosts than expected containing the combination of *Thelastoma* spp. with *L. appendiculatum* (Fig.3, $\chi^2=9.8$, $P<0.005$, df=3).

Abundance ranged from 0 to 29 gravid female pinworms per host, averaging 7.2 (SE 1.8). Overall mean intensity was 6.5 (SE 0.34), 2.9 (SE 0.17) and 1.9 (SE 0.20) gravid females per host for *Thelastoma* spp., *H. diesingi* and *L. appendiculatum* respectively.

Relative order of intensity did not vary whether hosts were infected with one, two or three species of pinworm. *Thelastoma* spp. had the highest mean intensity in all three categories of infected host (Fig.3). In hosts containing two species (Table 1) the overall mean intensity was 5.6 (SE 0.27), 2.6 (SE 0.28) and 2.2 (SE 0.32) for *Thelastoma* spp., *H. diesingi* and *L. appendiculatum* respectively. This order of relative intensity (*Thelastoma* spp. > *H. diesingi* > *L. appendiculatum*) was mirrored in the mean intensity of hosts containing three species (Fig.3).

Frequency distributions based on abundance were positively skewed for all species (Fig.4). *Thelastoma* spp. were found over a greater abundance range than the
Table 1. Number of mid-instar hosts (n=328) infected with 0, 1, 2, and 3 species of pinworm. Expected values are based on the independent distributions of the pinworm species.

<table>
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<tr>
<th>Host Class (number of pinworm species)</th>
<th>Number of Hosts</th>
<th>Observed</th>
<th>Expected</th>
<th>$\chi^2$</th>
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<tr>
<td>0</td>
<td></td>
<td>11 (3.4%)</td>
<td>19</td>
<td>3.54</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>110 (33.5%)</td>
<td>102</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>159 (48.5%)</td>
<td>150</td>
<td>0.50</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>48 (14.6%)</td>
<td>56</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Total $\chi^2=5.95$, $P>0.05$
Figure 3. Relative proportion of each combination of adult female pinworm recovered from mid instar hosts. Each section of the pie represents the percentage of mid instar hosts which harboured the specified combination of pinworms. The bar diagrams show the mean number of adult female pinworms within each category of host.

T- *Thelastoma* spp.
H-H. *diesingi*
L-L. *appendiculatum*
Figure 4. Frequency distribution of adult female pinworms from mid instar hosts. Abundance refers to the number of adult female pinworms in the host. Frequency is the number of hosts harbouring the specified number of pinworms.

A) *Thelastoma* spp.
B) *H. diesingi*
C) *L. appendiculatum*
other species. 102 hosts contained more than 5 gravid female Thelastoma spp. each, whereas 20 hosts contained more than 5 H. diesingi and only 8 contained more than 5 L. appendiculatum (Fig.4).

Effect of Host Size

Four categories of host were sampled and compared: adult female, adult male, late instar and early instar. Numbers of adult male and adult female pinworms were recorded.

**Thelastoma** spp.:

**Males**

Overall prevalence (i.e. prevalence produced by treating the four host groups as a single group) was 41% for male *T. bulhoesi* and 32% for male *T. periplaneticola*. Prevalence of male *T. bulhoesi* did not differ significantly among the four host groups (Fig.5, chi² =2.35, P>0.5, df=3). Adult female and adult male hosts did not differ significantly with respect to prevalence of male *T. periplaneticola*, although adult females were more commonly infected (Fig.5, chi² = 2.3, P>0.1, df=3). Prevalence was significantly greater in adult female hosts as compared to late or early instars (Fig.5, chi² = 5.28, P<0.025, df=1 for adult females versus late instars). Prevalence of *T. periplaneticola* was significantly lower in early instar hosts than in all other host classes (Fig.5, chi² = 5.63, P<0.025, df=1 for early instars versus late instars). Adult male and late instar hosts did not differ significantly with respect to prevalence of infection with male *T. periplaneticola*, although adult males were more commonly infected (Fig.5, chi² = 0.54, P>0.25, df=1). *T. periplaneticola* males were found over a much greater range of abundance than were the other species (Fig.6). 29 hosts contained 4 or more male *T. periplaneticola* and as many as 86 *T. periplaneticola* males were found in a single host. *T. periplaneticola* was the only male pinworm found
Figure 5. Prevalence of infection with adult male pinworms, showing the percentage of each host class infected with the specified species of male pinworm. For example approximately 70% of adult female hosts contained adult male *T. periplaneticola*.

AF-Adult female hosts
AM-Adult male hosts
LI-Late instar hosts
EI-Early instar hosts
Figure 6. Frequency distribution of adult male pinworms from early instar, late instar, adult male and adult female hosts combined. Abundance refers to the number of adult male pinworms in the host. Frequency is the number of hosts harbouring the specified number of pinworms.

A) *T. periplaneticola*
B) *H. diesingi*
C) *L. appendiculatum*
D) *T. bulhoesi*
in numbers greater than 10 per host (Fig.6). Mean intensity was 24.4 (SE 4.1) male *T. periplaneticola* per infected host, and the variance to mean ratio of 48.4 indicated overdispersion.

*T. periplaneticola* males were most numerous in adult female and late instar hosts (Fig.7). Approximately 80% of the *T. periplaneticola* males recovered were from adult female hosts. 17 of the 22 hosts containing more than 6 male *T. periplaneticola* were adult female roaches, 4 were late instar and one was an adult male. Male *T. periplaneticola* mean intensity was significantly higher in adult female hosts than in any of the other three host classes (Fig.7, Q statistic (proposed by Dunn 1964, used in non-parametric multiple comparisons where there are not equal numbers of data in each group being tested)=3.69, P<0.002 for adult female versus late instar hosts). Mean intensity was not significantly different in adult male and late instar hosts (Fig.7, Q=0.47, P>0.5) despite the high intensity in late instars.

*T. bulhoesi* was modally distributed at 1 male per infected host (SE 0.04) (Fig.6). Mean intensity was not significantly different in any of the host classes for male *T. bulhoesi* (Fig.7, Hc (Kruskal-Wallis nonparametric test statistic corrected for ties)=2.42, P>0.25).

Females

Overall prevalence of female *Thelastoma* spp. was 68%. Prevalence was significantly lower in early instar hosts than in other host classes (Fig.8, chi²=9.98, P<0.005, df=1 for adult male versus early instar hosts). Prevalence was not significantly different among adult female, adult male or late instar hosts (chi²=0.42, P>0.75, df=2).

*Thelastoma* spp. females were more abundant than the other pinworm species in all four host classes; this was particularly striking in adult female hosts where as many as 140 *Thelastoma* spp. females were found in a single host (Fig.9). This is compared to
Figure 7. Mean intensity of infection with adult male pinworms. For convenience the Y axis is represented as $\log_{10}$, but the actual mean values are shown above each bar. Mean intensity refers to the mean number of pinworms in infected hosts, discounting uninfected hosts.

AF-Adult female hosts
AM-Adult male hosts
LI-Late instar hosts
EI-Early instar hosts
Figure 8. Prevalence of infection with adult female pinworms, showing the percentage of each host class infected with the specified species of female pinworm. For example approximately 90% of adult female hosts contained adult female *Thelastoma* spp.

AF-Adult female hosts
AM-Adult male hosts
LI-Late instar hosts
EI-Early instar hosts
Thelastoma spp.
Figure 9. Distribution of adult female pinworms in each host class. The vertical line represents the range of abundances (pinworms per host). The thick horizontal line represents the mean, and the two thin horizontal lines the standard error.

AF-Adult female hosts
AM-Adult male hosts
LI-Late instar hosts
EI-Early instar hosts
A)-Thelastoma spp.
B)-H. diesingi
C)-L. appendiculatum
a maximum of 26 pinworms per single host for *H. diesingi* and 23 for *L. appendiculatum*, both of which occurred in adult female hosts (Fig.9).

There were significantly more female *Thelastoma* spp. in adult female hosts than in the other host classes (Fig.9a, Q=17.82, P<0.001 for adult female versus late instar). Adult male and late instar hosts did not differ with respect to *Thelastoma* spp. burden (Fig.9a, Q=1.86, P>0.5), but both contained significantly more female *Thelastoma* spp. than early instars did (Fig.9a, Q=3.81, P<0.001 for adult male versus early instar hosts).

*H. diesingi*

**Males**

49% of hosts contained male *H. diesingi*. Significantly more adult female and late instar hosts were infected than were adult males or early instars (Fig.5, chi² = 3.9, P<0.05, df=1 for adult female versus adult male hosts).

*H. diesingi* lay between *T. periplaneticola* and *T. bulhoesi/L. appendiculatum* in abundance range (0-10) and mean intensity (3.5,SE 0.25). Mean intensity was not significantly different in any of the host classes for male *H. diesingi* (Fig.7, Hc=7.60, P>0.05)

**Females**

Overall prevalence of *H. diesingi* was 53% and did not differ significantly among adult female, adult male or late instar hosts (Fig.8, chi²=2.87, P>0.1, df=2). Adult male and early instar hosts did not differ significantly (Fig.8, chi²=3.03, P>0.05, df=1) but prevalence was significantly lower in early instar as compared to late instar or adult female hosts (Fig.8, chi²=16.0, P<0.001, df=1 for early instar versus late instar).

Highest abundance occurred in adult female hosts, but abundances of adult female and adult male/late instar were not significantly different (Fig.9b, Q=0.65, P>0.5).

Adult male and late instar hosts were not different with respect to *H. diesingi* burden,
but both were significantly higher than early instar (Q=2.79, P<0.05 for late instar versus early instar).

**L. appendiculatum**

**Males**

24% of hosts contained male *L. appendiculatum*. Prevalence did not differ significantly among the four host groups (Fig.5, chi$^2 = 6.68$ P>0.05, df=3).

Male *L. appendiculatum* were modally distributed at 1 male per host (SE 0.03) (Fig.6). Mean intensity was not significantly different in any of the host classes (Fig.7, Hc=6.25 P>0.1). The variance to mean ratio of 0.88 was significantly less than 1 (P<0.05) indicating underdispersion.

**Females**

Overall prevalence of *L. appendiculatum* was 48% and was not significantly different among host classes (Fig.5, chi$^2=1.47$, P>0.5, df=3). *L. appendiculatum* was unique in that highest prevalence occurred in early instar hosts (Fig.5).

There were no significant differences in abundance among the host classes (Fig.9c, Hc=4.23, P>0.1). As with *H. diesingi* and *Thelastoma* spp., *L. appendiculatum* was found over the greatest abundance range in adult female hosts, and these hosts had the highest mean number of females per host (Fig.9).

**Species combinations**

Hosts were further categorized according to the species of female pinworms present in each. Of eight possible pinworm combinations the same three were most prevalent within adult-female, adult-male and late-instar hosts, although the relative order was different. These three host categories were *Thelastoma* spp. only, the combination of *Thelastoma* spp. with *H. diesingi* and the combination of all three species (Fig.10). This is consistent with the condition in mid-instar hosts (Fig.3).
Figure 10. The relative proportion of the various combinations of adult female pinworms within each host class.

Thel.-Thelastoma spp.
H.dies.-H. diesingi
L.append.-L. appendiculatum

A)-Adult female hosts
B)-Adult male hosts
C)-Late instar hosts
D)-Early instar hosts
Single-species infections were more common in early instar hosts than in other host classes. 27%, 22%, and 23% of adult female, adult male and late instar hosts respectively contained one pinworm species only, while 64% of early instar infections were single species (Fig.10).

Early instar hosts differed further in that they commonly contained L. appendiculatum alone, and rarely contained the Thelastoma spp./H. diesingi combination or all three species (Fig.10). The four host classes were not significantly different with respect to the proportion infected with Thelastoma spp. alone (Fig.10, chi²=5.2, P>0.1, df=3) or H. diesingi alone (Fig.10, chi²=1.73, P>0.5, df=3) but a significantly greater proportion of L. appendiculatum-only infections were found in early instars as compared to the other three host classes (Fig.10, chi²=14.5, P<0.005, df=3). Similarly, adult female, adult male and late instar hosts did not differ with respect to the proportion infected with the combination of Thelastoma spp./H. diesingi or the combination of three species (Fig.10, chi²=5.29 and chi²=4.71 respectively, P>0.1 for both) while there were significantly less early instar hosts containing these two combinations of pinworms (chi²=8.49, P<0.05 and chi²=11.73, P<0.01 respectively). The relative order of prevalence observed in mid-instar hosts (Thelastoma spp. > H. diesingi > L. appendix) was conserved within all host classes except early-instar, where more hosts contained L. appendix than contained H. diesingi or Thelastoma spp. (Fig.8).

Effect of Host Moult

There was no significant difference in mean adult female pinworm burden between recently moulted and control hosts in any of the species (Fig.11, Paired T-test: Thelastoma spp.: T=0.23, P>0.25; H. diesingi: T=0.6, P>0.25; L. appendix: T=0, P>0.25).
Figure 11. Comparison of mean adult female pinworm burden of newly moulted versus control hosts. Vertical lines represent standard error. Moulted roaches were selected based on their white colour and held with controls for three days before dissecting. Controls were selected based on their dark colour, thus controls were at least six days post moult.
In hosts that were completely white (i.e. hosts that had recently moulted) pinworms were found collected together into a tight mass located more posteriorly in the hindgut than usual. Flagellate protozoa cohabiting the anterior hindgut also congregated and moved down the hindgut with the nematodes. Hosts that were two days post-moult contained a mixed mass of pinworms and protozoa located at the colon-rectal sphincter. Pinworms and protists were located in their usual position in the anterior portion of the hindgut in hosts that were three or more days post-moult.

Pinworm Fecundity

*Thelastoma* spp. females contained considerably fewer eggs than did *H. diesingi* or *L. appendiculatum* (Table 2). The relative order of mean fecundity was *H. diesingi* > *L. appendiculatum* >> *Thelastoma* spp.

In all pinworm species there was a significant negative correlation between the number of conspecific females in the host and the mean number of eggs per female pinworm (Fig. 12, P<0.05 for all pinworm species). The rate of decrease of fecundity with increasing female pinworm density, as indicated by the slope (b) of the best fit regression line, was greater in *H. diesingi* and *L. appendiculatum* (Fig. 12, b=-0.034 and b=-0.037 respectively) than it was for *Thelastoma* spp. (b=-0.007). This difference is partly accounted for by the fact that *Thelastoma* spp. occurs over a greater abundance range than the other two species. When the analysis was restricted to *Thelastoma* spp. densities approximating those found for the other two species, the rate of decrease of *Thelastoma* spp. fecundity approached that found in *H. diesingi* and *L. appendiculatum* (Fig. 12d, b=0.018), although it was still lower.

The effect of pinworm density on fecundity was not apparent between species. No significant negative correlations were found between the number of non-conspecific females in the host and the mean fecundity of the pinworm species in question (Table 3).
Table 2. Mean eggs per female pinworm

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Eggs per Female (n,SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thelastoma spp.</td>
<td>32 (62, 2.1)</td>
</tr>
<tr>
<td>H. diesingi</td>
<td>172 (49, 10.3)</td>
</tr>
<tr>
<td>L. appendiculata</td>
<td>136 (47, 9.2)</td>
</tr>
</tbody>
</table>
Figure 12. The effect of intraspecific adult female pinworm density on mean fecundity. Each point represents the mean fecundity for pinworms from a single host. The straight line is the best fit regression of female pinworm numbers on Log_{10} mean fecundity.

A) *Thelastoma* spp. Regression equation: $Y = -0.007x + 1.58$ ($T=8.64$, $P<0.0001$)

B) *Thelastoma* spp., excluding those hosts which contained more than 14 adult female *Thelastoma* spp. females. $Y = -0.018x + 1.68$ ($T=7.57$, $P<0.0001$)

C) *H. diesingi*. $Y = -0.034x + 2.22$ ($T=6.23$, $P<0.0001$)

D) *L. appendiculatum*. $Y = -0.037x + 2.33$ ($T=5.59$, $P<0.0001$)
Table 3. The effect of interspecific adult female pinworm density on mean fecundity. Each predicted variable (Y) represents the number of eggs of the species in question. x1, x2 and x3 are the numbers of female H. disingi, L. appendiculatum and Thelastoma spp. respectively.

Thelastoma spp.: \[ Y = -1.20x_1 + 0.78x_2 + 33.77 \]
\[ x_1 = H. disingi \ (T=1.47, P>0.1) \]
\[ x_2 = L. appendiculatum \ (T=1.52, P>0.1) \]

H. disingi: \[ Y = -0.65x_3 - 1.35x_2 + 183.70 \]
\[ x_3 = Thelastoma spp. \ (T=1.83, P>0.07) \]
\[ x_2 = L. appendiculatum \ (T=0.73, P>0.4) \]

L. appendiculatum: \[ Y = 0.70x_3 - 2.92x_1 + 136.00 \]
\[ x_3 = Thelastoma spp. \ (T=1.14, P>0.2) \]
\[ x_1 = H. disingi \ (T=0.62, P>0.5) \]
Comparison of *L. appendiculatum* in Isolation with *L. appendiculatum* from the Multi-
species Colony

As in previous data sets, hosts were divided into four classes (adult female, 
adult male, late instar and early instar)

Male *L. appendiculatum* were more prevalent in the isolated colony (83%) than in 
the mixed colony (24%). In all four host classes prevalence was significantly higher 
in the isolated colony than in the mixed colonies (Fig. 13, adult female: \( \chi^2 = 5.5, \) 
P<0.025, df=1; adult male: \( \chi^2 = 20.2, P<0.001, df=1; \) late instar: \( \chi^2 = 22.0, P<0.001, \) 
df=1; early instar: \( \chi^2 = 3.95, P<0.05, df=1 \)).

Prevalence of females was 100% in all four host classes in the *L. appendiculatum*-only colony. This compares with the 40% prevalence found in mid instar 
hosts from the mixed colonies and the 48% found overall in the four host classes (adult 
female, adult male, late instar and early instar) from the mixed colonies.

As in the mixed colonies, male *L. appendiculatum* were modally distributed at 1 
per infected host in all host classes.

In contrast to the condition found for *L. appendiculatum* in the multi-species 
colony, differences did exist among the host classes with respect to pinworm 
abundance in the *L. appendiculatum*-only colony. There were significantly more adult 
female *L. appendiculatum* in adult female hosts than in the other three host classes in 
the *L. appendiculatum*-only colony (Fig. 14, \( Q = 3.19, P<0.01 \) for adult female versus late 
instar). Adult male and late instar hosts did not differ with respect to pinworm 
burden (Fig. 14, \( Q = 1.61, P>0.5 \)) but both contained significantly more pinworms than 
early instar hosts (Fig. 14, \( Q = 3.26, P<0.01 \) for adult male versus early instar).

*L. appendiculatum* from the isolate colony showed no significant differences in 
mean intensity when compared to *L. appendiculatum* from the mixed colonies in any of the 
host classes (Fig. 14, adult female: \( T = 1.19, P>0.05; \) adult male: \( T = 1.04, P>0.2; \) late 
instar: \( T = 2.01, P>0.05; \) early instar: \( T = 0.19, P>0.5 \)), although mean intensity was
Figure 13. Prevalence of infection with adult male *L. appendiculatum* in the colony containing hosts infected with four species of pinworm as compared to the colony of hosts infected with *L. appendiculatum* only.

AF-Adult female hosts
AM-Adult male hosts
LI-Late instar hosts
EI-Early instar hosts
Figure 14. Distribution of adult female *L. appendiculatum* from the colony containing hosts infected with *L. appendiculatum* only compared to the colony containing hosts infected with three species of pinworm. For comparative purposes data for *L. appendiculatum* from the mixed colony (Figure 9c) is here reproduced next to the corresponding data from the *L. appendiculatum*-only colony. The vertical line represents the range of abundances (pinworms per host). The thick horizontal line represents the mean, and the two thin horizontal lines the standard error.

AF-Adult female hosts
AM-Adult male hosts
LI-Late instar hosts
EI-Early instar hosts
I-Data from the *L. appendiculatum*-only colony
M-Data from the mixed (four pinworm species) colony (Fig.9c).
higher in the isolate colony in three (adult female, adult male and late instars) of the four host classes (Fig.14).

Female *L. appendiculatum* in the isolate colony contained an average of 92 eggs each (SE 4.9, range 28-154). This was significantly less than the average of 119 (SE 9.9, range 30-434) eggs per female *L. appendiculatum* found in the mixed colonies (F=4.05, p<0.001).

The negative effect of female pinworm density on mean fecundity observed in the mixed colonies was also observed in *L. appendiculatum* in the isolate colony (Fig.15). The correlation coefficient for the effect of density on fecundity in the isolate colony was not significantly different from that in the mixed colony (r²=0.33 and 0.35 respectively), and the common correlation coefficient for *L. appendiculatum* in mixed and isolated colonies was calculated at 0.35.

**Experimental Infections**

Figure 16 shows the average number of *L. appendiculatum* recovered from experimentally infected roaches dissected at various periods post-infection. Third and fourth stage larval males were found on days one and two in numbers ranging from two to twelve per host. By three to four days post-infection the males reached adulthood, and the number of males in a host declined to one by day five.

A similar phenomenon was observed in female *L. appendiculatum*, where no more than four adults were recovered from a single host, the number recovered usually being one or two. Juvenile females were found in numbers up to twelve per host. There were no noticeable changes in number or development of females when males reached adulthood.

Infections were performed using eggs from female *Thelastoma* spp. that had been collected from adult female hosts and early instar hosts. The hypothesis was that the two species of female *Thelastoma* might mimic the males. As shown earlier, *T. bulhoesi*
Figure 15. The effect of adult female *L. appendiculatum* density on mean fecundity in the colony containing hosts infected with *L. appendiculatum* only. Each point represents the mean fecundity for pinworms from a single host. The straight line is the best fit regression of female pinworm numbers on Log<sub>10</sub> mean fecundity.

Regression equation: $Y = -0.026x + 2.1$ (T=5.79, P<0.001)
Figure 16. Number of *L. appendiculatum* recovered from host hindgut on various days after experimental infection. Data points are means for repeated infections using varied initial doses of infective stage larvae. Vertical bars represent standard error, only half of which is shown for clarity.

Dark triangles—Juvenile female *L. appendiculatum*

Dark squares—Juvenile male *L. appendiculatum*

Open triangles—Adult female *L. appendiculatum*

Open squares—Adult male *L. appendiculatum*
preferred early instar hosts while *T. periplaneticola* predominated in adult female hosts. Thus eggs collected from female *Thelastoma* spp. occurring in adult female hosts should produce *T. periplaneticola* males, while eggs from females occurring in early instars should produce *T. bulhoesi* males. Results of experimental infections are shown in Table 4. Only *T. bulhoesi* males were recovered, regardless of the host from that the pinworm eggs were collected or the type of host infected.

*Thelastoma* spp. females develop more slowly than female *L. appendiculatum*. Adult *Thelastoma* spp. females were recovered only after 29 days at 29 C. (Fig.17). Male *T. bulhoesi* develop similarly to male *L. appendiculatum* in that development from hatching to adulthood took approximately three days at 29 C and the climax infrapopulation consisted of a single adult male per host (Fig.17).

As adult females were only recovered from one host it is difficult to assess the significance of the decline in pinworm numbers occurring over time, however only four adult female *Thelastoma* spp. were recovered from this host (Fig.17), and this is less than the mean of 9.0 (SE 0.62) larval *Thelastoma* spp. females recovered per experimentally infected host.
Table 4. Species of male *Thelastoma spp.* produced from eggs collected from female pinworms occurring in adult female and early instar hosts

<table>
<thead>
<tr>
<th>Source of Eggs (Host)</th>
<th>Host Infected (n)</th>
<th>Males Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Female</td>
<td>Adult Female (3)</td>
<td><em>T. bulhoesi</em></td>
</tr>
<tr>
<td>Adult Female</td>
<td>Adult Male (3)</td>
<td><em>T. bulhoesi</em></td>
</tr>
<tr>
<td>Adult Female</td>
<td>Early Instar (4)</td>
<td><em>T. bulhoesi</em></td>
</tr>
<tr>
<td>Early Instar</td>
<td>Adult Female (2)</td>
<td><em>T. bulhoesi</em></td>
</tr>
<tr>
<td>Early Instar</td>
<td>Adult Male (2)</td>
<td><em>T. bulhoesi</em></td>
</tr>
<tr>
<td>Early Instar</td>
<td>Early Instar (3)</td>
<td><em>T. bulhoesi</em></td>
</tr>
</tbody>
</table>
Figure 17. Number of *Thelastoma* spp. recovered from host hindgut on various days after experimental infection. Each point represents a single host.

Dark triangles-Juvenile female *Thelastoma* spp.

Dark squares-Juvenile male *Thelastoma* spp.

Open triangle-Adult female *Thelastoma* spp.

Open squares-Adult male *T. bulhoesi*
Discussion

Ecological theory holds that complete competitors can not coexist in the same niche, as over time the more efficient of the competing species will take over all niche space to the exclusion of others (Begon et al. 1986). The occurrence of several pinworm species parasitizing the same host organ thus leads one to speculate as to the factors permitting their coexistence. The explanation most commonly proposed for such a condition is that resource partitioning has occurred between species such that the species today occupy several "sub"-niches, and that by occupying these different niches the pinworm species avoid competition and are able to coexist within the same individual host.

An assumption of this model is that the organisms are existing at densities that approximate the carrying capacity of the host hindgut, and thus niche segregation results from competition between species for limited resources. It is the contention of this paper that, for most hosts in the present study, this assumption does not apply, and thus the coexistence of several species in the same host is permitted by the overabundance of resources relative to the pinworm burden. Only in very small hosts (early instar), where resources are limiting to individual pinworms, is competition important. The severe resource limitation of these small hosts, in fact the relative lack of space itself, has resulted in the development of intense intraspecific population limitation. A side effect of this is that in larger hosts infrapopulations are maintained well below the carrying capacity of the host hindgut.

The nematode guild was constant over a wide range of host sizes and over time. *Thelastoma* spp. was the dominant worm in both number of hosts infected (prevalence) and number of pinworms in infected hosts (intensity), particularly the latter. *L. appendiculatum* consistently placed lowest in these categories, while *H. diesingi* was in an intermediate position, skewed toward *L. appendiculatum*. There were unexplainable exceptions which appeared sporadically over time, particularly from
September 1988 to January 1989 when numbers of *Thelastoma* spp. females declined, but the overall pattern was clear: *Thelastoma* spp. was the most abundant pinworm followed by *H. diesingi* then *L. appendiculatum*. The absolute number of pinworms per host was also fairly constant for all species over a wide range of host size classes.

Prevalence and intensity reported in this study are typical of those reported from other locales (Gordon 1963, Hominick and Davey 1972a, 1972b, 1973, Khairul Anuar 1977, 1987, McCallister and Schmidt 1981, 1988, Perigrine 1974a, Todd 1943). While there are few specific accounts of *T. periplaneticola* or *T. bulhoesi*, there are many reports of other *Thelastoma* spp., as well as *H. diesingi* and *L. appendiculatum*, from *P. americana* and other cockroach hosts. These reports usually describe morphology only and omit the number of worms found. However, where prevalence and intensity are reported they are consistent with the numbers observed in our colony.

Khairul Anuar (1987) gives a break down of the numbers and species combinations of *H. diesingi*, *L. appendiculatum* and *Thelastoma malaysiense* in adult *P. americana* from various locals in Penang, Malaysia and reports mean intensities similar to those found in our study in both absolute and relative terms (approximately 9.3, 4.1 and 3.8 adult females per host for *T. malaysiense*, *H. diesingi* and *L. appendiculatum* respectively). Khairul Anuar (1987) also reports that the combination of *T. malaysiense* with *L. appendiculatum* is quite rare, occurring in only 8 of 155 hosts. This agrees with the negative correlation found between *Thelastoma* spp. and *L. appendiculatum* in the present study.

Khairul Anuar (1987) did not observe the high intensity of *Thelastoma* spp. found in adult female hosts in our colony, but other reports of high numbers of other *Thelastoma* spp. do exist. Leibersperger (1960) reports finding 168 *T. periplaneticola* in a single *P. americanus*, but does not differentiate between pinworm sexes or host age or sex; however his report of total pinworms recovered suggests that there were many male and female pinworms in some individual hosts. *T. periplaneticola* thus appears
unusual, as it occurs in relatively large numbers (100 and more) in a single host, a phenomenon not found in *T. bulhoesi, H. diesingi* or *L. appendiculatum.*

Hosts in this study were held in sealed containers, and this leads to the possibility of artefact in the pinworm distribution produced by this artificially high host density. This may be partially responsible for the high overall prevalence. However, studies from "wild" cockroach populations (often in and around human domiciles) report that prevalence of pinworm infection usually reaches 85% or more, indicating that our laboratory conditions were a fair approximation of the natural condition. "Wild" cockroaches are gregarious, be it in a rotting log or a student’s apartment, and the restrictions imposed by the containers in this study do not appear to alter the pinworm distribution.

Host moulting had no affect on the number of adult female pinworms for any of the species. Previous workers (Dobrovolny and Ackert 1934, Todd 1944) report slower development times for *L. appendiculatum* than were found in this study (30 days for females to attain adulthood at 30 C. as compared to 12 days at 29 C. reported here) and conclude that the pinworms were more likely to be found in older hosts, where the time between host moults was sufficient for the pinworms to reach maturity, because the larval pinworms did not survive the host moult. Larval worms were not counted in this study, but they were usually present in high numbers in hosts that had recently moulted, and the more rapid rate of development found for *L. appendiculatum* in our study casts doubt on this hypothesis.

The worms are displaced to the posterior hindgut for a short time during the host moult, then migrate back up to the anterior hindgut after the moult is completed. The hindgut protozoa show a similar response to the moult, indicating that the initial posterior displacement of the parasites is likely due to the increases in host hindgut peristalsis during moulting and the constrictions imposed by the shed hindgut lining. Ability to cope with the host moult is not surprising, as it would be fundamental to the
establishment and continuation of the parasitic association.

Thus it appears that each pinworm species maintains a relatively low and consistent intensity in hosts worldwide, despite probable differences in host diet and environment.

In attempting to explain such stability in guild structure, the effects interspecific competition are often invoked. Niche diversification in response to interspecific competition has been assigned a major role in shaping parasite communities (Holmes 1986, Schad 1963). This parasitic association is likely quite ancient (Morris, 1981) and thus past interspecific competition may have resulted in niche diversification that is today reflected in the pinworm species occupying distinct niches within the host hindgut at abundances which are determined by the carrying capacity of each individual niche. The resultant decrease in interspecific competition would then allow the species to coexist.

Previous studies suggest the presence of niche diversification in pinworms of turtles (Schad 1963) and of P. americana (Hominick and Davey 1972a). I was unable to find any evidence for niche segregation in our colony. All species seem to prefer the anterior portion of the hindgut. There is evidence that the pinworms location in the hindgut is affected by the composition of the host diet (Hominick and Davey 1972b, Hominick and Davey 1973, Peregrine 1974a, Peregrine 1974b). The apparent preference of both the nematode and protozoan parasites for the anterior portion of the hindgut probably means that this area contains more or better quality nutrients than the depauperate posterior hindgut. This seems reasonable as this area is nearest the source of incoming nutrients. The reported preference of H. diesingi for a more posterior position in H. diesingi-only infections (Hominick and Davey 1973) was not apparent in this study. Radial distribution was not measured in this study but, in the smaller hosts particularly, radial distribution is probably unimportant as the pinworms occupy such a substantial proportion of the hindgut. Microscopic examination
revealed no differences in intestinal contents between worm species. Rather than suggesting niche segregation, these data indicate that there is a high degree of niche overlap, or that there is a single niche within which the species coexist.

The existence of a single niche is supported by the negative effects that were observed between species. The best measure of the importance of these interspecific effects was obtained from the comparison of \textit{L. appendiculatum} in isolation with \textit{L. appendiculatum} in the multi-species colony. Results of this comparison show that while \textit{L. appendiculatum} prevalence was much lower in the mixed colony, the mean intensity of infection with \textit{L. appendiculatum} was not significantly different in the mixed versus the isolate colony. This was true for both sexes of \textit{L. appendiculatum} over all host size classes examined. These data suggest that the other pinworm species have a inhibitory effect on the initial establishment of \textit{L. appendiculatum}, but that there are no important interspecies effects on the resultant number of \textit{L. appendiculatum} once infection of the host is established.

Thus \textit{H. diesingi} and/or \textit{Thelastoma} spp. appear to confer some heterogeneity on the host population with regards to infectability with \textit{L. appendiculatum}. This is analogous to the host heterogeneity provided by varying host immunocompetence in other parasitic associations. In this case it is the presence of other worm species which is inhibiting the establishment of \textit{L. appendiculatum}. The negative correlation observed between female \textit{Thelastoma} spp. and \textit{L. appendiculatum} in the mixed colony may then be due to an inhibitory effect of the former on the establishment of the latter. This would be especially important in adult female hosts where \textit{Thelastoma} spp. can reach very high numbers and perhaps pose more of a problem to the establishment of \textit{L. appendiculatum}. Adult female hosts eat and presumably defecate more than other host classes, and thus represent the most prodigious source of pinworm generation. Denial of these hosts to \textit{L. appendiculatum} would further explain their scarcity in the mixed colonies.
The fact that *L. appendiculatum* in the mixed colony was unique in having a higher prevalence in early instar as opposed to the larger hosts may thus be a result of its being more likely to be able to secure sole possession of the hindgut in smaller hosts and thus exclude other pinworm species. This is supported by the disproportionately high number of early instars infected with *L. appendiculatum* only.

Thus interspecific competition for occupancy of the anterior hindgut may occur in early instars, but the evidence suggests that most worms are able to maintain themselves in larger hosts at a slightly more posterior position without any noticeable detrimental effects to their numbers or fecundity. These exclusion effects between pinworm species add support to there being one niche within which all the pinworm species coexist.

Interspecific competition is important in determining the combination of pinworm species present in a host, but its effects on the actual number of pinworms in the host appear for the most part negligible. There was a negative effect observed in the multi-species colony between *L. appendiculatum* and *Thelastoma* spp., but data from the *L. appendiculatum*-only colony indicates that *L. appendiculatum* prevalence is suppressed by *Thelastoma* spp., while intensity remains essentially unaffected. The effects of intraspecific competition must be examined to discern the factors governing pinworm density.

Experimental infections of mid to late instar hosts with *L. appendiculatum* and *T. bulhoesi* indicate that there is intense intraspecific population limitation such that the resultant infrapopulation consists of a single adult male and one or a few adult females. The occurrence of a single male *L. appendiculatum* or *T. bulhoesi* per infected host in the multi-species colony indicates that this process is ongoing in this colony also. Interspecific effects are not important in determining this mean intensity, as male *L. appendiculatum* maintained this modal distribution in the *L. appendiculatum*-only colony and experimental infections produced a single male per
infected host in the absence of other species. There is a strong intraspecific effect such that male *L. appendiculatum* and *T. bulhoesii* limit their infrapopulation densities to a single male per host.

Experimental infections were performed using mid to late instar hosts, where resources such as food and space are probably not limiting for the relatively minute males, and it is difficult to envision a resource which is in such abundance so as to consistently support exactly one male worm per host. Despite this, male infrapopulations are severely limited to one male worm per host in *T. bulhoesii* and *L. appendiculatum*, and three or four males per host in *H. diesingi*. The exception to this, *T. periplaneticola* in adult female hosts, appears to be an anomaly and is discussed later.

The resource competed for by males is likely female worms. The confines of the host gut limit the number of females that an individual male pinworm has access to, and this has probably favored development of intense interference competition in male worms.

Experimental infections indicate that *L. appendiculatum* and *T. bulhoesii* begin eliminating conspecifics only after sexual maturity is reached. Juvenile males tolerate each other such that several are found in a single host, whereas only a single adult male (*T. bulhoesii* and *L. appendiculatum*) or a very few (*H. diesingi*) coexist within an individual host. There may be a "race" between male worms to reach adulthood first, kill conspecifics and thus procure all females. This may explain the males small size and rapid rate of development in comparison to females.

Other pinworm species parasitic in the cockroach hindgut, such as *Blatticola monandros* in *Parellipsidion pachycercum* (Zervos 1988a), *Protrellis dixoni* in *Drymaplaneta variegata* (Zervos 1988b) and *Blatticola blattae* in *Blatella germanica* (Van Luc and Spiridonov 1990, personal observations) also severely limit their own infrapopulations such that most cockroaches contain one male and one female worm only.
Zervos (1988a) reviews the possible mechanisms that might generate such a distribution and suggests that this limitation is mediated via a sex-specific chemical toxin released by the pinworms which kills conspecifics. Active killing of conspecifics, resulting in very small infrapopulations, has been reported in other parasitic species including mites parasitic in marine mussels (Mitchell 1965) and hymenopterans parasitic in caterpillars (Salt 1959). Chemical mediation of this killing has been suggested in Gyrocotyle spp. (Platyhelminthes) parasitizing the gut of ratfish (Halvorsen and Williams 1968). Chemically mediated interference competition is responsible for allelopathy in many species of plant and microorganism (Rice 1974).

What these organisms share with the pinworm is a physically confining environment where there is little opportunity for dispersal away from conspecific competitors, or the parasites themselves occupy a substantial volume of the parasitized organ, such that density dependent intraspecific competition is intensified. There is undoubtedly a common selective significance to a sedentary lifestyle which promotes strong intraspecific competition by virtue of the inescapable proximity of conspecifics to each other and the subsequent value of local resources.

Conspecifics usually represent the strongest potential competitor to an organism. By eliminating conspecific rivals, an individual secures all of the available niche space and all potential mates. Furthermore, such an individual gains increased proportional representation in subsequent generations.

An important determinant as to whether an organism will actively kill conspecifics is likely the ease with which such action can be accomplished. Most organisms are fairly well equipped to deal with or escape from physical challenges from conspecifics as they are identical in structure to such a challenger; thus the cost of killing conspecifics, in terms of search time and reciprocal damage incurred, may outweigh the benefits for many organisms.
The confines of the cockroach hindgut facilitate intraspecific competition in these pinworms. Search time is minimal and the cost of this interaction to the killer also appears to be minimal, as it is probably mediated via a chemical which is indiscriminantly released into the hindgut. The hindgut also provides a vessel in which such a chemical can be concentrated. Free living organisms are not likely to employ this system, as the chemical would simply diffuse into the surrounding medium. Plants are the free living exception, their lack of mobility enhancing the effectiveness of a local toxin. This system could enhance territoriality in the free living environment, as chemicals could be released which would demarcate a territory and discourage conspecific competitors. It would be interesting to investigate territoriality in free living nematodes to see if this parasitic population limitation is an intensification of such a system.

The relative order of abundance of male pinworms is thus an inverse reflection of the severity of intraspecific population limitation. The occurrence in females of an identical relative abundance order (*Thelastoma* spp. >> *H. diesingi* > *L. appendiculatum*) suggests that a similar process may be determining their numbers.

Experimental infections indicate that infrapopulation limitation in females is similar, though less severe, to that seen in male worms. *L. appendiculatum* reduces its numbers to one or a few female worms per host after infection with twenty or thirty infective eggs. The occurrence of more than one adult female in the post-infective infrapopulation may in part be due to dilution of the responsible chemical agent in larger hosts, as smaller hosts from the infected colonies typically contained a single female worm.

The occurrence of many (twenty and more) female worms in larger hosts from the infected colonies is likely the result of overlapping infections. This indicates that while the males limitation of the number of conspecifics in the host is ongoing for the duration of the male worm's life, the females active killing of conspecifics may have a
shorter duration, a "window" that remains open long enough for the female to kill conspecifics which were ingested at the same time that she was, and then shuts such that females resulting from subsequent infection events (i.e. host ingestion of infective-stage pinworms) are not affected. It may also be that as females mature they become resistant to the effects of conspecifics, or that the effects of such a chemical vermicide are less severe on the diploid females than on the haploid males.

Whereas the male worms are probably competing for mates, it is not as clear what resources the female worms are competing for. Cockroaches such as early instar *P. americana* or adult *P. pachycercum* are themselves so small that they are able to maintain only a limited number of pinworms due to the size limitations of their hindgut. Parasite size and gut carrying capacity has been deemed important in determining the mechanisms of population limitation in *Ascaris lumbricoides* infections of humans (Keymer 1982). The adult female pinworm occupies a substantial proportion of the cockroach hindgut, so that space itself may be the limiting factor (Fig. 18a). This is supported by the occurrence of modal distributions of one parasite per host in other nematodes infecting small insects (Adamson and Buck 1990, Zervos 1988a, Zervos 1988b). The actual space over which the worms are competing may be even smaller than the entire hindgut, for pinworms show a marked preference for the anterior part of the hindgut (Adamson and Buck 1990, Hominick and Davey 1973, McCallister and Schmidt 1981). Thus space itself, in particular the anterior section of the hindgut of early instar hosts, may be the limited resource over which the worms compete. However late instar cockroach nymphs and adults occasionally support more than a hundred worms (Fig. 18b). Despite this, the majority of these hosts harbour far fewer worms than the available space would permit (Fig. 19). The continuation of this active interference competition for space in larger hosts, where space is no longer limiting, would then seem to violate the tenet of limited resources being necessary for ongoing competition, as the result is populations that are apparently well below the carrying capacity of their
Figure 18. Relative position and size of adult male and female *Thelastoma* spp. in situ.

A-First instar host  
B-Adult female host  
F-Female pinworm  
M-Male pinworm  
mt-Malphigian tubules  
il-Ileum  
r-Rectum
Figure 19. Adult female pinworm density (bars) and hindgut volume (squares with connecting line) in the various host classes.

EI-early instars
MI-mid instars
LI-late instars
AM-adult male
AF-adult female
The graph shows the density (adult females/mm³) of Thelastoma spp. and L. appendiculatum, as well as the volume of the hindgut (mm³) across different host classes: EI, MI, LI, AM, and AF.

- **Thelastoma spp.** is represented by dark bars.
- **L. appendiculatum** is represented by white bars with diagonal lines.
- **H. diesingi** is represented by white bars.
- **Hindgut Volume** is represented by a line graph.

The density and volume increase with the host class from EI to AF.
environment. Of course the tenet remains inviolate as resources are limited for a portion of the pinworm suprapopulation, namely those infecting early instar hosts.

These pinworms occupy a niche which is distinctly separated into discrete patches of very constant dimension. Once "assigned" to a given patch (i.e. a particular instar) there is no opportunity for an individual pinworm to disperse out of that patch. One would predict that competition would be most severe in the patch that represents the most limited supply of resources. Resources (indeed space itself) are most limiting in smaller hosts, and small hosts (be they small roach species or early instars of a larger species) probably represents the main focus of selective pressures which favour population limitation, providing the impetus that maintains this limitation in larger hosts where space is no longer limiting.

All cockroaches go through early developmental stages which are quite small in relation to the pinworm. Furthermore, newly emerged first instar hosts represent the only host population which is guaranteed uninfected, and a pinworm which gains sole possession of such a host might be able to then exclude subsequent pinworms from establishing or at least avoid being itself excluded. The unusually high occurrence of L. appendiculatum-only infections in early instar hosts suggests that this is the case for this species. P. pachycercum is a relatively small cockroach, and B. monandros occupies a substantial portion its hindgut. This is analogous to Thelastoma spp., H. diesingi and L. appendiculatum that occur in early instar P. americana. This study demonstrates that this population limitation continues over a wide range of host sizes, the result being larger hosts which harbour far fewer nematodes than they could potentially. This availability of niche space in larger hosts has facilitated the co-occurrence of multi-species infections within a single host species. This is supported by the preponderance of single species infections within small insect species where all "patches" are of limiting dimension.
Another manifestation of intraspecific competition was the negative effect of pinworm density on fecundity. Competition for limited nutrients would help explain the negative fecundity affects of female conspecifics on one another, as egg production is likely affected by nutrient uptake (Peregrine, 1974).

The negative effect of increased worm burden on mean fecundity was species specific. Only conspecific females suppressed each others fecundity. The suppression of fecundity caused by the presence of even a single conspecific female was significant, whereas the presence of many worms of the other two species had no apparent effect. This was true for all three species. Thus if competition for nutrients is a causal factor in suppressing fecundity, it is necessary that these worm species feed on three absolutely distinct components of the hindgut microflora. As previously stated, there is no evidence for niche segregation in these pinworms, and thus exploitative competition for nutrients is probably not the cause of the density-dependent fecundity suppression.

Furthermore, there was a significant difference in fecundity (approximately 30% for all species) in female pinworms from one versus two pinworm infections of large hosts. It is difficult to envision a nutrient supply which is capable of supporting 150 worms and yet is limiting when only two worms are present.

A further point against nutrients as the factor governing the effect of crowding on fecundity is the exponential, as opposed to linear, decrease in mean fecundity as worm density increases. The geometric decrease in fecundity seen in all three species indicates that the relative effects of crowding on fecundity are relatively stronger at the lower worm densities and then become less marked at higher worm densities. This is not consistent with nutrient supply as the factor determining fecundity.

A chemical factor released by females, which reduces conspecific fecundity, is consistent with these data. This implies a mechanism similar to that proposed for the active killing of conspecifics which occurs when adulthood is reached. The chemical
factor may be the same one responsible for the conspecific limitation but excreted in a
diluted or modified form. This is supported by the co-occurrence of reduced population
limitation with a reduced rate of conspecific density-dependent fecundity suppression
in *Thelastoma* spp..

Thus classical exploitation competition for resources does not appear to be
important in the majority of infrapopulations. It appears that the strong
intraspecific population limitation keeps the pinworms below the carrying capacity of
the hindgut of most hosts. Only in smaller hosts, where space is limiting, would
exploitation competition be important, and it is probable that this population
limitation is a response to conditions imposed by small hosts.

The potential carrying capacity of the hindgut of larger cockroaches is
exemplified in one anomalous case where population limitation is not apparent: adult
female cockroaches infected with *Thelastoma* spp. females and *T. periplaneticola* males.

There were many more *Thelastoma* spp. recovered than were *H. diesingi* or *L. appendiculatum* (total worms recovered of each species: 5991, 1210 and 640
respectively), and the occurrence of large numbers of *Thelastoma* spp. in adult female
hosts seems the best explanation for this numerical dominance. In adult female hosts
there is apparently a break down in the intraspecific limiting affect of *Thelastoma*
spp. such that a hundred or more can coexist in a single host. Male *T. periplaneticola*
revert to a low intensity infection in other hosts, becoming modal at one worm per host
in early instars. This indicates that *T. periplaneticola* retains its ability to limit
its population size, but that this is somehow repressed in adult female hosts.

These high numbers of pinworms probably approximate the potential carrying
capacity of the host, as there appears to be little space for more than about 150
pinworms in a single adult female host (personal observation). Very high numbers of
these worms were also found in a few late instar hosts. Late instars were not
identified as to sex, and these few individuals may represent female roaches. No adult
male hosts were found to contain such high numbers of Thelastoma spp., indicating that it is some inherent difference between male and female roaches that allows this coexistence. Some affect of host hormones is possible, as the cockroach ovary is known to produce hormones, while cauterization of host neurosecretory cells has been shown to affect the population of H. diesingi parasitizing Blatta orientalis (Gordon, 1968).

Perhaps female host hormone in the gut interacts with the pinworm antihelminthic so as to make it inert. Alternatively, the environment of the adult female hindgut may alter the worms so as to stop them releasing the antihelminth or provide them with a resistance to its action. Thus the reduction of population limitation in adult female hosts provides a population "sink" from which Thelastoma spp. can numerically dominate the supraguild.

Thelastoma spp. numerical dominance in our colony may seem attributable to the inclusion of two worm species (T. periplaneticola and T. bulhoesi) in a single group (Thelastoma spp.). The subsequent comparisons with H. diesingi and L. appendiculatum would then seem unjustified as data from two worm species were summed and compared to single species data. To some extent this is true and unavoidable, as I am at present unable to distinguish two morphological types in female Thelastoma spp.. However, overall there were five and eight times respectively as many female Thelastoma spp. recovered than were H. diesingi or L. appendiculatum, and thus if it truly was two species then one or both of them was in some way peculiar from H. diesingi or L. appendiculatum.

The evidence from male worms indicates it was one form (T. periplaneticola) that may have been particularly responsible for the increased numbers of Thelastoma spp. pinworms. The frequency distribution for male T. periplaneticola is very similar to that found for female Thelastoma spp.. Male T. bulhoesi, with its modal distribution of one, is most like L. appendiculatum, and if T. bulhoesi females behave as H. diesingi and L. appendiculatum females do (and mirror their respective male’s
distribution) then female *T. bulhoesi* may be rare and difficult to distinguish in a background of many female *T. periplaneticola*.

Confounding this issue was the inability to produce male *T. periplaneticola* from experimental infections, with all males produced being *T. bulhoesi*. More infections with *Thelastoma* spp. eggs are necessary to satisfactorily explain this result, however it suggests a male polymorphism. This would mean that the *T. bulhoesi* morph retains its strong self-limiting effect while the *T. periplaneticola* morph does not and coexists in high numbers. A similar phenomenon has been reported for two pinworm species infecting skinks and geckos (Ainsworth, 1990), where one morph maintains a mean intensity of one male per host while the other exists at numbers of up to seven per host. As both morphs would be competing for the same females, and there were eighteen times as many male *T. periplaneticola* recovered as *T. bulhoesi*, the *T. periplaneticola* morph would be at a distinct advantage in procuring a mate. Experimental infections indicate it is probably not an environmentally determined polymorphism, as male *T. bulhoesi* developed in hosts of varying size and sex.

Whether it is actually only one species of pinworm in *Thelastoma* spp. with a male polymorphism, or a combination of a more populous species (*T. periplaneticola*) and a rarer species (*T. bulhoesi*), the comparison of *Thelastoma* spp. with *H. diesingi* and *L. appendiculatum* is justified and does not in itself explain the dominance of *Thelastoma* spp. Rather the explanation presumably lies in the reduced population limitation occurring in adult female hosts.

In conclusion the factor that determines and maintains the stability of the guild structure in our colony is intraspecific interference competition. This interference competition is likely mediated via a species and sex-specific chemical toxin released by the pinworms when adulthood is reached. The relative abundance of pinworms through time (*Thelastoma* spp.>> *H. diesingi* > *L. appendiculatum*) is thus an inverse reflection of the degree of intraspecific limitation within each species.
Interspecific effects are unimportant in determining infrapopulations, but are important in determining prevalence. Thus while interspecific competition affects the supraguild by determining the species combinations present in each infrapopulation, intraspecific population limitation dictates the number of individuals of each species present in each infrapopulation.

There is some debate as to the relative importance of interspecific competition in structuring communities. The occurrence of site-specific parasites has been used as evidence of niche diversification due to interspecific effects (Holmes 1973, Schad 1963), while reviews of field experiments and studies on free living organisms differ in their assessment of the importance of interspecific effects in maintaining community structure (Brooks 1980, Connell 1983, Schoener 1983, Shorrocks et al. 1984). This study indicates that species may not compete even in the presence of substantial niche overlap. However, all systems are different and the debate over the importance of interspecific competition is somewhat artificial, as what is true for one group of organisms may not be for another. Pianka (1981) describes "a gradient in the intensity of (interspecific) competition, varying continuously between the endpoints of a complete competitive vacuum (no competition) to a fully saturated environment with demand equal to supply...". These pinworms then represent a system biased toward the former of these two extremes. The continuing co-existence of these non-competing species is facilitated by intense intraspecific population limitation in small hosts that prevents parasite densities from reaching levels sufficient to cause resources to be limited in the majority of larger hosts.

These effects are likely direct adaptations to the parasitic lifestyle, where the small gut size of early instar hosts relative to the size of the pinworm limits the availability of space for female pinworms and the availability of mates for male pinworms, resulting in intense intraspecific competition.
Each species maintains its own population without regard to the other species present. Populations are maintained well below the apparent carrying capacity of the majority of hosts, and thus competition for resources is probably of little importance, particularly in the larger hosts. However, the best nutrients probably occur most anteriorly in the host hindgut, so some exploitation competition (both inter- and intra-specific) may occur in this area.

*Thelastoma* spp. (in particular *T. periplaneticola*) appear to reduce this intraspecific interference competition in adult female hosts, thus providing a population "sink" from which *Thelastoma* spp. can numerically dominate the population despite its very low fecundity. This phenomenon may be the result of a peculiar interaction between the pinworm-produced antihelminth of *T. periplaneticola* and some moiety, perhaps hormonal, in the hindgut of the adult female cockroach.
REFERENCES


