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ABSTRACT

The lastoma bulhoesi (Magalhães, 1900), Leidynema appendiculatum (Leidy, 1850), and Hammerschmidtella diesingi (Hammerschmidt, 1838), (Order Oxyurida), parasitize the colon of the American cockroach, Periplaneta americana (Linnaeus). Individual hosts may harbour 1, 2, or all three species simultaneously. These helminths inhabit the same region of the host gut, are transmitted the same way, and have similar feeding behaviours. Such similar life histories lead to the expectation that communities within hosts will be interactive, with such interactions being mainly competitive.

Using 3 replicate cockroach colonies containing all species of parasite, as well as single colonies of hosts infected with either L. appendiculatum alone, or T. bulhoesi alone, I have looked for evidence of interspecific interactions in the distributions of females of each species among hosts. I examined 72 hosts from each colony, and found evidence of negative interaction between T. bulhoesi and L. appendiculatum, and positive interactions between these two species and H. diesingi. The first two species were found together less often than expected under an assumption of independent distribution, while H. diesingi was found with both of these species more often than expected. L. appendiculatum was more prevalent among small, immature hosts than either T. bulhoesi or H. diesingi, indicating possible niche segregation based on host size. Examination of effects of number of females of each species in an infracommunity on female fecundity provided further evidence for both negative and positive interactions among the species. Fecundity of both L. appendiculatum and T. bulhoesi females was reduced in the presence of H. diesingi, while that of H. diesingi was enhanced in the presence of either of these species. Analysis of dietary
preference based on size of food item revealed complete overlap among the three species, though *L. appendiculatum* and *T. bulhoesi* consumed substantially more of the model food items than did *H. diesingi*, indicating that differences in interactions among the species may be based upon differences in diet. Lastly, larval resistance to expulsion from the host gut during host moult for *T. bulhoesi* and *L. appendiculatum* indicated that *L. appendiculatum*’s apparently greater ability to exploit young hosts is not based on greater resistance to expulsion from these hosts. I conclude that the three pinworm species form interactive infracommunities, but that there is evidence of both positive and negative interactions.
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INTRODUCTION.

The study of community ecology seeks, among other things, to determine the nature of the factors structuring communities. Are the various populations in a community acting independently of one another, or do they form some sort of coordinated whole? The former depiction goes back to H.A. Gleason, who proposed that communities are composed of species acting solely in their individual interests. He presented this as an alternative to F.E. Clements's idea of communities being tightly-coordinated groupings of species acting together to maintain a sort of homeostasis within the community (McIntosh, 1995). Since these two models were proposed, community ecologists have debated the importance of each, with no conclusion having yet been reached.

At first glance, a community seems a simple thing to define; it is a collection of individuals of two or more species living in the same place, at the same time. The simplicity is, as with so many things in ecology, deceptive, and the hidden complexity can create difficulties for community ecologists. The difficulties are both conceptual—what does "community" mean?—and physical. The former difficulty is likely insoluble, but the latter may not be.

The physical difficulty lies in putting a reasonable limit on the "place" in which the community to be studied exists. For example, migratory birds pass through enormous amounts of space in the course of a season, encountering an enormous variety of other species along the way. Where is the community to which they belong? Even something as apparently well-bounded as a small pond has, when one begins to look closely, boundaries that "bleed" into the surrounding terrain. Dragonflies, for
example, live out part of their lives as inhabitants of the aquatic environment, and the rest in the terrestrial environment, though still closely connected to the aquatic. The spatial boundaries of communities of free-living organisms are forced to be arbitrary to at least some degree.

Even when spatial boundaries have been successfully defined, there is likely to be an enormous number of confounding factors involved. Fluctuating temperatures, humidity, wind, and light may contribute in unpredictable ways to any patterns found among the species in a community.

Lastly, all of the above factors make it difficult to obtain replicate communities. It is impossible to find two, let alone multiple, identical sites for study.

The above problems complicate the study of communities of free-living organisms, but they can be overcome to great extent with studies of intestinal helminths. In such studies the spatial boundaries of a community are easily defined: the host's intestine constitutes these limits. This is particularly true in the case of helminths that are directly transmitted through the ingestion of infective eggs by the host individual. There are no free-living stages whose activities must be taken into account. Furthermore, there will be much less variation in environmental factors from one host to another. The gut physiology of one host individual should be very similar to that of another host individual of the same species. This similarity can be enhanced by raising host individuals under as similar conditions as possible, eliminating possible differences in the intestinal environment due to diet or level of hydration.

The ability to clearly delineate the boundaries of each community, and make the environment within those boundaries as similar as possible means that replicate
communities can be easily obtained. These replicates can be examined at both the within-host level, and at the host population level. This is the great advantage of studying laboratory communities of intestinal helminths over similar studies of free-living organisms.

Naturally, such laboratory-reared communities may seem highly artificial compared to free-living communities, even parasitic communities within free-living hosts. That, however, is precisely the point. These model communities exist to reduce the complexity of natural communities to a few, essential (one hopes) factors. For example, parasites of free-living hosts may fluctuate in number, not through any interactions among individuals within a single host individual, but because recruitment is seasonal. Within laboratory colonies of hosts, infective stages of the parasite are constantly available. Host nutrition can be expected to fluctuate in the wild, whereas it should be constant in the laboratory.

The main drawback to parasite/host systems is the inability to directly observe the parasites non-destructively. Where the observer of free-living organisms can employ a variety of techniques to keep track of their number on a daily, weekly, or yearly basis, or sit and watch the different members of a community interact, the parasitologist is forced either to use indirect methods of assessment, or to rely on snapshots showing a system’s state at a moment in time. The former might involve something like counts of eggs emerging from a host followed over a period of time, while the latter comes from dissecting the host and directly counting the number of helminths within. These counts can then be used to determine whether or not individuals of different species are independently distributed, how individuals of each
species are distributed (randomly, evenly, or contagiously), and whether or not a species shows a characteristic prevalence or mean intensity within a host community. All of these factors, and others, can then be used to make inferences about the possible level, and effect, of interactions among the species within host individuals.

For species that do interact the question then involves determining the nature of the interaction, whether it is positive, or negative. Does one species facilitate the success of another, or do the species compete with one another for some resource?

The helminth species I have looked at all have very similar life histories. Because they cannot emigrate from a host if conditions within the host become less than ideal, and because hosts are constantly exposed to the possibility of new infections, each species would seem to be under considerable pressure to develop some means of coping with the presence of conspecifics and individuals from other species. As stated above, a species may choose to cooperate, or compete with other species. Broadly speaking, for this latter strategy, there are two paths a species can take. Some choose to maximize their efficiency at exploiting a common resource, ignoring the presence or absence of competitors, while others may actively defend their access to the resource, blocking the access of competitors. The former strategy is referred to as "exploitation" competition, while the latter is known as "interference" competition (Keddy, 1991).

Before competition can be invoked as a structuring force, it is necessary to show first that the species involved require the same resource. In the present study, the first level of required resource is, of course, host individuals. If there is a limit on how many helminths a host can harbour, there will be competition among the species for
access to hosts. Within an individual host, the possible resources are space and food. Chapter 3, in which model food items have been supplied for the worms, addresses this question with regard to feeding within a host. Once it has been demonstrated that the species do require the same resources, competition becomes a plausible structuring factor. If, and how, mutually-required resources become limiting, these limitations may then act to shape within-host communities.

Chapter 1 looks for evidence of a division of resources at the host population level. Do the helminth species all exploit the host population equally, or is there evidence that a species might focus on a particular stage, or sex, of host? Do the species interact with each other, and, if so, are the interactions positive, or negative?

Based on results from chapter 1 indicating that two of the species, *Leidynema appendiculatum* and *Thelastoma bulhoesi*, appear to exploit hosts of different ages, with *L. appendiculatum* appearing to focus on juveniles while *T. bulhoesi* focuses on adults, I have looked, in chapter 2, for indications of differences in their ability to resist expulsion from immature hosts when these hosts moult. I expected *L. appendiculatum* to be more resistant than *T. bulhoesi*, but found that the opposite was the case.

Finally, in chapter 4 I have looked at the effects of competition, or facilitation, on each species *per capita* fecundity.

I conclude that the communities are, in fact, interactive in nature, but that there is evidence for both positive and negative interactions among the species.
DEFINITION OF TERMS.

The fields of parasitology and ecology are rife with jargon, and it may be helpful to begin with a set of definitions of terms that will be used throughout this paper. For the most part, these will follow those laid out in Bush et al. (1997).

Unfortunately, Bush et al. omit a definition of the thing around which all their other definitions revolve—parasitism. The most common definitions of parasitism involve some sort of harm done to the host by the parasite. For example, Schmidt and Roberts (1996), in a commonly-used introductory textbook of parasitology, give the following definition, “Parasitism is a relationship in which one of the participants, the parasite, either harms its host or in some sense lives at the expense of the host.” Such definitions render the status of an organism as a parasite contingent on a trait that may, in fact, be governed by factors external to that organism. A large literature on the subject of the evolution of virulence exists, within which there are numerous examples of organisms that fit Schmidt and Roberts’s definition in some hosts, but don’t in others, and, worse still, may or may not at different times in the same host individual (e.g. Ewald, 1994; Bull, J.J., 1994; Lenski, R.E. and May, R.M., 1994). Obviously a term purporting to describe one organism that is dependent on that organism’s host organism, or current state of the host organism, is unsatisfactory. The definition of “parasite” that I intend is similar to one proposed by Dogiel (1964):

“Parasites are those animals which use other living animals as their environment and source of food, at the same time relinquishing to their hosts, partly or completely, the task of regulating their relationships with the external environment.”
This definition, though it omits mention of parasites other than those that parasitize animals, makes clear the point that the concept of parasitism is one that describes an ecological relationship; the host is an environment in the same sense that a forest, lake, or plain is, and a parasite is an organism that colonizes and exploits that environment. “Pathogenic” becomes simply a description of the current state of the relationship between the environment and its inhabitants, one that is not a necessary component of the concept. I bring up this point in order to forestall any question as to the nature of the pinworms treated in this study. There is no evidence that they are pathogenic. McCallister (1988) found that individual *Periplaneta americana* hosting either, or both, of two of the three species covered in the current study, *Thelastoma bulhoesi* and *Hammerschmidtella diesingi*, were, in fact, significantly larger than those that were uninfected. While the difference was most likely due to his finding that females were more commonly parasitized than males, and that females were the larger sex, his evidence suggests that there are no detrimental effects on host growth due to infection.

Parasites are commonly quantified using three terms: prevalence; intensity; and abundance. “Prevalence” is the percentage of hosts infected with one or more parasites, among all hosts sampled. “Intensity” is the number of parasites, usually of a particular species, within an infected host. “Mean intensity” is this same quantity, averaged over all infected hosts. Finally, “abundance” is the number of parasites within an individual of the host species, whether or not the individual is actually infected. Unlike intensity, therefore, abundance can have a value of zero. “Mean abundance” can be calculated as prevalence times mean intensity.
“Infrapopulation” refers to the population of a single species of helminth within a host individual. The sum of all of a parasite species’ infrapopulations is known as the “component population”.

The next level is that of the community. An “infracommunity” is the community of parasite species within a host individual, and the “component” community is the sum of these over all hosts.

The term “host” refers to an individual of the host species, whether actually infected or not. While Bush et al. do not explicitly define the term in this manner, they do use it this way. Such a broad use of the term does not seem unwarranted in the current case. It is unlikely that there are individual P. americana that are immune to infection with these worms throughout their entire lives, and so all are at least potential hosts, and thus it seems warranted to use the term in what philosophers refer to as the “timeless” sense. On a more prosaic level, this usage also avoids awkward phrases such as, “potential host individual,” or, “individual of the host species”.

THE PARASITES AND THEIR HOST.

The Parasites.

The parasites considered in this study come from three genera within the order Oxyurida: Thelastoma bulhoesi (Magalhães, 1900), Leidynema appendiculatum (Leidy, 1850), and Hammerschmidtiiellia diesingi (Hammerschmidt, 1838) (Fig. 1).
Figure 1: From top: Anterior 2/3’s of adult female *H. diesingi, L.appendiculatum, and T. bulhoesi*. Length for each species ranges between 2.0 and 3.5 mm.

Oxyuridans are commonly referred to as pinworms due to the possession by individuals of most species within the group, particularly female individuals, of an extended, sharply-pointed tail. While the phylogenetic relationships between the Oxyurida and their free-living relatives have yet to be completely worked out (Blaxter, *et al.*, 1998), the Oxyurida share ecological affinities with soil-dwelling, bacteriophagous nematodes such as *Caenorhabditis elegans* (Adamson, 1989). Pinworms, similarly, feed on the bacteria found within the posterior intestine of their hosts (Fig.2).
Figure 2: Typical position of pinworms in host hindgut. The top illustration represents an average juvenile host, the bottom an average adult. Scale bars = 2 mm.

A common feature of these hosts is the modification of this region of the intestine to a fermentation chamber (Adamson, 1989). Invertebrate hosts are inhabitants of rotting logs, or areas of decaying vegetation, while vertebrate hosts are either omnivores, or herbivores. Oxyuridans are presumed to have originated among either millipedes, or cockroaches (Adamson, 1989), possibly through the modification of an originally phoretic relationship. Phoretic relationships involve one species utilizing another, usually larger, species for transportation. Such relationships are not obligatory for either species.
Figure 3: Infective eggs of (from left) *L. appendiculatum*, *T. bulhoesi*, *H. diesingi*.

All pinworms are directly transmitted via the fecal/oral route. Females of the species in this study release their eggs into the lumen of the host intestine. Eggs (Fig. 3) are then deposited within a host fecal pellet when the host defecates. Hosts are infected when they consume part, or all of a fecal pellet. Fecal pellets are very solid, and resistant to mechanical breakup. Containment within these pellets may offer the eggs protection from both desiccation, to which pinworm eggs are quite susceptible (Adamson, 1989), or host-induced mechanical destruction.

Pinworms are haplodiploid; males are produced from unfertilized eggs (Adamson, 1989). Adamson and Ludwig (1993) have shown that haplodiploidy can enhance the colonization success of organisms that colonize new habitats before mating. In such cases immature females colonize new habitats, and may, upon maturation, find themselves without potential mates. Females in this situation can nevertheless produce eggs that will, upon hatching, produce males with which the female can then mate to produce female offspring. Hosts for pinworms are often residents of restricted habitats — rotting logs, piles of dead vegetation, etc.; this being the case, there is at least some possibility of Oedipal encounters, and successful colonization of a new host population.
The Hosts.

The American cockroach, *Periplaneta americana* (Linnaeus), is distributed worldwide, though it is believed to have originated in tropical Africa (Roth, 1982). Wherever humans have gone, *P. americana* has gone along. It is found in restaurants, homes, caves, and other less savoury places, and feeds on virtually anything, including cockroach feces (pers. obs.).

A female cockroach deposits oöthecae (egg cases) containing, on average, sixteen eggs, and produces as many as two oöthecae per week, with total production recorded ranging from 10 to 84 oöthecae in a female’s lifetime (Roth, 1982). Embryos escape, literally, from the oötheca after approximately 30 days at 29°C. All embryos must cooperate to pry open the oötheca, which has a “spring-loaded” hinge. If readiness for eclosion is not synchronized among the majority of the embryos present, or if some have died, the resistance in the oötheca hinge is sufficient to trap all embryos inside, where they will die (Roth, 1982).

Once hatched, juvenile cockroaches moult approximately 10 times during their development to adulthood, taking 5 – 6 months to mature (Roth, 1982). A mated female lives, on average, for just under a year, though longer lifespans have been recorded (Roth, 1982). Allowing one oötheca per week over this lifetime, a female will produce just over 700 offspring. A constant supply of naive hosts is fairly certain (Fig.4).
As mentioned above, coprophagy is common among these hosts. Alexander (1993) has shown that, for animals relying for much of their energy requirements on extensive bacterial fermentation of nutrients in a hindgut modified for this purpose, coprophagy is a strategy that helps the animal regain nutrients that would otherwise be lost in the form of undigested bacteria. Thus, for these hosts, coprophagy is, if not obligatory, certainly useful, and is, therefore, a reliable means of transmission for intestinal helminths such as the ones considered in the present study.

In addition, Bell et al. (1973) demonstrated that *P. americana* individuals excrete an aggregation pheromone in their feces. Cockroaches use this pheromone to find other cockroaches, or places others have been. Cockroaches also tend to remain in areas in which there are high concentrations of the pheromone. This aspect of their feces can also be expected to contribute to their exposure to helminth eggs.
Within the field of parasitology, the question of whether communities of species are aggregates of coevolved species, each occupying its niche with few or no restrictions presently placed upon it by the others, or chance collections of species, that may or may not compete, has most commonly been addressed through studies of communities of parasitic intestinal helminths. These helminth/host systems make particularly good community models because of the availability of replicate environments – hosts. Conditions within the hindguts of different individuals of the same sex and age of a host species, should, however, be much more similar to one another than the environments of free-living organisms.

Holmes and Price (1986) reviewed three hypothetical means by which the distribution of multiple parasitic helminth species within individual hosts may be effected: interactive site segregation (Holmes, 1973), population concentration (Rohde, 1979), and individualistic response (Price, 1980). The first of these hypotheses posits an interactive process whereby parasite communities have been, and continue to be, structured by interspecific interactions. Species involved have a high probability of successfully colonizing a host, resulting in large infrapopulations. Multiple, large infrapopulations can be expected to lead to frequent interactions between species, resulting in competitively-enforced resource partitioning. Realized niches (a restricted portion of the fundamental niche) should be significantly smaller than fundamental niches (the niche space a species would inhabit in the absence of competitors).

The second and third hypothesized community types are isolationist in nature, in that species in these communities do not interact, and inhabit their particular niches
for reasons intrinsic to them. Isolationist species are species with a low probability of colonizing a host, resulting in small infrapopulations with a low probability of interaction. Species in these communities will be distributed independently of one another within a host, each occupying a characteristic niche. There are two proposed mechanisms by which such communities might arise. First, individuals may be more influenced by the necessity of finding a mate than by acquisition of resources. Their spatial restriction, on or in a host, may make finding a mate a more reliable process. This hypothesis was proposed by Klaus Rohde (1979, 1982) in order to explain the high level of site specificity among monogeneans, ectoparasites of marine fish. The second mechanism was proposed by Price (1980), and suggests that parasite species are, of necessity, highly specialized, adapted independently to very narrow niches. Under both of these hypotheses interactions between species in the past were unimportant.

This thesis, and studies of similar cockroach/pinworm systems before it, attempts to determine the level of interaction among *T. bulhoesi*, *L. appendiculatum*, and *H. diesingi*. Nature, of course, is seldom so tidy as to divide neatly into interactionist/isolationist halves, leading some researchers (e.g. Goater *et al.*, 1987) to suggest that “interactionist,” and “isolationist,” are best thought of as the two poles of a continuous spectrum of community types, with different communities showing different levels of each. I have, therefore, looked for the level, and kind, positive or negative, of interactions among these three species.

The pinworm communities with which the present study is concerned should fall toward the interactionist end of the spectrum. Their hosts exist in a very limited
environment, something not found in most studies of parasite communities as most have been done on hosts in the wild. The host environment, furthermore, is virtually saturated in each pinworm species’ transmission stages, so probability of transmission for each can be expected to be high. And, because each is transmitted in the same manner, their transmission probabilities, upon ingestion of a species’ infective egg, should be similar. This being the case, there would be a high likelihood of interspecific encounters. Whether these encounters lead to any meaningful effects on one or another of the species involved will depend on the level of similarity of resource use among them, and to what extent they push the limits of a mutually-necessary resource.

Schad (1963) examined the community of Oxyuroid nematodes parasitizing the hindgut of the European tortoise, *Testudo graeca*, looking for signs of niche diversification. Though the models of Holmes, Price, and Rohde did not exist at the time, his study was an attempt to determine whether these communities were isolationist, or interactive. Of ten or more species of oxyurids found in *T. graeca*, the intestinal distributions of eight well-described species were examined. On first examination, these showed high levels of spatial overlap along the length of the anterior hindgut. Further sampling, however, in which radial, as well as longitudinal distribution, was examined indicated that those species most similar in their longitudinal distributions showed little overlap in their radial distributions. Schad concluded that differences in radial and longitudinal distribution were enough to account for these species’ coexistence in single hosts. Differing trophic preferences between two of the species showing the most overlap were cited as further evidence of
niche diversification, and differing buccal structures among other species were assumed also to indicate differences in trophic preference, and resulting niche separation.

Hominick and Davey (1972a, b, 1973, 1975), using methods of analysis very similar to Schad’s (1963), looked for evidence of evolved niche segregation between two of the species of pinworm parasitizing the hindgut of P. americana: H. diesingi; and L. appendiculatum. They found evidence of longitudinal and radial segregation within the hindgut, with L. appendiculatum taking the anterior-most position, as well as the most central. H. diesingi, in contrast, was found slightly further back in the hindgut, and closer to the gut wall. These preferences, based on evidence from hosts infected with only one species, or with both species, were felt to represent the species’ fundamental niches, and therefore represented evidence of evolved niche segregation, rather than ecologically-enforced segregation. Furthermore, each species showed differing trophic preferences; L. appendiculatum consumed a diet composed of both large and small particles, while H. diesingi only consumed small particles, thus, according to Hominick and Davey, demonstrating even greater total niche segregation, and easily explaining their coexistence.

Noble (1991), and Adamson and Noble (1992, 1993) examined a similar community to that of Hominick and Davey’s, with the addition of one (or possibly two) more species, T. bulhoesi (based on the existence of two morphologically-distinct male types, a second species of Thelastoma, T. periplaneticola, may be present. However, there are no discernible differences among the females found, so it is also possible that there are two male morphs within T. bulhoesi). The studies done by Adamson and Noble attempted to replicate the findings of Hominick and Davey regarding evidence of
spatial and trophic segregation, but were unsuccessful in both. While evidence of a certain amount of longitudinal separation was found (Adamson and Noble, 1992) in coincident infections, it was equivocal at best. Median longitudinal position for both *L. appendiculatum* and *H. diesingi* was anterior to that of *T. bulhoesi*, but *T. bulhoesi* was nevertheless present in all positions in the inhabited portion of the hindgut due to its greater intensity. The median positions of *L. appendiculatum* and *H. diesingi* did not differ. Longitudinal overlap, therefore, was complete, with *T. bulhoesi* being less specialized with regard to position than the other two species. Adamson and Noble were unable to duplicate Hominick and Davey’s (1973) technique for assessing radial distribution, and could reach no conclusion in this regard. They were also unable to detect trophic differences among the species based on examination of gut contents using either optical, or scanning electron microscopy, and so could neither confirm nor refute Hominick and Davey’s earlier finding. Adamson and Noble did, however, make the point that, if *L. appendiculatum*’s diet does consist of both large and small particles, and *H. diesingi*’s consists of small particles, *H. diesingi*’s diet would then constitute a subset of *L. appendiculatum*’s, and would not be considered as evidence of trophic segregation.

Adamson and Noble (1992) found that the three species were not distributed independently among hosts. *T. bulhoesi* and *L. appendiculatum* were found together less often than expected, and alone more often, given their prevalences, while both were found more often than expected with *H. diesingi*. Furthermore, intensities of *L. appendiculatum* and *T. bulhoesi* were negatively correlated, while intensity of *H. diesingi* was positively correlated with both species. Competitive interactions
among the species, then, would seem to be confined to those between

*L. appendiculatum* and *T. bulhoesi*.

Adamson and Noble (1993) reported a consistent order of numerical dominance among the three species. Mean intensity for *T. bulhoesi* was higher than that of *H. diesingi*, with *L. appendiculatum* being least intense. Furthermore, their data indicated that this same order of dominance applied to the species’ prevalences. While the former seems plausible, the latter does not. A consistent order of dominance in intensity may imply a consistent superiority in some trait expressed by a species within hosts – superior ability to obtain a mutually-necessary resource, for example. Or, it may be an indication that hosts may have different carrying capacities for each species, and that a given species’ intensity of infection is established independently of other species that may also be present. In order to consistently dominate a host population in terms of prevalence, however, a parasite species would have to possess some trait that made it more likely to infect hosts than other species. All three species are acquired by hosts in the same manner, through ingestion of eggs contained in contaminated feces. Even if one species were to develop such a trait, an attractant pheromone for example, it would not take many lucky coinfections with one, or both, of the other species for these to become hitchhikers on the first species’ pheromonic efforts.
CHAPTER 1: PATTERNS IN COMMUNITY ORGANIZATION.

Introduction.

Laboratory colonies of *Periplaneta americana*, the American cockroach, are commonly infected with combinations of the pinworms *Leidynema appendiculatum*, *Thelastoma bulhoesi*, and *Hammerschmidtiiella diesingi*. Worms inhabit the hindgut of the cockroach, where they are presumed to feed on resident bacteria.

The following study looks for evidence of interspecific interactions in the infracommunities of these species in three separate host colonies. Five host colonies have been used; three of these contain all three species of pinworm, while the remaining two contain only one species, *L. appendiculatum* in one, *T. bulhoesi* in the other.

Adamson and Noble (1992) suggested that relative prevalence among species in a mixed-infection colony is stable. Using a cross-sectional sample of the three mixed-infection colonies rather than following one community over time, and sampling at fixed intervals, as Adamson and Noble (1993) did, I have looked for evidence of stability in a species’ prevalence. I used the cross-sectional sample rather than sampling at repeated intervals as this latter procedure could disrupt transmission patterns, perhaps affecting the outcome. If relative prevalence is stable, then prevalence for each species from one mixed-infection colony to the next should be approximately the same. For this to be the case, each species would have to have a fairly constant transmission rate, implying that the presence or absence of another species within a host is of no consequence to a species’ chances of successfully
establishing in that host. If, on the other hand, relative prevalence is not stable, each species should have a different prevalence in each colony.

Pinworms are transmitted directly, via the fecal/oral route. Females release their eggs into the lumen of the host intestine to be carried along with other gut contents “downstream,” until they are finally released into the host environment contained in a fecal pellet. Packaging of eggs in this manner has implications for both infrapopulation, and infracommunity structure, as well as the likelihood of competitive interactions among worms. Pellets deposited by a host infected with more than one species of worm are expected to contain a mix of eggs from those species. Hosts live 1.5 to 2 years (Roth, 1982), with that time approximately equally divided between the maturation period, and adulthood. Female pinworms, on the other hand, begin producing eggs at approximately 3 to four weeks of age. Provided contact with infective stages is frequent enough, as the number of multiply-infected hosts increases, hosts should encounter, with increasing frequency, fecal pellets containing multiple species, and, after only a few generations of worms, most hosts should harbour all three species. Once one or more hosts harbour, for example, T. bulhoesi and L. appendiculatum, and others harbour one of these along with H. diesingi, it will not be long before hosts begin to encounter fecal pellets from each of these infracommunities. If, during establishment or larval development, each species succeeds or fails independently of the others, then most hosts should harbour all three species of pinworm, and all three species should have prevalences in these mixed-infection colonies similar to those they would have in isolation. To test this, comparisons are
made between the prevalence of *T. bulhoesi* and *L. appendiculatum* in the mixed colonies, and their prevalence in colonies harbouring only one or the other species.

I have used the multiple-infection colonies, plus the single-infection colonies, to examine the possibility that mean intensity of infection is a constant trait of a species. If species were to have approximately the same mean intensity in each mixed-infection colony this would suggest that their populations were being regulated by some means. These may be host-generated, or due to intra-, or interspecific interactions. Using analysis of variance, I compare the mean intensities of each species between colonies. For *L. appendiculatum* and *T. bulhoesi*, single-infection colonies of each of these species allow an examination of the possible effects of interspecific competition on this trait by comparing their mean intensity in these colonies to that in the mixed-infection colonies. Approximately equal mean intensities in all four colonies for either of these species would suggest that interspecific influences were unimportant in regulating that species' population size. Consistency of population size would then have to be ascribed to either host-generated factors or intraspecific factors. On the other hand, a significant difference in mean intensity between the mixed colonies and a species' single-infection colony would be indicative of interspecific interaction.

As a further test for interspecific interactions, I have used presence/absence data in a 2x2x2 contingency table to test for independence of distribution of each species among hosts. If species are independent of each other, they should be found in single, double, and triple infections in proportion to their different prevalences. Interactions should be detectable in the form of a species being found alone, or in combination with one or another of the other species more, or less often than predicted by their individual
prevalences. This test will detect both negative, and positive interactions between species (as well as no interaction), both of which are of interest. Given the similarity of the species’ life histories, negative interactions are expected, but similarity, at least in some aspects of life history, could be based on a positive association of one species with another.

Finally, there is disagreement between Noble (1991), and Hominick and Davey (1972a) regarding the success of *L. appendiculatum* and *H. diesingi* in young hosts compared to that in mature hosts; the former study found *L. appendiculatum* to be more prevalent than *H. diesingi* in young hosts, while the order was reversed in mature hosts, whereas the latter study found the opposite result. Each sample of 72 hosts from each colony in the following study has been subdivided into early, mid, and late instar, and adult hosts in order to address this question of differential host use based on host developmental stage. Obviously, should there prove to be differences among the species in terms of host use based on host age, this would represent a means of partitioning the resource represented by the host colony, and thereby facilitate coexistence among the species.

**MATERIALS AND METHODS.**

Three colonies of cockroaches (*Periplaneta americana*), containing hosts infected with one to three species of pinworm (*Thelastoma bulhoesi, Leidynema appendiculatum, Hammerschmidtiiella diesingi*), were maintained in 80L, plastic garbage cans with food (ground Purina Dog Chow), and water in constant supply. Two of the three mixed colonies have existed at the University of British Columbia for more than 25 years, with regular transfer of cockroaches between them during that time; the
third colony was created using hosts from these colonies in 1993, over a year before this study began. It is thus unlikely that any significant differences existed between roaches in different colonies; a cockroach from colony 1 should be no less susceptible to infection with pinworms, based on its being from colony 1, than a cockroach of equivalent age and sex from either of the other colonies. All of the colonies in the study had been isolated from one another for a minimum of one year at the time of sampling.

Samples of 72 hosts, based on sex and size, were drawn from each colony. Hosts were chosen so that equal numbers of male and female hosts were obtained. Each sample was broken down so that 18 hosts were classified as early-instar, 18 mid-instar, 18 late-instar, and 18 adults (9 male and 9 female). As a measure of host size, the length of the hind tibia was determined for each host, following, with modification, Noble (1991) who used hind femur length. I found the hind tibia easier to measure accurately.

At the same time, similar samples were drawn from a colony infected only with *L. appendiculatum*, and another infected only with *T. bulhoesi*, for a total of 72 hosts each. Each of these samples was classified in the same manner as those from the mixed colonies. These colonies were maintained in the same manner as the mixed colonies. In addition to numbers of females and males per host, larvae were counted in 48 of the *L. appendiculatum* hosts, and 72 of the *T. bulhoesi* hosts. These data were not collected in the mixed colony samples because it proved impossible to reliably distinguish between larvae of the two species.
Mean abundance (total number of females/total cockroaches sampled), mean
intensity (total number females/infected hosts), and prevalence (percent of all hosts
infected) for each species were calculated.

Analysis of variance (ANOVA) was done to determine whether colony, worm
species, host sex, or host stage has any effect on mean intensity. Host sex and stage
were coded under a single variable for this test. Count data were log-transformed prior
to analysis to bring the data closer to a normal distribution.

Chi\(^2\) contingency analysis was performed to check for independence of
pinworm species' distributions among the hosts within each colony, using a 2 x 2 x2
contingency table assessing the presence or absence of each of the 8 possible species
combinations.

Variance to mean ratios (VM) were calculated for females of each of the species
in each colony to give a measure of their degree of aggregation. Aggregation of species
has been shown to be a facilitating factor in species coexistence, reducing the
opportunities for interspecific competitive interactions (Dobson, 1985). While less
commonly used than \(k\), the aggregation parameter of the negative binomial distribution,
VM is more sensitive to changes at the extreme right tail of a species' distribution
(Scott, 1987), and thereby gives a better indication of the presence of hosts with
particularly high infrapopulations. \(k\) is fairly insensitive to these values, and reflects
changes in distribution close to the mean. As it is the most-heavily infected hosts
within which competition can be expected to be most intense, and that will be most
likely to suffer any ill effects of infection, I considered VM to be the more appropriate
statistic.
RESULTS.

Mixed Colonies.

A total of 338 female *T. bulhoesi*, 185 female *L. appendiculatum*, and 118 *H. diesingi* were collected from the 216 hosts (72 hosts per colony) from the mixed-infection colonies.

Prevalence of infection, counting both male and female worms, regardless of species, was 97% for colony 1, 96% for colony 2, and 100% for colony 3, with an overall prevalence of 98%. Considering female worms only, prevalence in colony 1 was 64%, colony 2 was 75%, and colony 3 was 79%; overall prevalence was 73%.

*L. appendiculatum* was the most prevalent species overall, infecting 104 hosts (48%), with *T. bulhoesi* the next most, at 70 hosts (32%), and *H. diesingi* last, at 60 hosts (28%). The prevalence of each species varied between colonies (Figure 1.1). Chi² analysis indicates that the difference in prevalence between colonies of *L. appendiculatum* and *H. diesingi* is statistically significant ($\chi^2 = 20.5$, d.f. = 2, $P < 0.05$, and $\chi^2 = 8.45$, d.f. = 2, $P < 0.05$), while that of *T. bulhoesi* is not ($\chi^2 = 2.07$, d.f. = 2, $P > 0.05$). *L. appendiculatum* was significantly more prevalent in colonies 2 and 3, while *H. diesingi* was significantly more prevalent in colony 3 than in 1 or 2.

Overall prevalence of infection was lower in immature hosts than adults for both *T. bulhoesi*, and *H. diesingi* in each of the mixed-infection colonies ($P < 0.05$), while *L. appendiculatum* showed no such difference ($P > 0.05$) (Table 1.1).
Figure 1.1: Prevalence of females of each species within each of three mixed-infection colonies.

Chi$^2$ goodness of fit tests indicated that females of all species were aggregated in distribution, except in colony 1, wherein the distributions of \textit{L. appendiculatum} and \textit{H. diesingi} were best described as Poisson. Degree of aggregation is indicated by the value of the variance-to-mean ratio (VM). Calculation of VM indicates that \textit{T. bulhoesi} is the most aggregated of the three species, followed by \textit{H. diesingi}, and \textit{L. appendiculatum}, except in colony 1, wherein the order is reversed for \textit{L. appendiculatum} and \textit{H. diesingi} (Table 1.2).
<table>
<thead>
<tr>
<th>Colony</th>
<th><em>T. bulhoesi</em></th>
<th></th>
<th><em>L. appendiculatum</em></th>
<th></th>
<th><em>H. diesingi</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult (n = 54)</td>
<td>Nymph (n = 162)</td>
<td>Adult (n = 54)</td>
<td>Nymph (n = 162)</td>
<td>Adult (n = 54)</td>
<td>Nymph (n = 162)</td>
</tr>
<tr>
<td>1</td>
<td>67 %</td>
<td>30 %</td>
<td>$\chi^2 = 7.79$, d.f. = 1, P &lt; 0.05</td>
<td>33 %</td>
<td>24 %</td>
<td>$\chi^2 = 0.60$, d.f. = 1, P &gt; 0.05</td>
</tr>
<tr>
<td>2</td>
<td>61 %</td>
<td>19 %</td>
<td>$\chi^2 = 11.85$, d.f. = 1, P &lt; 0.05</td>
<td>24 %</td>
<td>57 %</td>
<td>$\chi^2 = 0.08$, d.f. = 1, P &gt; 0.05</td>
</tr>
<tr>
<td>3</td>
<td>67 %</td>
<td>17 %</td>
<td>$\chi^2 = 16.33$, d.f. = 1, P &lt; 0.05</td>
<td>67 %</td>
<td>57 %</td>
<td>$\chi^2 = 0.48$, d.f. = 1, P &gt; 0.05</td>
</tr>
</tbody>
</table>

Table 1.1: Prevalence of females of each pinworm species in adult compared to immature hosts in each mixed-infection colony.
Table 1.2: Values of the variance-to-mean ratio for each species in each of three mixed colonies. ("*" indicates best fit is Poisson, "**" indicates best fit is Negative Binomial.)

<table>
<thead>
<tr>
<th>Species</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T. bulhoesi</strong></td>
<td>8.60</td>
<td>14.6</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>($\chi^2 = 610.0, \text{d.f.} = 71, \ P &lt; 0.05**)</td>
<td>($\chi^2 = 75600, \text{d.f.} = 71, \ P &lt; 0.05**)</td>
<td>($\chi^2 = 766.0, \text{d.f.} = 71, \ P &lt; 0.05**)</td>
</tr>
<tr>
<td><strong>L. appendiculatum</strong></td>
<td>1.61</td>
<td>1.66</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>($\chi^2 = 114.0, \text{d.f.} = 71, \ P &lt; 0.05$)</td>
<td>($\chi^2 = 118.0, \text{d.f.} = 71, \ P &lt; 0.05**$)</td>
<td>($\chi^2 = 122.0, \text{d.f.} = 71, \ P &lt; 0.05**$)</td>
</tr>
<tr>
<td><strong>H. diesingi</strong></td>
<td>1.30</td>
<td>3.42</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td>($\chi^2 = 92.1, \text{d.f.} = 71, \ P &lt; 0.05$)</td>
<td>($\chi^2 = 243, \text{d.f.} = 71, \ P &lt; 0.05**$)</td>
<td>($\chi^2 = 265.0, \text{d.f.} = 71, \ P &lt; 0.05**$)</td>
</tr>
</tbody>
</table>

A 2x2x2 contingency table indicates that species were not independently distributed among hosts in any of the mixed colonies (colony 1: $\chi^2 = 11.43, \text{df} = 4; \ P < 0.05$; colony 2: $\chi^2 = 6.68, \text{df} = 4, \ P < 0.25$; colony 3: $\chi^2 = 10.69, \text{df} = 4, \ P < 0.05$).

Though the overall effect was statistically significant in only two of three colonies, the pattern was the same in each. Sample sizes for each species combination within colonies were too small to allow separate tests of each combination, however *L. appendiculatum* and *T. bulhoesi* were paired less often than expected in all three colonies. *H. diesingi*, on the other hand, was found alone less often than expected, and in common with one or both of the other species more often than expected. At the same time, hosts infected with all three species were more common than expected in both colonies 1 and 3. In none of the colonies was the number of uninfected hosts different than expected based on the three species’ prevalences.

These patterns in prevalence are also seen in the distribution of female worms of each species among hosts. One third of *T. bulhoesi*’s, and nearly one half of *L. appendiculatum*’s total populations (i.e. summed over the samples from all three
mixed-infection colonies) were found in hosts in which each was the only species present. The next largest proportions of their populations, 31% and 26% respectively, were found in hosts shared only with *H. diesingi*. In contrast, only 10% of *H. diesingi*’s total population was found in single-infection hosts, with the remaining 90% split fairly evenly among hosts shared with either, or both, of *T. bulhoesi*, and *L. appendiculatum*.

Mean tibia length of hosts harbouring *L. appendiculatum* alone was less than that of hosts harbouring either *T. bulhoesi* or *H. diesingi* alone, or any combination of the three species (Figure 1.2). This was true regardless of host sex; male and female hosts parasitized by *L. appendiculatum* alone were smaller than those harbouring other species, or combinations of species.

Figure 1.2: Infracornmunity composition and its relation to host size, using hind tibia length as an indicator of host size.
There is no statistically significant difference in intensity of infection for any of the species between the mixed colonies. However, mean intensity of infection did vary significantly between the species. *T. bulhoesi* had the highest mean intensity of infection in each of the three mixed colonies (Figure 1.3), followed by *H. diesingi* and *L. appendiculatum*. The difference was statistically significant in 2 of the three colonies (colony 1, ANOVA, \( F = 10.8, \text{df} = 2, P < 0.01 \); colony 2, \( F = 4.88, \text{df} = 2, P < 0.05 \) ) In colony 3, the same basic pattern is continued, though the differences between the species are not significantly significant (ANOVA, \( F = 2.68, \text{df} = 2, P > 0.05 \) ).

![Figure 1.3: Mean intensity of infection for females of each species in each mixed colony.](image)

Each species has a characteristic pattern of host exploitation (Figure 1.4). Adult hosts tend to harbour the largest populations of worms, particularly adult female hosts. This effect is particularly strong in the case of *T. bulhoesi*, whose infrapopulations in these hosts are much larger than in other host types, and than the other two species' in any host type in all mixed-infection colonies.
Figure 1.4: Mean female intensity by host stage and sex in Colonies 1, 2 and 3.
Single-infection Colonies.

Prevalence of infection with female worms in single-infection colonies infected with either *T. bulhoesi* or *L. appendiculatum* alone, was 67% and 88% respectively. There was no significant difference in mean size of the roaches sampled from each colony when infection status was ignored. However, hosts infected with *L. appendiculatum* in isolation were smaller than hosts infected with *T. bulhoesi* in isolation [(8.28 mm (s.d. = 2.68) compared to 9.62 mm (s.d. = 2.66), t = 2.62, df = 109, P < 0.05)]. As in mixed colonies, prevalence of infection was higher among adult hosts than among juveniles in the colony in which *T. bulhoesi* was the only species present (Fisher’s Exact test, P < 0.05), while prevalence of infection was fairly evenly distributed among adults and juveniles in the colony in which *L. appendiculatum* was the only species present (Fisher’s Exact test, P > 0.05).

Host size was a significant predictor of species’ abundance for both species. The model for *L. appendiculatum* is:

\[ \ln (\text{females} + 0.5) = -0.49 + 0.20(\text{tibia}); (r^2 = 0.38, P < 0.01), \]

and for *T. bulhoesi* is:

\[ \ln (\text{females} + 0.5) = -1.20 + 0.22(\text{tibia}); (r^2 = 0.48, P < 0.01). \]

These slopes are equivalent (ANCOVA, F = 0.21, df = 1, P > 0.5). There is, however, a significant difference in the intercepts (*t*-test: \( t = 5.09, df = 141, P < 0.05 \)). *T. bulhoesi* ‘s being the lower, indicating that *T. bulhoesi* begins infecting hosts later in their lives than *L. appendiculatum*. 
Worms were distributed among hosts in an aggregated fashion, with *L. appendiculatum* females being more aggregated (VM = 18.13) than *T. bulhoesi* (VM = 6.58).

There was no difference in prevalence for either species based on host sex (*T. bulhoesi* $\chi^2 = 0.11$, df = 1, $P = 0.93$; *L. appendiculatum* $\chi^2 = 0.32$, df = 1, $P = 1.00$; both tests continuity-corrected). However, intensity of *T. bulhoesi* was significantly higher in female hosts, (5.92, s.d. = 5.96, compared to male hosts, 2.38, s.d. = 1.44; independent samples $t$-test, $t = -2.69$, df = 37.43, $P < 0.05$). *L. appendiculatum* showed no significant difference in intensity between host sexes.

Mean intensity of larvae in the *L. appendiculatum* colony was positively related to increasing numbers of female adults ($P < 0.05$, $r^2 = 0.29$). Host size had a positive, though not significant, effect. In the *T. bulhoesi* colony, increasing host size had a significant effect on the number of larvae present ($P < 0.05$, $r^2 = 0.17$). Number of adult females present was also positively, though not significantly, associated with increased numbers of larvae.

Data from single-infection colonies, when compared to that from mixed colonies, indicate that *L. appendiculatum* suffers substantial reductions in both prevalence (88% in isolation compared to 26%, 58% and 60% in each of the mixed colonies), and intensity in the mixed colonies, as well as a reduction in aggregation. While the decrease in prevalence was not statistically significant (2-sample $t$-test, $t = 2.0$, df = 2, $P > 0.05$), the change in mean intensity was (2-sample $t$-test, $t = -13.17$, df = 2, $P < 0.05$). *T. bulhoesi* similarly has its prevalence reduced (67% in isolation compared to 39% in colony 1, and 29% in colonies 2 and 3), but intensity remains the same, regardless of colony (Figure...
1.4). In *T. bulhoesi*’s case, the reduction in prevalence was statistically significant (2-sample t-test, \( t = 5.03, P < 0.05 \)).

![Figure 1.1: Comparison of intensity of infection for *T. bulhoesi* and *L. appendiculatum* in single- (T: *T. bulhoesi*; L: *L. appendiculatum*), compared to mixed-infection colonies(TM: *T. bulhoesi*, mixed colony; LM: *L. appendiculatum*, mixed colony).](image)
DISCUSSION.

The present study extends earlier studies by Hominick and Davey (1972a), Noble (1991), and Adamson and Noble (1992, 1993). Where their studies used a single mixed-infection colony, and, in the studies by Noble (1991), and Adamson and Noble (1992, 1993) a single *L. appendiculatum* colony, I have examined three mixed-infection colonies and, in addition, compared data from these with data from both a *L. appendiculatum* colony, and a *T. bulhoesi* colony. Addition of these colonies has lead to a clearer picture of the interactions between at least these two species, as well as clarifying which properties of infrapopulations may be said to be consistent species traits, and which are simply random effects.

I found that prevalence varies for all three species among the mixed colonies. Adamson and Noble (1992) found *T. bulhoesi* was the most prevalent species in 9 of 14 samples collected over a period of 2.5 years, while *H. diesingi* had the highest prevalence in the remaining 5. *L. appendiculatum* was the least prevalent in all. In their conclusions they imply that this pattern, *T. bulhoesi > H. diesingi > L. appendiculatum*, is a stable one. However, *T. bulhoesi* was the most prevalent species in only one of three mixed colonies in the present study, *L. appendiculatum* being the most prevalent in the other two. In addition, prevalence of all three species varied among colonies, though in *T. bulhoesi*'s case the differences were not statistically significant.

Successful infection of hosts through coprophagy is a product of three factors—the host's decision to consume part or all of a fecal pellet, the presence or absence of infective eggs within that pellet, and the ability of the parasite in question to establish within the host. This latter factor depends on other factors, including a larva's ability to
hatch successfully, feed, and resist the occasional posteriad motion of gut contents, as well as the availability of resources within the potential host, which may, in turn, be affected by factors intrinsic to the host, and the presence and number of previously-established worms. Prevalence will then depend on the proportional distribution of each species’ eggs among fecal pellets spread throughout the host environment. Gradual changes in prevalence will come about due to "errors" in sampling among these fecal pellets by hosts that can result in a higher proportion of new infections with a particular species than would be expected from that species eggs’ frequency among fecal pellets. Prevalence is thus more likely to be a property that changes slowly over time, barring sudden and drastic changes in the size of the host population. Adamson and Noble’s (1992) study may be a demonstration of how slow this process can be.

While prevalence, and therefore abundance, of a given species varied from colony to colony among the mixed-infection colonies, mean intensity of infection did not. Within these colonies, mean intensity seems to be roughly constant for each species. In all three mixed-infection colonies the order of numerical dominance was *T. bulhoesi* > *H. diesingi* > *L. appendiculatum*, and none showed significant variation in intensity among colonies. This finding accords with that of Adamson and Noble (1992). *T. bulhoesi*’s intensity levels were also approximately equal between mixed and single-infection colonies, however *L. appendiculatum* suffered drastic reduction in intensity between single-infection, and mixed colonies.

In mixed-infection colonies, *L. appendiculatum* and *T. bulhoesi* were found paired within hosts, without *H. diesingi*, less often than expected, given their prevalences. On the other hand, *H. diesingi* was found with one, or both, of these species much more often
than expected in each colony, and, in addition, its intensity was shown to be positively associated with increasing numbers of the other two species. Only 2% of its population was found in hosts in which it was the only species present, while the remaining 98% was divided fairly evenly among the combinations possible with the other two species. In fact, the greatest portion of *H. diesigi*’s population, 36%, was found in hosts carrying all three species. This contrasts with 14% of *L. appendiculatum*’s population, and 18% of *T. bulhoesi*’s in such hosts. These proportions for *L. appendiculatum* and *T. bulhoesi* were approximately equal to the proportions of these species in hosts harbouring only these two, 16% and 19%, respectively. Furthermore, large proportions of the populations of both of these species were found in hosts they shared only with *H. diesigi* (26%, and 31% respectively).

Connor and Adamson (1998) showed that all three species’ diets overlap substantially, but that *H. diesigi* appears to consume less food than the other two. This may be an artifact of the means of modelling food items. Fluorescent latex beads were used, of sizes presumed to approximate bacteria inhabiting the host gut (1 – 10μm); these beads may not accurately model *H. diesigi*’s food source. Hominick and Davey (1973) used light microscopy to directly examine the gut contents of female *L. appendiculatum* and *H. diesigi*. They observed that the gut of *L. appendiculatum* contained both fine and coarse particles, while *H. diesigi* contained only fine material. *H. diesigi* may rely for at least part of its diet on pre-digested material excreted by individuals of the other two species. While the data above indicate that *H. diesigi* can survive alone in a host, its greater numbers in hosts harbouring one, or both, of the other species suggests that their
presence either makes it easier for \textit{H. diesingi} individuals to establish in a host, or to thrive.

\textit{T. bulhoesi} and \textit{H. diesingi} were more prevalent in adult hosts than in nymphs, while \textit{L. appendiculatum} showed no difference. In addition, \textit{T. bulhoesi}'s intensity among adult female hosts was much higher than among nymphs of either sex. \textit{L. appendiculatum} and \textit{H. diesingi} also showed higher intensities of infection among adults, but not to the same extent as \textit{T. bulhoesi}. That all three species did better in these hosts than others may indicate an increase in carrying capacity in these hosts. That \textit{T. bulhoesi} did markedly better in adult females than the other species, and than it did in any other type of host, suggests that there is something more than just an increase in carrying capacity at work. Hormonal differences between adult females and other host-types may be a factor. Hominick and Davey (1972b) examined this hypothesis using female hosts, manipulating their hormonal balance through surgery, but were unable to reach any conclusion on the matter.

Contrary to the findings of Hominick and Davey (1972a), \textit{L. appendiculatum} was better able to exploit young hosts than either of the other species, within both single-infection, and mixed-infection colonies. Forty-four percent of \textit{L. appendiculatum}'s total population in the mixed-infection communities is found within hosts in which \textit{L. appendiculatum} is the only species present, and these hosts are significantly smaller than hosts harbouring any other form of infracommmunity (Figure 1). The mechanism by which \textit{L. appendiculatum} achieves greater prevalence in young hosts has yet to be clearly demonstrated. However, \textit{L. appendiculatum} may exclude other species by outgrowing them, and thus controlling the available space within the colon, and the resources therein.
*L. appendiculatum* matures in approximately 12-18 days at 25°C (Noble, 1991; Connor, unpublished data), while *T. bulhoesi* takes approximately 32 days, also at 25°C (McCallister and Schmidt, 1983). This much more rapid growth rate could allow *L. appendiculatum* to completely dominate the spatial aspect of the gut of the smallest hosts, which was often barely larger than a single female worm. *L. appendiculatum*'s failure to dominate among older, larger hosts in the mixed-infection colonies may be due to the gradual diminishment in importance of spatial dominance as the host gut becomes large enough to accommodate many worms.

The large difference in infection intensity of *L. appendiculatum* between mixed and single-infection colonies appears to be due primarily to the presence of *T. bulhoesi*. These two species are seldom found in the same host individual, and when they are, it is most often in adult hosts. Even in adult hosts, however, there is evidence of a negative relationship between the two. There is no evidence of a similar negative interaction between *L. appendiculatum* and *H. diesingi*. In light of the failure of *L. appendiculatum* to achieve intensities of infection in adult hosts in the mixed colonies that approach its intensity in similar hosts in its own colony, success in young hosts may be key to *L. appendiculatum*'s continued presence in the mixed colonies, and represent a form of competitively-enforced niche segregation. It is interesting to note that in colonies 2 and 3, in both of which *L. appendiculatum*'s prevalence is higher than in colony 1, the difference resulting from its greater prevalence among immature hosts; its prevalence in mature hosts remained essentially unchanged. *T. bulhoesi* in these two colonies shows the opposite pattern, with a lower prevalence among immature hosts, and no real change.
in prevalence among mature hosts. It appears, then, that *T. bulhoesi* may have a consistent advantage in contests for mature hosts.

Perhaps the strongest indication that some sort of competitive interactions are taking place within the mixed colonies comes from the finding that, despite 2 of 3 of these mixed-infection colonies having existed for over 25 years, no species was found at a prevalence above 60%. Both *L. appendiculatum* and *T. bulhoesi* show significantly lower prevalences in these colonies than in their single-infection colonies, where prevalence of *T. bulhoesi* females was 67% and that of *L. appendiculatum* was 88%, indicating that each is being denied use of some hosts in the mixed-infection colonies. Furthermore, given the mode of transmission for these helminths — eggs packaged in fecal pellets — one would expect a feedback effect such that once one host was infected with two or more species, the number of similarly infected hosts would rise as hosts encountered what would be, in effect, pre-packaged communities that fecal pellets from such a host could be expected to contain. If success or failure for a given worm in a host were independent of the presence or absence of other species, I would expect to see, among adult hosts at least, many more three species communities.

Within all colonies, pinworms were aggregated in distribution. In keeping with the constancy each species displayed with regard to mean intensity, aggregation levels, too, seem to be fairly constant for each species among the mixed colonies. Aggregation of parasites may be due to a number of factors including differences in host susceptibility to infection, whether behavioural or immunological, and clumping in the distribution of infective stages within the host environment (Anderson and Gordon, 1982; Shaw and Dobson, 1995). Data in chapter 4 indicate that this latter possibility may account for
much of the clumping of adults. Immunological differences are unlikely to play a role in aggregation of these species, for two reasons. First, cockroaches have fairly simple immune systems, relying primarily on mechanical exclusion of parasites by their external cuticle, and the peritrophic membrane lining much of the gut (Dunn, 1986), which, of course, would have no effect on inhabitants of the lumen of the hindgut, which is not protected by the peritrophic membrane. Secondly, even the more-complex immune responses of vertebrates have a difficult time coping with lumen-dwelling intestinal parasites such as helminths (Playfair, 1995), that are simply too large to be vulnerable to the immune system’s defense mechanisms.

*L. appendiculatum* is the least aggregated of the three overall in the mixed-infection colonies, and is much less aggregated than it is within its single-infection colony. A reduced level of aggregation may result from within-host competitive interactions that eliminate a species’ chances of achieving high infection intensities, or of parasite intensity-dependent pathogenic effects that increase host mortality and eliminate heavily-infected hosts (Anderson and Gordon, 1982). In mixed-infection colonies either of these mechanisms may be playing a role in reducing *L. appendiculatum*’s level of aggregation. Multiply-infected hosts are also the most heavily infected, particularly those infected with *T. bulhoesi*, and one or both of the other species. Such heavily infected hosts may suffer a degree of parasite-induced mortality not experienced by more lightly infected hosts, though there is no evidence that these pinworms are, in fact, pathogenic at any level.

The continued existence of each of these three species of pinworm in mixed-infection colonies, then, is likely due to a combination of three factors. First, none has a
consistent competitive advantage in all host stages, or either host sex, over any of the others. Though *L. appendiculatum* may be able to exclude the others from small hosts, perhaps through its more-rapid growth rate, its advantage apparently fades as hosts increase in size. Further, all species are aggregated in their distribution among hosts, a pattern that tends to reduce opportunities for competitive interactions among species, thereby allowing otherwise intense competitors to coexist at the component community level (Dobson, 1985). Lastly, there is a continuous supply of new, uninhabited “islands” being produced, allowing a species the opportunity to colonize and exploit a competition-free environment.

There may also be a component of facilitation at work, at least in the case of *H. diesingi*. As mentioned above, the majority of individuals of this species are found in hosts harbouring at least one of the other two species, with an insignificant proportion found in hosts harbouring only *H. diesingi*. The mechanism by which this facilitation, if such it is, might be mediated is unknown.
CHAPTER 2: DIFFERENTIAL EFFECTS OF HOST MOULT ON LARVAL SURVIVAL OF TWO SPECIES OF INTESTINAL HELMINTHS IN THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA*.

**Introduction.**

Colonies of *Periplaneta americana* maintained at the University of British Columbia contain two-, to three hundred cockroaches each, with an average prevalence of infection with gravid females of one or more pinworm species of 72% (Chapter 1). Hosts, therefore, live under a substantial “rain” of pinworm eggs at all times, suggesting that the probability of encountering an infective stage during any given instance of coprophagy is very nearly 1. And yet, rather than picking up an infection early on in host development, prevalence of infection increases with host age, at a markedly different rate for *L. appendiculatum* compared to *T. bulhoesi*, and *H. diesingi* (Noble, 1991; Chapter 1).

*L. appendiculatum* reaches just over 60% prevalence, its maximum in these mixed-infection colonies, at a time in host development when *T. bulhoesi* and *H. diesingi* infect only approximately 20% of hosts. It is only in the later juvenile stages, and in adults, that these latter two species equal *L. appendiculatum*’s prevalence. Furthermore, at the transition from juvenile to adult host, *L. appendiculatum* actually experiences a reduction in prevalence. In addition to mixed-infection colonies, I also have colonies of hosts singly-infected with either *L. appendiculatum*, or *T. bulhoesi*, and this same pattern holds, suggesting that this difference in utilization of host stages is a trait of each species (Chapter 1) and not due to competition between the species in coincidental infections.

Cockroaches go through a number of moults as they mature, with the time interval between moults gradually increasing from approximately one week to forty days (Willis
et al., 1958). Moulting, of course, allows the cockroach to increase in size by shedding the restraining exterior cuticle. The cuticle is ectodermally-derived, as are the linings of the foregut, hindgut and trachea, which are also shed when the cockroach moults.

In the hindgut, old tissue separates from the new tissue beneath, and begins to shrink, rather like a slowly-deflating balloon. Constrictions at the ileal, and rectal ends serve to close the shrinking tube. Lumenal contents undergo changes in osmotic pressure during this process which have been shown to result in changes in hydrostatic pressure within the worms (Lee, 1960). Worms rely on their hydrostatic skeleton for both mobility and proper feeding. Significant changes in hydrostatic pressure could impair their ability to resist expulsion, or lead to starvation. The old gut lining is expelled when the host defecates. Though some disintegration of the tissue occurs, anything trapped within the old gut is at risk of being expelled when the old gut passes out of the cockroach’s body.

The present study examines a possible mechanism accounting for the differences between *L. appendiculatum*, and *T. bulhoesi* in prevalence in young hosts. The current study asks whether or not *L. appendiculatum*’s greater prevalence in young hosts is due to a greater resistance on its part to expulsion of larvae from the host gut during moulting by comparing the effects of host moult on larvae of *L. appendiculatum* and *T. bulhoesi*. In conducting the study, host selection was not restricted to any particular stage, unlike Hominick and Davey’s (1972a) study in which only final instar, and adult hosts were compared. This step was taken in light of both my own data (Chapter 1), and that of Noble (1991), suggesting that *L. appendiculatum*’s advantage lies in younger hosts than those examined by Hominick and Davey (1972a).
Lee (1960), and Noble (1991) conducted studies of the effects of moulting on adult female pinworms, and found nothing to indicate that it had any negative effects on these worms’ survival. Neither, however, looked at the larval pinworm stage, which, due to its much smaller size, may be more susceptible to either the changes in osmotic pressure within the host gut associated with the moulting process, or to entrapment within the collapsing tube of moulted gut tissue. For this reason the focus of this study is on differences in the mean number of larvae for each species before, and after host moult.

MATERIALS AND METHODS.

Cockroaches (Periplaneta americana) were maintained in 80l, plastic garbage cans, and fed a diet of ground Purina Dog Chow, and water, ad libitum.

Freshly-moulted cockroaches (recognizable by their bright white coloration) from either of two colonies, one infected with L. appendiculatum, the other with T. bulhoesi, were isolated in 8x6 inch ice cream buckets from which the bottoms had been removed and replaced with wire screen (0.3 x 0.3 mm mesh). The wire screen allowed fecal pellets to pass through to collecting trays below, thus preventing further infection of the isolated roach. At the same time a slightly smaller, fully-tanned roach was selected as a match to the freshly-moulted roach. The intent was to select a roach from the instar previous to the one the freshly-moulted roach had just left. Hosts were isolated for a period of twenty-four hours, to allow passing of the shed hindgut lining. No gut remnants were found within any of the moulted hosts.

Hosts were dissected, their hindguts removed and placed in 0.075% saline. These were then teased apart using dissecting pins. Any worms within were classified as adult
female, male, or larva, and counted. Only singly-infected hosts were used due to the difficulty of distinguishing the larvae of the two species.

A paired-samples t-test was performed, using log-transformed data (\( \ln(y + 0.5) \)), to test whether or not there was a significant difference in the number of larvae between freshly-moulted, and non-moulted roaches for each of the worm species. For completeness, data were also gathered on the number of adult female worms, and adult male worms in moulted vs. non-moulted hosts.

RESULTS.

Freshly-moulted hosts of \( L. \) appendiculatum harbored significantly fewer larvae than hosts that had not recently moulted (\( n = 47 \) pairs, Not-moulted, untransformed mean = 4.23, \( s.d = 4.57 \); Moulted, untransformed mean = 2.23, \( s.d = 2.42 \); \( t = 2.94, df = 46, P < 0.05 \)). There was no significant difference in number of larvae harbored by moulted vs. non-moulted hosts of \( T. \) bulhoesi (\( n=23 \) pairs, “Non-moulted” untransformed mean = 5.17 larvae, \( s.d = 8.03 \), compared to “Moulted” untransformed mean = 4.04 larvae, \( s.d. = 5.87 \); \( t = -0.25, df = 22, P > 0.05 \)) (Figure 2.1). Figures 2.2 and 2.3 show the equivalent data for adult female worms, and males of each species. In neither of these cases was there a significant difference in number of individuals present.
Figure 2.1: Difference in larval abundance before (No) and after (Yes) host moult for each species.

Figure 2.2: The difference in female abundance before (No) and after (Yes) host moult for each species.

Figure 2.3: The difference in male abundance before (No) and after (Yes) host moult for each species.
DISCUSSION.

The results suggest that host moult may be an important source of juvenile mortality for *L. appendiculatum*, but it does not seem to be significant for *T. bulhoesi*. Mean number of larvae in the “No,” sample for *L. appendiculatum* was twice the mean number in the “Yes,” group (4.23, s.d. = 4.57, compared to 2.23, s.d. = 2.42). The means for these groups in the *T. bulhoesi* colony samples were 4.04, s.d. = 5.87, and 5.17, s.d. = 8.01

A comparison of host use by host developmental stage between *L. appendiculatum*, and *T. bulhoesi* in their respective single-infection colonies, shows that *L. appendiculatum* infects a higher percentage of small hosts than does *T. bulhoesi* (53% compared to 17%; Chapter 1). This pattern holds in all three mixed infection colonies maintained in our laboratory. Within these colonies *L. appendiculatum* is much more prevalent in young hosts than either *T. bulhoesi*, or *H. diesingi*.

Two previous studies have examined the effect of host moult on worm survival with regard to adult worms. Lee (1960) noted that cockroach moulting involves a certain amount of water loss from the hindgut, with a subsequent increase in osmotic pressure within the contents of the gut. He examined pinworms found within the moulted gut lining and found them shrunken and inactive. He then tested the effects of changes in osmotic pressure, *in vitro*, on adult female *H. diesingi* to assess the worms’ likely response to similar changes in osmotic pressure *in vivo*. Pinworms rely on hydrostatic pressure within their pseudocoelom to maintain their shape, and give locomotory muscles something upon which to act. Changes in osmotic pressure within the host gut will lead to changes in the internal hydrostatic pressure of any worms therein. These changes in
hydrostatic pressure could interfere with locomotion, and perhaps feeding. Either of these effects could result in the worm being expelled, either through an inability to move out of the moulted gut lining, or through starvation. Lee found that there were indeed changes in the worms, but that while such changes were potentially detrimental to the worm, the effects were temporary, and not likely to cause the adult females any difficulty in breaking free of the old gut lining as it decayed within the host.

Noble (1991), finding a similar pattern of difference in prevalence based on host stage to the one prompting the current study, tested the hypothesis that host moult might negatively affect adult female numbers, and found that it had no effect on any of the three species in his study.

Neither of these studies examined the potentially more vulnerable larvae. A study by Hominick and Davey (1972a) did address this question with respect to apparent differences in host use, based on host developmental stage, between *L. appendiculatum* and *H. diesingi*, however, contrary to Noble (1991), and to my own data (Chapter 1) they found that *L. appendiculatum* was less successful than *H. diesingi* in immature hosts. This reversal of the pattern detected by Noble (1991) and myself (Chapter 1) could be explained by Hominick and Davey's (1972a) choice of the instar just preceding the final moult to adulthood as their measure of an immature host. Data from both Noble (1991), and myself (Chapter 1) indicate that *L. appendiculatum* declines in prevalence at this point in host development, but these same data also indicate that *L. appendiculatum*’s prevalence in still-earlier stages is higher than that of the other two species, *H. diesingi* and *T. bulhoesi*, in equivalent hosts, and higher than its own prevalence in adult hosts.
Hominick and Davey, however, also concluded that larvae of *L. appendiculatum* were more susceptible to expulsion during host moult than were those of *H. diesingi*.

Larvae of all species are considerably smaller than adult females, suggesting that they might be more vulnerable to the effects of osmotic changes, or to becoming entangled within the moulted gut tissue, and therefore may be more likely to be expelled with the old lining. A similar line of reasoning, however, should apply to adult males of both species, which are also considerably smaller than females. *L. appendiculatum* males, as Figure 2.1c indicates, do seem to suffer a decline in number after a host moults. This decline, though not statistically significant (*P* = 0.09), also suggests that worm size might be playing a role, but neither *T. bulhoesi* larvae, nor *T. bulhoesi* males, suffer as great a loss, despite being similar in size, suggesting that there is some other factor than size at work.

Of the two species, *L. appendiculatum* has the shorter development time, taking approximately 18 days to reach maturity at 29°C (Noble, 1991; Chapter 5), while *T. bulhoesi* takes approximately 32 days under similar conditions (McCallister and Schmidt, 1983). Such rapid growth as that shown by *L. appendiculatum* must require substantial food intake. Perhaps a drop in bacterial populations during, or shortly after, host moult, results in starvation for some of the *L. appendiculatum* larvae. *T. bulhoesi*, with its slower growth rate, and therefore likely lower food requirements, would not be as susceptible to starvation during brief food shortages.

*L. appendiculatum*'s rapid maturation rate may allow it to gain control over the resources available in smaller hosts by crowding out competing species. The greater potential for larval death during brief periods of food shortage is offset by the larger
number of larvae present in the host. Newly-acquired infections are likely composed of larvae from only two or three females, and many are therefore closely related. The loss of a few sister larvae from a host should not impose too great a negative impact on a female’s reproductive success, considering the large number of larvae that never get as far as a new host.

*T. bulhoesi,* on the other hand, may be a victim of its own success at resisting expulsion during moult. From a host’s perspective, intestinal helminths, due to their large size, present a difficult challenge to the immune system, even the complex, adaptive immune systems of vertebrates (Playfair, 1995). For many insects the main line of defense against alimentary tract parasites is the peritrophic membrane lining the midgut, which prevents penetration into their hemocoel from the gut. Obviously, this will have no effect on helminths that inhabit the hindgut, where there is no peritrophic membrane. In addition, as pinworms are non-invasive they may completely escape detection by the immune system. There is, then, little the host can do to control these intestinal helminth populations. As egg-free fecal pellets are rare in these colonies (Chapter 4), a host is likely to further infect itself each time it consumes a fecal pellet, and if gut bacterial densities are high enough to be effectively non-limiting, there is nothing, in principle, to limit the number of worms per host save the host's rate of consumption of fecal pellets, and the worms’ intrinsic death rates, without a mechanism such as clearance during moulting. The susceptibility of *L. appendiculatum* to expulsion during the host moult could, coincidentally, result in hosts keeping worm numbers, at least for this species, below a potentially pathogenic level; this would be particularly important in small hosts.
*T. bulhoesi*’s resistance to expulsion may result in worm burdens sufficiently high in smaller hosts to prove fatal, and thus account for its low prevalence in these hosts.
CHAPTER 3: TROPHIC NICHE OVERLAP AMONG THREE SPECIES OF PINWORM PARASITIC IN THE HINDGUT OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA.

Introduction.

_Hammerschmidtia diesingi, Leidynema appendiculatum, and Thelastoma bulhoesi_ are common pinworm (Nematoda: Oxyurida) parasites of the anterior region of the hindgut of the American cockroach, _Periplaneta americana_; their diet consists of the bacterial flora within the host's hindgut. Individual hosts in laboratory colonies of _P. americana_ often harbor 1, 2, or all 3 of these species. This study asks how much overlap there is among these three species in terms of their trophic niche.

Using light microscopy, Hominick and Davey (1973) concluded that _H. diesingi_ and _L. appendiculatum_ utilized different food resources; the diet of _L. appendiculatum_ consisted of a mix of large and small particles, while that of _H. diesingi_ consisted solely of small particles. However, their conclusions were not supported by any more-precise assessment of the difference. Adamson and Noble (1992) pointed out that, as characterized by Hominick and Davey (1973), _H. diesingi_'s food preferences appeared to be a subset of _L. appendiculatum_'s, and thus the difference in size of food item would not constitute evidence of differentiation of trophic niche. In an unpublished study they attempted to duplicate Hominick and Davey's (1973) results using scanning, and transmission electron microscopy to examine gut contents of the worms, but were unable to distinguish any variation in gut contents. In the present study I used polystyrene beads of known sizes as marker "food" items, and examined the extent of niche overlap among
the 3 species of pinworm in terms of particle size, and looked for differences in amount consumed.

MATERIALS AND METHODS.

Cockroaches (Periplaneta americana) were maintained in 80l plastic garbage cans, and fed a diet of ground Purina Dog Chow and water, ad libitum. They were kept at a constant air temperature of 30°C.

Adult female roaches were removed from the colony, placed in individual 100 mm x 15 mm Petri dishes, and held without food for 48 hours. They were fed agar (0.3 g agar:15 ml H₂O) containing fluorescent polystyrene beads (Polysciences Inc., Warrington, Pennsylvania). Beads of 1, 3, 6, and 10 μm were used as these cover the range of common bacterial sizes. The 1 μm and 6 μm beads contained a yellow-green dye that emitted light of 540 nm, and the 3 μm and 10 μm beads contained a red dye that emitted light of 657 nm. These different dyes were used to facilitate discrimination among the different sizes of beads when viewed with the microscope.

Twenty-four hr after being fed, roaches were anesthetized by refrigeration at 4°C for 20 minutes, then dissected. The colon and rectum were transferred to a Syracuse dish containing 0.75% saline. Worms were collected by dissecting the colon, and placing adult female worms in separate dishes for each species. Worms were then fixed in hot glycerol. Only worms from hosts containing all 3 species were collected. Seven hosts were surveyed; 43 T. bulhoesi, 25 L. appendiculatum, and 18 H. diesingi were collected and scored from these hosts.
Fixed worms were placed on slides and examined using a Leitz Dialux 22EB microscope, equipped with the Leitz 3-λ Ploemopak vertical fluorescence illuminator.

Total numbers of each bead size in each worm of each species in a given host were determined and summed to give a total resource-usage figure for each species in that host. These data were used to calculate niche overlap indices, and to look for differences in consumption among the species.

Proportional availability of each size of bead was determined by taking a sample of agar, re-melting it, and counting the differently-sized beads using a haemocytometer. 2 mixtures of agar were used, differing in their proportional bead availability. The first mixture, A1, contained beads in proportions of 46, 16, 28, and 10 percent for sizes 1, 3, 6, and 10 μm, respectively. The second mixture, A2, contained these same sizes in proportions of 75, 18, 5, and 1 percent.

2 niche overlap indices were calculated: Horn’s index-

\[ R_o = \frac{\sum (p_{ij} + p_{ik}) \log (p_{ij} + p_{ik}) - \sum p_{ij} \log p_{ij} - \sum p_{ik} \log p_{ik}}{2 \log 2} \]

and Hurlbert’s-

\[ L = \sum (p_{ij} p_{ik} / a_i) \]

In both indices, \( p_{ij} \) and \( p_{ik} \) are the proportions resource \( i \) is of the total resources utilized by each species \( j \) and \( k \) respectively. In the formula for Hurlbert’s index, \( a_i \) is the proportion of the total resources available represented by resource \( i \) (\( \sum a_i = 1 \)).

Horn’s index varies from 0, no overlap, to 1.0, complete overlap. Hurlbert’s index extends the scope of overlap indices by taking into account the proportional availability of each resource. A value of 0 indicates no overlap, and 1 indicates complete overlap.
with usage of each resource by each species in proportion to that resource's abundance. Values of Hurlbert's index greater than 1 occur when 2 species utilize the same resource to a greater extent than its proportional availability, indicating their convergence on a common resource.

Niche overlap indices were calculated using software available as a supplement to Krebs (1989). Analysis of variance was used to test for differences among the 3 species in quantities of beads consumed. Analysis of variance was calculated using Systat for Windows, v. 5.05.

RESULTS.

*H. diesingi* ingests fewer beads than the other 2 species. This is particularly evident at the 1 μm size (Fig. 3.1). *H. diesingi* differs significantly from the other 2 species in its consumption of 1 μm beads (ANOVA: *H. diesingi* vs. *L. appendiculatum*: \( P < 0.01 \); *H. diesingi* vs. *T. bulhoesi*: \( P = 0.05 \)), and from *L. appendiculatum* in the 3 μm range (\( P < 0.01 \)). Differences in consumption between *L. appendiculatum* and *T. bulhoesi* are not statistically significant. All 3 species use 6 μm particles to the same low extent, and none were found to have consumed 10 μm beads.

Values of niche overlap indices for all species are virtually identical within each of the 2 agar/bead concentrations (Table 3.1). Both Horn’s and Hurlbert’s indices indicate substantial niche overlap among the species. Values of Hurlbert’s index obtained indicate proportional use of particular sizes of particle in excess of their proportional availability, by all three species, preference being shown for the smaller 1 μm and 3 μm beads. Differences in values of Hurlbert’s index between the 2 agars are
presumably due to the differences in the proportional availability of the differently-sized beads.

Figure 3.1: Mean consumption per female per particle size was first computed for each species in each host, these were then averaged to obtain the data shown.

<table>
<thead>
<tr>
<th></th>
<th>Index</th>
<th>H. diesingi/ L. appendiculatum</th>
<th>H. diesingi/ T. bulhoesi</th>
<th>L. appendiculatum/ T. bulhoesi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar 1</td>
<td>Horn</td>
<td>0.97 (+/- 0.02)</td>
<td>0.987 (+/- 0.01)</td>
<td>0.99 (+/- 0.00)</td>
</tr>
<tr>
<td>(N = 3)</td>
<td>Hurlbert</td>
<td>1.61 (+/- 0.04)</td>
<td>1.65 (+/- 0.07)</td>
<td>1.68 (+/- 0.06)</td>
</tr>
<tr>
<td>Agar 2</td>
<td>Horn</td>
<td>0.98 (+/- 0.01)</td>
<td>0.99 (+/- 0.01)</td>
<td>0.98 (+/- 0.01)</td>
</tr>
<tr>
<td>(N = 4)</td>
<td>Hurlbert</td>
<td>1.2 (+/- 0.10)</td>
<td>1.13 (+/- 0.07)</td>
<td>1.23 (+/- 0.11)</td>
</tr>
</tbody>
</table>

Table 3.1: Mean index values for each species pair at two bead concentrations (+/- S.E.).
DISCUSSION.

Each bead size comprised a similar proportion of all 3 species' diets (Figure 3.1), with the 1 and 3 μm sizes making up the largest portion. A study of the intestinal bacteria harbored by *P. americana*, done by Bracke et al. (1979), found that the vast majority were well within this size-range. Anatomical studies of the worms show their buccal cavities have a maximum diameter of less than 10 μm, and that all three species share a similar average body size of approximately 2.5 x 0.22 mm (Chitwood, 1932; Lee, 1958).

Hominick and Davey (1973) detected, through the use of light microscopy, qualitative differences in food items contained in the intestines of specimens of female *L. appendiculatum* and *H. diesingi*. Intestines of specimens of *L. appendiculatum* contained a mixture of coarse and fine particles, whereas those of *H. diesingi* contained only fine particles. They interpreted these observations as evidence of trophic segregation between the 2 species, allowing them to coexist within the same host. As Adamson and Noble (1992) pointed out however, both species consume fine material and, therefore, it seems more reasonable to conclude that *H. diesingi*’s diet is a subset of *L. appendiculatum*’s.

My findings offer some support for Hominick and Davey’s (1973) findings of qualitative differences in the diets of *H. diesingi* and *L. appendiculatum*, though I disagree with their interpretation of these differences. Females of *L. appendiculatum* did contain substantially more large particles than did female *H. diesingi*. *H. diesingi*, however, consumed substantially fewer beads of all sizes, compared to the other 2 species, though the proportion of its diet each size comprised was similar. Larger
particles may have been present in insufficient quantity in specimens of *H. diesingi* examined by Hominick and Davey (1973) to be detectable using light microscopy.

My model only addresses the worms' consumption of spherical particles; it does not examine the possibility that the worms may differ in their ability to handle, for example, long chains or filaments. However, I have not found such material in the ingesta of any of the worms examined. Neither do I consider the possibility that the worms are capable of finer distinctions among food particles than those with which I presented them. Based on studies by Mapes (1965) and Roggen (1973) I have assumed that all three species are indiscriminate feeders, and that any apparent size-discrimination is governed by buccal cavity size, not actual choice. Furthermore, by examining only hosts harboring all 3 species of worm, I have focused on differences in the realized niches of the worms so that differences among species would have been maximized.

I have shown that there is no significant difference between *T. bulhoesi* and *L. appendiculatum* in the number of particles of each size consumed. Adamson and Noble (1992) reported a significant negative interaction between these species, finding that they occurred together less often in single hosts than expected, and that when they were found together there was a negative correlation between their numbers. Data in chapter one indicate that it is among younger, smaller hosts that this conflict is most apparent. A Mantel-Haenzel chi² test for independence of distribution indicates that *L. appendiculatum* and *T. bulhoesi* are not independently distributed with respect to each other over five host size classes (P < 0.01). They are found together in smaller hosts less often than expected given their prevalences. *H. diesingi*, on the other hand, was shown to be unaffected by the presence of either of the other 2 species, and actually co-occurred
more often than expected with both *T. bulhoesi*, and *L. appendiculatum* (Adamson and Noble, 1992). *H. diesingi* consumes very little compared to the other 2 species (Figure 3.1).

The current study suggests that one source of competitive interaction in this system, at least between *L. appendiculatum* and *T. bulhoesi*, may be food. At the same time, it sheds some light on the data from chapter 1 indicating that *H. diesingi* females are actually more numerous in hosts harbouring either of the other two species. The much lower quantities of beads found in the intestine of *H. diesingi* females indicates that they may be feeding on a resource not modeled by the beads. A study of the microbial flora of the cockroach hindgut by Bracke *et al.* found substantial bacterial growth in the form of long filaments attached to the wall of the hindgut. These filaments were on the order of tens of micrometers in length, and formed a dense tangle. Hominick and Davey's (1973) finding that *H. diesingi*'s mouth was found most often near the hindgut wall, combined with my finding of few free-floating model food items in the gut of females of this species, offers a strong indication that *H. diesingi* may graze on these filamentous bacteria. This would explain the lack of beads in the worms I examined. The beads may have remained suspended in the more-fluid portion of the hindgut contents, and not penetrated down into the tangle of filamentous bacteria, in which case *H. diesingi* would be unlikely to encounter them.

Alternatively, the other resource could take the form of predigested nutrients, excreted by females of the other two species. Finally, the other two species could be feeding on organisms that are competition for *H. diesingi*'s preferred food. In hosts without females of *T. bulhoesi* or *L. appendiculatum*, these organisms may exclude, or
sufficiently reduce the numbers of *H. diesingi*’s preferred food to the point that *H. diesingi* individuals have a hard time finding enough to survive. In either case, rather than offering competition for *H. diesingi*, *T. bulhoesi* and *L. appendiculatum* seem to play a significant role in facilitating *H. diesingi*’s success.
CHAPTER 4: CONSEQUENCES OF INTERSPECIFIC AND INTRASPECIFIC INTERACTIONS ON EGG PRODUCTION.

Introduction.


There is substantial niche overlap among all three species with regard to size of food item consumed (Connor and Adamson, 1998). There is also considerable overlap in the species’ spatial niches, though there is some disagreement as to the extent of this overlap (Adamson and Noble, 1992; Hominick and Davey, 1973). Extensive overlap can be expected to lead to competitive interactions within, or between, species in individual hosts should food or space prove insufficient for maintenance of all individuals present. Nematodes continue to grow after their final moult to adulthood (Malakhov, 1994), and competition may be expected to show its effects in the form of reduced growth. Alternatively, competition may affect fecundity, reducing the number of eggs produced by individual females. It is this latter possibility that the following study addresses.

Adamson and Noble (1993) examined the effect of intra-, and interspecific competition on the potential fecundity of *T. bulhoesi*, *L. appendiculatum*, and *H. diesingi* by counting the number of in utero eggs in females taken from hosts of varying worm burden. They found that, for each species, the number of conspecific females present in a host had a significant negative relation to the number of eggs present in utero, while number of females from other species had no significant relation to this quantity. Their
study did not, however, address the question of the rate at which eggs were produced by individual worms. Without this information it is difficult to say exactly what high, or low, numbers of in utero eggs mean. For example, few eggs may mean simply that the female in question is producing, and releasing eggs rapidly enough that there is no large build-up of in utero eggs, rather than indicating impeded egg production. And a large number of in utero eggs could actually be a result of inhibition of egg release, rather than high fecundity.

The following study examined the actual output of eggs from hosts, and looked for evidence of inter-, and intra-specific competitive effects.

Despite an individual female's best efforts, the nature of the flow of materials through the cockroach hindgut is very irregular (Bignell, 1982) and can be expected to create variation in the number of eggs per fecal pellet. Such variation, if it exists, should lead to aggregation of eggs among fecal pellets. This pre-existing aggregation may then contribute to the aggregation of worms among hosts. I calculated the variance-to-mean ratio for eggs of each species among fecal pellets in order to assess the level of aggregation of each.

The following study assesses the number of eggs per female per day that actually exit a host in fecal pellets. Are there any signs of inter-, or intraspecific interactions affecting this number, either enhancing or diminishing the average number of eggs a female lays? What is the frequency of infective fecal pellets, and the mean number of eggs in each? What is the level of aggregation of eggs among fecal pellets? Is it similar to the level of aggregation of worms among hosts? Do hosts harbouring a particular infracommunity produce fecal pellets containing a similar "proto-infracommunity?"
MATERIALS AND METHODS.

Egg production and release.

Ten adult male hosts were collected from a colony infected only with *T. bulhoesi*, 13 from a colony infected only with *L. appendiculatum*, and seventeen adult male and fifteen adult female cockroaches were collected from a colony of cockroaches infected with three species of pinworm. Only male hosts were selected from single-infection colonies in order to eliminate any effects of host sex on egg production. In collecting hosts from the mixed colony, however, this procedure proved impractical; though *L. appendiculatum*, and *H. diesingi* were found together in male hosts with sufficient regularity, only one out of seventeen male hosts was found to harbour both *L. appendiculatum*, and *T. bulhoesi*.

Cockroaches were isolated in individual white plastic ice-cream buckets, 20cm diameter by 15.25cm deep. The bottom of each bucket was removed and replaced with a wire mesh to allow fecal pellets to pass through to a collecting tray below. Roaches were held in these containers for five days, and their fecal output collected. At the end of the five day period all hosts were dissected, their hindguts removed, and opened to determine the number of males and females of each species of worm present. Dissections were done in 0.75% saline.

Fecal pellets were placed in 0.75% saline in individual 35x10 mm Petri dishes, broken up, and the number of eggs of each species of worm present was counted.

Counts of pinworm eggs (eggs/female/day) were log-transformed using the equation $y = \ln(\text{count} + 0.5)$ before being analyzed in order to obtain an approximately normal distribution. Stepwise multiple regression was used to test for effects of
interactions on egg production. Aggregation of eggs among fecal pellets is reported in terms of the variance-to-mean ratio (VM). This value was calculated for each species over all fecal pellets from all hosts infected with a given species, rather than averaged for each within hosts, and then calculating the mean of those values. I took this approach because this is how hosts will encounter fecal pellets in their environment, which is the feature of interest for this parameter.

For ease of expression, I refer to the hosts collected from the colonies infected with either *L. appendiculatum* alone, or *T. bulhoesi* alone as the “*L. appendiculatum* colony,” or the “*T. bulhoesi* colony.” While every effort was made to ensure uniformity of these colonies in all characteristics except the species of pinworm infecting hosts, there were no replicate colonies available, and it is therefore possible that differences in fecundity of pinworms between the two colonies were due to some factor other than the species of pinworm harbourered.

**RESULTS:**

**Egg production and distribution: Single-infection colonies.**

Host fecal pellets containing eggs contained an average of 60.53 (SE = 26.22, *n* (hosts) = 12) eggs in the *L. appendiculatum* sample, and 7.70 (SE = 2.42, *n* = 10) eggs in the *T. bulhoesi* sample. Though all hosts harbouring gravid females, the frequency of egg-containing fecal pellets was 93% in the *L. appendiculatum* sample, and 81% in the *T. bulhoesi* sample.
Hosts within the *L. appendiculatum* sample produced an average of 5.00 (SE. = 0.65) fecal pellets over five days, while those from the *T. bulhoesi* sample produced an average of 16.6 (SE = 1.49) pellets.

There was no significant difference in per capita egg production per day between the two species (Independent samples *t*-test on log-transformed data, *t* = -1.45, df = 20, *P* > 0.05). *L. appendiculatum* produced an average of 11.82 (SE = 2.78, n = 12) eggs per capita per day, compared to *T. bulhoesi*’s average of 7.36 (SE = 1.29, n = 10).

Stepwise regression analysis of eggs per day per female against number of females present, and the number of fecal pellets produced per day by a host, indicates that egg output per worm for *L. appendiculatum* was significantly reduced by the presence of increasing numbers of conspecifics. The number of fecal pellets released per day by a host had a positive, though insignificant, relation to the number of eggs produced per capita per day. The resulting model is:

\[
\ln (\text{eggs/day/female} + 0.5) = 3.15 - 0.10(\text{females}); \quad (r^2 = 0.42; \quad P < 0.05, \quad n = 13).
\]

For the *T. bulhoesi* sample, there was a negative (*b* = -0.12), but statistically insignificant, relation between fecundity and increasing infrapopulation. Unlike the case with *L. appendiculatum*, the number of fecal pellets produced by a host in a day had a significantly positive relation to *T. bulhoesi*’s per capita egg production. The resulting model is:

\[
\ln (\text{eggs/day/female} + 0.5) = 1.55 + 0.28(\text{pellets/day}); \quad (r^2 = 0.70; \quad P < 0.01, \quad n = 10).
\]

There was no significant difference in intensity of infection of hosts between the *L. appendiculatum* sample and the *T. bulhoesi* sample (5.15, SE = 1.14, n = 13, compared
Mean eggs per pellet for *T. bulhoesi* was 7.70 (n = 10), with variance = 58.41. For *L. appendiculatum* these values were 60.53 (n = 12), and 8251.56. Each species’ eggs were distributed among fecal pellets in an aggregated manner, and levels of aggregation were essentially equal between the two, with *T. bulhoesi*’s VM being 34.19, and *L. appendiculatum*’s being 34.04.

**Egg production and distribution: Mixed-infection colony.**

Host fecal output in the mixed-infection colony averaged 16.5 (SE = 1.34, n = 32) pellets per capita. Total host fecal output over five days was significantly greater for male hosts at 19.88 (SE = 1.87) pellets compared to 11.93 (SE = 1.42) pellets for female hosts (t = 3.01, df = 30, P < 0.01).

From 32 hosts sampled, 526 fecal pellets were collected and examined. Thirty-three percent of these contained *L. appendiculatum* eggs, 45% *T. bulhoesi*, and 51% *H. diesingi*.

Eggs of each species were aggregated in distribution among fecal pellets (VM: *T. bulhoesi* = 38.10, *L. appendiculatum* = 24.23, *H. diesingi* = 31.21).

Mean number of eggs per pellet for each species was:

- *T. bulhoesi*: 17.64 (n = 20, SE = 4.04)
- *L. appendiculatum*: 6.45 (n = 14, SE = 1.60)
- *H. diesingi*: 10.37 (n = 22, SE = 2.67)
Daily per capita egg production for each species was:

\[ T. \text{bulhoesi}: \ 4.94 \ (n = 20, \ s.d. = 3.35) \]
\[ L. \text{appendiculatum}: \ 15.09 \ (n = 14, \ s.d. = 17.32) \]
\[ H. \text{diesingi}: \ 10.27 \ (n = 22, \ s.d. = 9.49) \]

Stepwise multiple regression of eggs/female/day for each species (Table 4.1) indicates that \( L. \text{appendiculatum}'s \) per capita output was negatively related to increasing number of conspecifics, \( T. \text{bulhoesi} \) females, and \( H. \text{diesingi} \) females, but not statistically significantly. \( T. \text{bulhoesi}'s \) was significantly related, negatively, only to increasing numbers of \( H. \text{diesingi}; \) all other factors showed a positive relationship. \( H. \text{diesingi}'s \) fecundity was positively related to increasing numbers of both of the other species, and negatively related to increasing numbers of conspecifics, though none of these relationships was significant.

<table>
<thead>
<tr>
<th>Species</th>
<th>Regression</th>
<th>( r^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T. \text{bulhoesi} )</td>
<td>( y = 2.39-0.05(H. \text{diesingi}) )</td>
<td>0.31</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>( L. \text{appendiculatum} )</td>
<td>( y = 3.63-0.12(T. \text{bulhoesi})-0.34(H. \text{diesingi})-0.03(L. \text{appendiculatum})-0.2(\text{pellets/day}) )</td>
<td>0.62</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>( H. \text{diesingi} )</td>
<td>( y = 1.75-0.05(H. \text{diesingi})+0.21(L. \text{appendiculatum})+0.01(T. \text{bulhoesi})+0.05(\text{pellets/day}) )</td>
<td>0.15</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 4.1: Multiple regression equations of per capita egg production in terms of eggs/female/pellet/day for each species. All data log-transformed.

<table>
<thead>
<tr>
<th>Host Sex (n)</th>
<th>( T. \text{bulhoesi} )</th>
<th>( L. \text{appendiculatum} )</th>
<th>( H. \text{diesingi} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (17)</td>
<td>29.4%</td>
<td>64.7%</td>
<td>58.8%</td>
</tr>
<tr>
<td>Female (15)</td>
<td>100%</td>
<td>33.3%</td>
<td>80.0%</td>
</tr>
</tbody>
</table>

Table 4.2: Prevalence of each pinworm species by host sex.
Females of *T. bulhoesi*, *L. appendiculatum*, and *H. diesingi* were present in 62.5%, 50.0%, and 68.8%, respectively, of hosts taken from the mixed-infection colony. All three species showed a bias in prevalence between the host sexes, but this bias was significant only for *T. bulhoesi* (Fisher’s Exact test, $P < 0.01$) (Table 4.2).

Intensity of infection with females for each species was: *T. bulhoesi* = 9.25 (s.d. = 6.82), *L. appendiculatum* = 1.81 (s.d. = 1.33), and *H. diesingi* = 3.41 (s.d. = 3.45).

Intensity of infection was divided evenly between male and female hosts for *L. appendiculatum* (male hosts = 1.82, SE = 0.46, n = 11; female hosts = 1.80, SE = 0.37, n = 5; independent samples $t$-test, $t = -0.29$, df = 14, $P > 0.05$), however, both *T. bulhoesi* and *H. diesingi* showed significantly higher intensities in female hosts than in male hosts (*T. bulhoesi*, 11.2, SE = 1.72 females per female host (n = 15) compared to 3.4, SE = 1.25, per male host (n = 5); $t = -3.70$, df = 18, $P < 0.05$; *H. diesingi*, 4.92, SE = 1.18 females per female host (n = 12) compared to 1.6, SE = 0.27 per male host (n = 10), $t = -3.03$, df = 20, $P < 0.05$).

Differences in infection intensity according to host sex for both *T. bulhoesi*, and *H. diesingi* led to significant differences in the mean number of eggs per pellet for each species between the host sexes (Table 4.3). *L. appendiculatum*, despite showing no difference in intensity between host sexes, nevertheless showed a similar significant difference, though in this case the male host showed the greater egg production.
### Table 4.3: Mean eggs per pellet per host (* significant difference between male and female hosts, Independent samples *t*-test, *P* < 0.05).

<table>
<thead>
<tr>
<th>Species:</th>
<th>Host Sex (n = Hosts)</th>
<th>Eggs per Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thelastoma bulhoesi</em></td>
<td>Male (5)</td>
<td>5.41 (6.93)</td>
</tr>
<tr>
<td></td>
<td>Female (15)</td>
<td>21.72 (18.93)</td>
</tr>
<tr>
<td><em>Leidynema appendiculatum</em></td>
<td>Male (10)</td>
<td>7.72 (6.15)</td>
</tr>
<tr>
<td></td>
<td>Female (4)</td>
<td>3.27 (4.74)</td>
</tr>
<tr>
<td><em>Hammerschmidtiiella diesingi</em></td>
<td>Male (10)</td>
<td>3.90 (2.31)</td>
</tr>
<tr>
<td></td>
<td>Female (12)</td>
<td>15.77 (15.02)</td>
</tr>
</tbody>
</table>

### Table 4.4: Mean per capita eggs per host fecal pellet.

<table>
<thead>
<tr>
<th>Species:</th>
<th>Host Sex (n = Hosts)</th>
<th>Counts (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thelastoma bulhoesi</em></td>
<td>Male (5)</td>
<td>1.52 (0.78)</td>
</tr>
<tr>
<td></td>
<td>Female (10)</td>
<td>2.09 (1.50)</td>
</tr>
<tr>
<td><em>Leidynema appendiculatum</em></td>
<td>Male (10)</td>
<td>5.70 (6.24)</td>
</tr>
<tr>
<td></td>
<td>Female (4)</td>
<td>1.34 (1.41)</td>
</tr>
<tr>
<td><em>Hammerschmidtiiella diesingi</em></td>
<td>Male (10)</td>
<td>2.93 (2.19)</td>
</tr>
<tr>
<td></td>
<td>Female (12)</td>
<td>4.25 (4.73)</td>
</tr>
</tbody>
</table>

Differences between male and female hosts in mean number of eggs per pellet for each species were not due merely to differences in intensity of infection. There were differences in per capita egg production for all three species in male, compared to female, hosts. The difference was most pronounced for *L. appendiculatum* (Table 4.4).

Neither *L. appendiculatum* nor *T. bulhoesi* showed significant differences in per capita daily egg output between single-infection and mixed colonies. However, for both these species, intensity of infection in the mixed colony was significantly different than that in their single-infection colonies. *L. appendiculatum*'s mean intensity was greater in its isolate colony than in the mixed colony (5.15 (SE = 1.14) females per host compared to 1.81 (SE = 0.33), independent samples *t*-test with unequal variances: *t* = 2.93, df =
17.82, P < 0.05). *T. bulhoesi*, on the other hand, had a higher intensity in the mixed colony, 8.00 (SE = 1.44) females per host, than in the single-infection sample with 4.00 (SE = 1.32) females per host (independent samples t-test: \( t = -2.22, df = 23, P < 0.05 \)).

Presence of a given species in a host was not necessarily followed by the presence of that species' eggs in fecal pellets from that host (Figure 4.1). For example, though all hosts were infected with at least one species of worm, 12.5% of fecal pellets contained no eggs, and, while 16.5% of fecal pellets were from hosts infected with all three species, only 1.7% of pellets actually contained all three species' eggs. On the other hand, though only 4.8% of fecal pellets came from hosts infected only with *H. diesingi*, 13.3% of pellets had only *H. diesingi* eggs in them. Consumption, therefore, of a single fecal pellet from a host with a known infracommunity will not guarantee transfer of a similar community to the new host, or even transfer of infection.
Figure 4.1: Comparison of percent of fecal pellets from hosts containing specific infracommunities with percent of those pellets containing eggs from specific species.
DISCUSSION.

Findings reported herein both confirm and contradict an earlier study by Adamson and Noble (1993), in which the in utero egg number of all three species was shown to be most strongly related to intensity of infection with conspecifics, compared to interspecific effects. In each case the effect was a negative one. Their study differed significantly from the current study in examining the effects of competition on in utero egg counts, rather than counts of eggs in fecal pellets, which represent the actual infective dose reaching the outside environment, produced by an infrapopulation of females. The present study, examined egg numbers in host fecal pellets, and thus measured actual per capita egg production, found this to be true for both L. appendiculatum and H. diesingi; daily egg production of T. bulhoesi females, however, was actually positively, though not significantly, related to increased number of conspecifics. Adamson and Noble (1993) also found that L. appendiculatum females were less fecund in their single-infection colony than in the mixed colony, whereas the current results indicate no difference in fecundity between these situations.

The current results indicate an asymmetrical relationship between T. bulhoesi and L. appendiculatum on the one hand, and H. diesingi on the other. Both of the former species showed reductions in fecundity in the presence of increasing infrapopulations of H. diesingi, but H. diesingi's fecundity showed a positive relationship to increasing L. appendiculatum and T. bulhoesi numbers. As shown in Chapter 1, infrapopulations of H. diesingi tend to be highest in the presence of either of these species, and furthermore, that H. diesingi is found in conjunction with either of these species more often than expected compared to instances of its being the only species present.
Infection intensity for all three species, as well as average number of species present, have been shown in Chapter 1 to increase with host size, indicating a general increase in carrying capacity within hosts. Hosts in this study were all adults, the largest hosts, therefore carrying capacity is expected to be maximal. The asymmetry demonstrated indicates that *H. diesingi* reduces resources required by *T. bulhoesi*, and *L. appendiculatum*, while apparently gaining from the presence of these species.

Hominick and Davey (1973), using light microscopy, noted differences in gut contents of *L. appendiculatum* and *H. diesingi*, the former containing a combination of coarse and fine particulate material, while the latter contained only fine particles. In Chapter 3 I found that both *L. appendiculatum*, and *T. bulhoesi* contained many more fluorescent beads, that had been used as model food items, than did *H. diesingi*. These differences suggest a resolution to the origin of the asymmetrical relationship among these species. *H. diesingi* may rely to a large extent on the other two species to provide it with pre-digested food. All three species are indiscriminate feeders, eating whatever comes into their mouths, so, though *H. diesingi* may “prefer” the pre-digested material ejected from the other species, it will also consume some of the food used by *T. bulhoesi* and *L. appendiculatum*, accounting for its negative effect on their fecundity.

Alternatively, *T. bulhoesi* and *L. appendiculatum* may reduce population levels of some bacterial species, allowing still other species, normally held at low population levels by these competitors, to increase in number. These “ecologically released” species may actually constitute *H. diesingi*’s preferred diet. Model food items used in chapter 2 would have mimicked only those bacteria found floating free in the gut lumen. A substantial proportion of the bacterial population of the cockroach hindgut is found attached to the
gut wall (Cruden and Markovetz, 1987), a region that Hominick and Davey (1973) found

\textit{H. diesingi} preferred as a feeding site while \textit{L. appendiculatum} preferred the gut lumen. It may be that \textit{L. appendiculatum} and \textit{T. bulhoesi} reduce lumen-dwelling populations, allowing wall-dwellers to increase in number, thus enriching \textit{H. diesingi}'s food supply.

A comparison of worm clutch size between host sexes indicates that host sex also plays a role in determining clutch size for each of the three species. Though intensity of infection with \textit{L. appendiculatum} was not significantly different between the host sexes, \textit{per capita} egg production, as well as total egg production was higher in male hosts than in female hosts. \textit{T. bulhoesi} and \textit{H. diesingi}, on the other hand, showed significantly higher intensity, and higher \textit{per capita} egg production in female hosts. These differences indicate a difference in carrying capacity for these species between the host sexes.

Differences in fecundity between host sexes for \textit{L. appendiculatum} females shown in the current study may be due to the effects of interspecific competition; total number of female worms was much larger in female hosts than in males.

Exploitation competition could be responsible for the reduction in fecundity of females with increasing infrapopulation size. Connor and Adamson (1998) showed that all three species share the same trophic niche, at least to some extent, with regard to prey size, and that \textit{L. appendiculatum} and \textit{T. bulhoesi} consume similar quantities of food, while \textit{H. diesingi} consumes much less. \textit{H. diesingi}, however, may also be making use of a food resource unavailable to \textit{T. bulhoesi} and \textit{L. appendiculatum}, as indicated by its negative effect on the fecundity of these two species, while there seems to be no reciprocal effect. In addition, Adamson and Clease (1989) presented data showing that
both *T. bulhoesi* and *L. appendiculatum* produce much larger eggs than *H. diesingi*, and as the current study shows, *L. appendiculatum* produces many more than either of the other species. As all three species continue to grow after reaching reproductive maturity (Malakhov, 1964), while at the same time producing and releasing eggs, competition for food could eventually force female worms to reduce either growth or egg production. *L. appendiculatum* females grow faster, and can be expected to place a greater strain on host resources. This may explain their stronger response to both intra-, and interspecific population increases.

Data from Adamson and Noble (1992), as well as data in chapter 1 show that *L. appendiculatum* and *T. bulhoesi* are found together less often than expected, and it is reasonable to conclude that exploitation competition for food is the mediating factor in intra-, and inter-specific competitive interactions among all species, with *L. appendiculatum*, and *T. bulhoesi* the most intensely competing, and unable to coexist except in large hosts with a rich bacterial population in their hindgut.

For each species, whether all three in the mixed-infection colony, or *T. bulhoesi*, and *L. appendiculatum* in their single-infection colonies, there is substantial variation in the number of eggs per fecal pellet that remains unexplained by regression analysis. Presumably, this variation is ascribable to the vagaries of the flow of materials within the host hindgut. Total transit time for food through the alimentary canal of *P. americana* has been shown to average 20.6 hours (Snipes and Tauber, 1937), with the majority of that time being spent in the hindgut. Gut movements within the hindgut are dominated by the random formation and relaxation of annular constrictions (Bignell, 1982), resulting in temporary segmentation of the gut, and longitudinal compressions and relaxations.
Neither of these pushes gut contents in any particular direction. Peristalsis and anti-peristalsis also take place, but these movements are much less frequent. The picture of the fate of an individual pinworm egg released into the gut is thus one in which the egg is carried back and forth within the gut, with only an overall rearward tendency. This being the case, the final number of eggs packed into a host fecal pellet will be quite variable. An individual female of a given species can only influence this number to a limited extent.

As a result of the irregularity of flow within the host gut, eggs of all three pinworm species are distributed in fecal pellets in an aggregated manner in the host environment, though the distribution of *L. appendiculatum*’s eggs within its single-infection colony is less so than in the mixed-infection colony, and less so than either of the other two species. The aggregated nature of the distribution of eggs within fecal pellets may account, at least in part, for the aggregated distribution of worms among hosts.

Examination of individual fecal pellets from mixed-infection hosts has also shown that, though two or three species of female may be present in a host, fecal pellets from that host will not necessarily reflect this mixture, but instead may contain eggs of one, two, three, or none of the species present in the host. Discounting fecal pellets from triply-infected hosts, the proto-community contained in fecal pellets did match that of the originating host 75% of the time. Transfer of a particular infracommunity from one host to another, therefore, has a good chance of occurring. The exception was pellets from triply-infected hosts. Figure 1 shows that, while 16.5% of all fecal pellets collected in this study came from such hosts, only 1.7% of all fecal pellets contained eggs of all three
species. Hosts harbouring all three species, then, have likely experienced multiple
infection events, rather than to have acquired such an infracomunity from a single fecal
pellet.
CHAPTER 5: FINAL DISCUSSION.

The preceding chapters have examined the population structure of the pinworm community harboured by colonies of the American cockroach, *P. americana*, at the University of British Columbia. Three species of pinworm, *L. appendiculatum*, *T. bulhoesi*, and *H. diesingi* coexist in these communities, and have done so for over 25 years (Adamson and Noble, 1992). All three species have very similar life histories; they live in the same part of the host intestine, all are bacteriophagous, all use the same method of transmission to, and colonization of new hosts, and all achieve similar adult sizes. Such apparent similarity in life histories leads to the expectation of intense competition among the species, and a low probability of coexistence based on the familiar idea that complete competitors cannot coexist. One species should show itself to be better at exploiting a niche than its competitors, and come to dominate that niche. It is, therefore, surprising to find the three species continuing to coexist, both at the level of the host population, and within individual hosts.

In chapter 3, I have demonstrated that there is nearly 100% overlap among the three species in terms of size of food item consumed, suggesting that food could become a limiting resource for these worms, and thus a focus of competition among them.

It is also reasonable to conclude that space within the host intestine represents a limiting resource. Bracke *et al.* (1979) have shown that the highest bacterial densities are found within the anterior third of the cockroach hindgut, with numbers dropping off precipitously beyond this. All three species have been shown (Adamson and Noble) to inhabit this region of the hindgut in singly-infected hosts, and to cede position within it under conditions of crowding in a predictable manner. In hosts in which all three species
are present, \textit{L. appendiculatum}, maintains the anterior-most position, followed by \textit{H. diesingi}, which is followed by \textit{T. bulhoesi}. Space problems will become critical in small hosts. Many of these were found to be capable of holding no more than a single female. These small hosts represent, periodically, a large proportion of the host population, and, therefore, a valuable resource.

Evidence of negative interactions between species was shown in the present study by the substantial reductions in prevalence of both \textit{T. bulhoesi} and \textit{L. appendiculatum} in mixed-infection colonies compared to single-infection colonies harbouring one or the other of these species. In addition, \textit{L. appendiculatum} showed a substantial reduction in average intensity of infection in the mixed-infection colonies, compared to its single-infection colony. This decrease was due to \textit{L. appendiculatum}'s failure to achieve intensities in large hosts in mixed-infection colonies comparable to those achieved in its single-infection colony. \textit{T. bulhoesi}, on the other hand, showed no significant difference in average infection intensity between the two situations, presumably because it achieves its greatest intensities in adult female hosts, regardless of colony, and it dominated this class in the mixed-infection colonies. Thus it would seem that \textit{T. bulhoesi} may be denying \textit{L. appendiculatum} the chance to fully exploit the range of hosts available in the mixed-infection colonies.

The role of \textit{H. diesingi} in the mixed-infection colonies appears to be that of exploiter of both the host, and the other two species. In chapter 1, I showed that \textit{H. diesingi} was found more often than expected in hosts with either of the other two species, and that the greatest proportion of the total number of individuals of \textit{H. diesingi} found, was found in these hosts. In chapter 4, I showed that \textit{H. diesingi} had a negative
impact on the fecundity of both of these species, while experiencing greater fecundity in their presence than alone, indicating that it was utilizing a resource required by the other two species, while somehow benefiting from their presence.

In the niche overlap study in chapter 3, while all three species showed similar proportions of the different model food items in their intestines, *H. diesingi* showed far fewer than either *T. bulhoesi* or *L. appendiculatum*, suggesting that the beads used as model food items might not have successfully represented *H. diesingi*’s preferred food source. This offers support for Hominick and Davey’s (1973) finding that *H. diesingi* and *L. appendiculatum* exploited different trophic niches. They found that *H. diesingi* specialized on much-finer food particles than did *L. appendiculatum*.

The results in the preceding two paragraphs suggest that perhaps *H. diesingi* relies on the other two species to release pre-digested nutrients, which *H. diesingi* then consumes. Alternatively, *T. bulhoesi* and *L. appendiculatum* may prey on a food source that somehow competes with, and reduces the numbers of, another food source that *H. diesingi* prefers to utilize. *T. bulhoesi* and *L. appendiculatum* may graze down this competitor, allowing *H. diesingi*’s food supply to increase.

If *H. diesingi*’s success in hosts is facilitated by either, or both, of the other two species, its continued existence in the colonies is understandable. The continued existence of *L. appendiculatum* and *T. bulhoesi* is, under the usual assumptions of competitive interactions, more puzzling. As noted above, their life histories are very similar. Their fundamental spatial niche is the anterior portion of the host hindgut, they show complete overlap in trophic preference, and their mode of transmission is the same. This would appear to be a case of complete competitors coexisting, which is not supposed
to be possible without other factors acting to reduce the effects of their mutual competition.

One factor that may contribute to these species' coexistence is the lack of consistency in inter-parasite (both within species, and between) interactions in terms of the number, and species composition of competitors faced each time. An essential assumption of most competitive scenarios is the repetition of competitive interactions over multiple generations within a specific habitat, each generation refining the competitive strategy of the generation before. The strategy chosen must depend, at least in part, on how similar the roster of competitors is from one competing generation to the next. Species that inhabit a given space over multiple generations will be exposed repeatedly to similar competitors, and success will depend on developing a strategy to minimize the impact of those competitors. Each new generation of intestinal helminths, however, finds itself in a new competitive situation. Conspecifics may be present, or not; competing individuals from other species may be present, or not. Competitors may be present in large, or small numbers, and whichever situation obtains at one point in a helminth's life in a host, it may change drastically later should a host reinfect itself, or as possibly older competitors simply die off. Because of these constantly-changing conditions, it is unlikely that any strategy based on interference with competitors would successfully develop. With no consistency in the levels, or nature, of competition to be faced, it would surely be more likely that an individual, and thereby a species, would concentrate on obtaining resources by focusing on the resources, and ignoring competitors.
Thus, exploitation competition would be the most likely form that competitive interactions would take, and these would result in competitive exclusion only when the limits of these resources were reached. The most likely place this would occur in the present system would be in the smallest hosts, which presumably have the lowest carrying capacity, and it is here that I have found most hosts to be infected by single individuals. Most of these are from only one species. *L. appendiculatum*’s prevalence in these hosts was higher than that of either of the other two species in the mixed-infection colonies, though its prevalence declined, while that of the others increased, with host age. *L. appendiculatum* was also more prevalent among these hosts in its single-infection colony than *T. bulhoesi* was in these hosts in its single-infection colony.

*L. appendiculatum*, then, appears to have a greater ability to exploit small hosts than either *T. bulhoesi*, or *H. diesingi*, allowing it to exclude these two species in these hosts. It’s decline in prevalence in more mature hosts, as the prevalences of *T. bulhoesi* and *H. diesingi* increase, indicates that this ability declines in effectiveness with increasing host size. Why this should be so is unknown, however it would appear to be an ecological, rather than an evolved, restriction of *L. appendiculatum*’s ability to fully utilize all available hosts. *L. appendiculatum* in isolation showed itself capable of successfully exploiting hosts in any age class, and in fact was more prevalent, and reached higher intensities, in a broader spectrum of host sizes in its single-infection colony than did *T. bulhoesi* in its single-infection colony. It would appear, then, that *T. bulhoesi*, perhaps in conjunction with *H. diesingi*, manages to restrict *L. appendiculatum*’s ability to exploit larger hosts in mixed-infection colonies.
As data from chapter 4 indicate, host fecal pellets contain, on average, five or more helminth eggs, which leads to the question of why infrapopulations of worms aren’t larger than they are. One explanation for this apparent regulation of populations of helminths within their cockroach hosts was proposed by Zervos (1988); her suggestion was later taken up by Adamson and Noble (1993) in regard to the system in this thesis. Zervos (1988) suggested a toxin, secreted by individual worms, as a means of explaining what appeared to be strong population regulation among the worms she studied, and made a claim for population self-regulation. Adamson and Noble (1993) attempted to explain limited infrapopulations, fecundity-reduction, and each species’ predilection for the anterior-most position in the host hindgut as products of a toxin. The first two were direct effects of the toxin, while the latter was due to the supposition that an extreme anterior position, in an environment with an upstream and a downstream end, would represent a sheltered position in the hindgut.

The suggestion of population regulation through the effects of a species-specific, and sex-specific toxin (as opposed to a merely inhibitory secretion) has a number of flaws. The first is its contention that a species will act to regulate its own population numbers through something as drastic as culling. This is unlikely. The progeny of an individual that “decided” to rebel against the program through the development of resistance to the toxin would swiftly replace those of the individuals that continued to participate in the regulatory process.

One could, perhaps, argue that each infrapopulation, at least at first colonization, will be near-clonal as founding individuals would likely come from one infection event, consisting of the eggs in a single fecal pellet, which would stand a good chance of all
originating with a single female (many small hosts harbour only one female). In this case, a species-specific toxin would simply be culling genetically-unnecessary individuals. However, unless such a toxin had interspecific effects as well, the result for the secreting species could be the ceding of unnecessarily large amounts of resource to competing species, perhaps to the point of regulating its own population into extinction.

Lastly, there is the problem of a secretor avoiding its own toxin. Adamson and Noble's (1993) suggestion that the extreme anterior portion of the host colon might offer a refuge due to it's being at the upstream end of the colonic flow was based on the assumption that there is, indeed, a consistent direction, rearward, to the flow of gut contents. This is not the case. Cook and Reinecke (1973), studying the movements of the hindgut of the Madeira cockroach, *Leucophaea maderae*, found that the most common gut movement was one of compression during which longitudinal muscles contract, resulting in an overall shortening of gut length. The next most common activity was one involving segmentation. Muscles encircling the gut contract, then relax, but cause no advancement of gut contents. Least frequent were motions involving peristalsis, and anti-peristalsis. These results show that there is no reliably rearward direction to the flow of gut contents, and, therefore, no position of privilege allowing shelter from the effects of any helminth-secreted toxin.

Data from chapter 1 regarding larval intensities for all three species show that there is a positive relation between host age and larval intensity, as well as between larval intensity and the number of adult females present, regardless of species. This positive relationship between larvae and adult females suggests that the adults have either a facilitatory effect on larvae, perhaps by providing pre-digested nutrients for them, or, at
least, no negative effects. Neither of these is necessarily the case. The presence of adults could act to inhibit the maturation of larvae, resulting in a build-up of larval numbers, held in immaturity until space among the adults opens up, or until they fail to mature, and die.

Inhibition of larval maturation would allow a species to regulate its population without requiring unrealistic demands on the interests of individuals. A mutation that provided an individual with resistance to the inhibitory substance would likely prove disadvantageous as that individual’s progeny could quickly overwhelm the resources in a host individual’s gut. This is particularly true of *L. appendiculatum*, with its higher fecundity. Individuals that succumbed to the inhibition would not necessarily be sacrificing their reproductive interests, and might, in fact, be enhancing them by biding their time until older individuals within the host die, leaving them room to mature and reproduce in their turn. Both larvae, and adults could benefit through a likely separation of trophic niches; larvae, being much smaller than adult females would almost certainly be relying on a different food source than that of the adults.

The question in most studies of helminth infracommunities often centers around the question of whether the communities are interactionist, or isolationist in nature. Is the population structure we see in infracommunities the result of interactions among parasite species in ecological time, or the result of evolutionarily-based specialization (Holmes and Price, 1986)? As with most proposals with either/or answers, it was quickly realized that this scheme was too rigid, and that these were simply the extreme ends of a range of possibilities (e.g. Goater *et al.*, 1987). Studies of helminth communities to date seem to confirm this more-flexible approach (reviewed in Sousa, 1994).
The helminth infracommunities I have studied fall toward the interactionist end of
the spectrum. In mixed-infection colonies I have shown that *T. bulhoesi* and
*L. appendiculatum* appear to be in conflict with one another, with *L. appendiculatum*
excluding *T. bulhoesi* (and *H. diesingi*) from smaller hosts, while *T. bulhoesi* (and
possibly *H. diesingi*) restricts the ability of *L. appendiculatum* to fully exploit mature
hosts. *H. diesingi*, in contrast, appears to benefit from the presence of either of the other
two species in a host, both in terms of its own intensity of infection and fecundity, while
having a negative impact on this latter trait for both of the other species. Thus, while the
community is interactive, the nature of the interaction is not consistent among the species.
Neither is it necessarily what one would expect given the apparent similarity of the three
species' life histories.

Interactions among species, even when they are negative, do not necessarily lead
to competitive exclusion of one, or more species. Macroparasites of host species’
intestines have commonly been found to be aggregated in their distributions (Kennedy *et
al.*, 1986; Dobson, 1985). Levels of aggregation that increase with host age, as were
found in this study, are suggested to be indicative of a lack of density-dependent effects
on the host population due to the parasites, as well as on the parasites themselves
(Anderson and Gordon, 1982). Most of the studies on which this conclusion is based,
however, dealt with hosts that were substantially larger than their parasites, and did not
take into account hosts such as those in this study, the smallest of which are barely larger
than their parasites. In this case, a single helminth could represent a fatal parasite density
for the host. Density-dependent host mortality would then be taking place at the opposite
end of the host life cycle from that normally assumed. Regulatory effects of the parasite
on the host population could take place among juvenile hosts, and actually diminish as hosts matured. Mature hosts must harbour larger bacterial populations, and provide more space for worms, perhaps to the extent that these seldom become limiting. Thus, aggregation levels could steadily increase with host age, while, at the same time, parasite density-dependent regulation of the host population goes on.

As mentioned, the aggregation level of *L. appendiculatum* within the mixed-infection colonies is reduced from the level in its single-infection colony. Reduction in aggregation levels can come about in a number of ways. One of these is through differential host mortality due to differing levels of intensity of infection; heavily-infected hosts die, thus eliminating the few instances in which high infrapopulation levels would have contributed to substantial increases in the variance of intensity. However, this same effect could be achieved through the effects of interspecific competition whereby a species is denied the opportunity to reach high levels of infection intensity through the negative effects of competing species. This appears to be the case for *L. appendiculatum*. Within its own colony there are a few hosts infected with substantial numbers of females, however, within the mixed-infection colonies such hosts are absent.

Aggregation has also been shown in numerous simulations to promote coexistence of competing species (Ives and May, 1985). Shorrocks *et al.* (1984) used both laboratory populations, and modeling to study the effects of aggregation on the coexistence of competing species of *Drosophila*, and found that, even in the face of intense competition, a suitable level of aggregation would allow all species to coexist indefinitely. Aggregation, then, is a powerful force for promoting the coexistence of multiple species.
Aggregation can arise from a number of sources: differential host susceptibility due to either behavioural or immunological differences among hosts; differences in the tolerance of hosts of large infections; and aggregation of infective stages (Shaw and Dobson, 1995). In the case of the pinworm/cockroach system, pre-existing aggregation of infective eggs is certainly a likely source of the worms' aggregation within hosts. Further aggregation is likely made possible by the increasing ability of hosts to tolerate larger infections as they grow. Keymer and Anderson (1979) have shown that host feeding behaviour can also play a critical role in determining levels of aggregation. They found that the distribution of larvae of *Hymenolepis diminuta* among *Tribolium confusum* hosts was over-dispersed even when infective stages were evenly distributed throughout the host environment.

*P. americana* individuals do indulge in coprophagy (pers. obs.). There are at least two good reasons for their doing so. Immature cockroaches that have been cleared of the bacterial flora in their hindguts have been shown to suffer a retardation of weight-gain, and underdevelopment of their hindgut (Bracke and Markovetz, 1980). As there is bound to be some loss of the bacterial population each time a developing nymph moults, coprophagy may represent a quick way to replenish the gut bacterial population. In addition, Cruden and Markovetz (1987) report findings showing that hindgut bacteria contain storage products such as glycogen that would represent a valuable source of energy for the host. Recovery, through coprophagy, of this energy that would otherwise be lost at defecation could represent a valuable alternative source of nutrition for the hosts.
Theoretical work by Tilman (1994) showed that it is impossible for all of the species in a sub-divided community such as the one considered in this thesis, to occupy all of the available patches simultaneously. Tilman’s paper is interesting in that it applies to sessile species. Intestinal helminths, while not sessile in the usual sense, do share sessile organisms’ lack of ability to move from one habitat to another. Both types of organism rely on the release of propagules into their environment for dispersal. Weaker competitors from both types rely on their propagules finding an uninhabited, or under-inhabited, patch of suitable resource upon which to establish. Scattering of suitable habitat patches of varying size can, then, also promote the coexistence of multiple species, some of which would certainly exclude the others were the habitat not so subdivided. In the present study, host individuals represent such patches. Open patches are continuously generated through the birth of new hosts, ensuring that a parasite species will always have a reasonable probability of encountering an open patch. Other patches may open up through the death of the parasites they harbour. Similar results can be found in Hanski’s models of metapopulation dynamics (e.g. Hanski and Gyllenberg, 1993; Hanski and Gilpin, 1991).

Each colony of hosts in my study represents a large habitat that has been subdivided into numerous patches of varying sizes among which each helminth species can disperse. Each colony also represents a closed system in that there can be no movement between them, thus making it impossible for helminth immigrants from outside a colony to rescue a helminth population threatened with extinction. Nevertheless, all three species, despite evidence of negative interactions among two of them at the infracommunity level, have persisted in these host populations for over 25 years.
All of the above indicates that population structure may be more important to a community's diversity, whether parasitic or free-living, than interactions among its member species. Independent aggregation of species, and many patches of suitable habitat that vary in size, and resource level, may be the real determinants. Should this prove to be the case, there are obvious implications for conservation ecologists attempting to develop a plan to maximize species diversity in the face of increasing habitat subdivision. Suitable habitat patches, and connecting corridors appropriate to the scale of the organisms involved will need to be maintained if less-able competitors are not to be driven to extinction through confinement with superior competitors.
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APPENDIX: TRANSMISSION OF LEIDYNEMA APPENDICULATUM AND THELASTOMA BULHOESI, PINWORM PARASITES OF THE AMERICAN COCKROACH PERIPLANETA AMERICANA.

Introduction.

Individual cockroaches in laboratory colonies of Periplaneta americana, the American cockroach, are often parasitized by one or more of Leidynema appendiculatum, Thelastoma bulhoesi, and Hammerschmidtella diesingi. These pinworms inhabit the host colon, and feed on resident bacteria.

Transmission of each of these species is via infective eggs contained in cockroach fecal pellets. Infection of new hosts results when a cockroach consumes an infective egg. However, the process is apparently not quite so simple as that. The rate at which new infections are established depends on the frequency with which hosts consume fecal pellets, number of eggs per fecal pellet, and ability of larvae to establish within the host. These three factors combined constitute the parasite's transmission rate.

There is no data on the first parameter, and chapter 3 gives the mean number of eggs per fecal pellet for each of the three species. The final parameter, the ability of larvae to establish within the host, at first glance, seems straightforward enough — a larva that hatches from an ingested egg should have a fairly high likelihood of establishing. After all, the probability of an individual egg gaining access to a host is quite small; it is not unreasonable to expect that selection would have acted to ensure that each opportunity given is fully realized. The situation is not, however, so simple.
Noble (unpublished data) attempted to estimate establishment rate by feeding uninfected hosts 30 infective eggs from either of two species, *T. bulhoesi* or *L. appendiculatum*. Eggs were obtained from females that had been dissected out of hosts, and fed to new hosts on small pieces of apple. At intervals of approximately forty-eight hours thereafter, he dissected a sample of these hosts and counted the number of larvae present. For both species, the proportion of larvae/eggs decayed over two weeks from a maximum of 0.66 to zero. Presumably Noble’s method failed to capture some essential element of the transmission process.

In order to examine transmission for two of the species, *L. appendiculatum*, and *T. bulhoesi*, I introduced uninfected cockroaches into populations of hosts harbouring one or the other species for a limited period of time, and counted the number of new infections per day, both in terms of prevalence, and mean intensity, for each. Hosts were examined after 48 hours exposure, and after allowing sufficient time for female worms to mature.

**MATERIALS AND METHODS.**

Four groups of ten cockroaches, five adult males, and five adult females, were taken from a colony of uninfected cockroaches, and marked with silver ink using a Pilot Super Color pen. Two groups were released, allowing a period of four days between the end of the first group’s period of exposure and the second group’s introduction, into a host colony infected only with *L. appendiculatum*. Following a similar procedure, the remaining two groups were released into a colony infected only with *T. bulhoesi*. Each group was exposed to the infective colony for a period of forty-eight hours. At the end of this time they were removed and dissected in 0.075% saline. The hindgut of each
cockroach was removed, and dissected, and the contents examined for larvae, which, if present, were counted.

Following this, in order to determine how many adult females result from an exposure of this duration, a further twenty uninfected hosts per colony, 10 adult males, and 10 adult females, were again marked and released into either the *L. appendiculatum* colony, or the *T. bulhoesi* colony. These were again left for forty-eight hours, then removed. At this point cockroaches were transferred to a 20l plastic bucket.

Cockroaches exposed to the *L. appendiculatum* colony were held in these, with food and water constantly available, at 28C, for 18 days to allow female worms time to mature (Noble, 1991). Those exposed to the *T. bulhoesi* colony were held under the same conditions, for 33 days, as *T. bulhoesi* females mature more slowly (McCallister and Schmidt, 1983).

At the end of the appropriate time period all hosts were dissected under the same conditions as above. Any worms found were counted and designated adult females, adult males, or larvae, as appropriate.

Transmission rate was calculated as total number of worms found among all exposed hosts/number of hosts exposed/2, the number of days hosts were exposed, and expressed as worms per susceptible host per 24 hours.

All source colonies were maintained in 80l plastic garbage cans, with ground Purina Dog Chow, and water constantly available, at 28C.

For analysis, all count data were log-transformed using the formula $y' = \ln(y + 0.5)$. 
RESULTS.

Sixty, and seventy-percent of hosts introduced into the *L. appendiculatum* colony in each of the first two trials, became infected. Newly-infected hosts in the first trial harboured a mean of 3.83 larvae (s.d. = 2.71), while those in the second trial harboured a mean of 9.43 (s.d. = 12.65). This difference is not statistically significant (independent-samples t-test, P > 0.05).

Transmission to new hosts was much lower in the *T. bulhoesi* colony. Twenty, and forty-percent of hosts, in trials 1 and 2, became infected. Infected hosts in trial 1 harboured a mean of 2.00 larvae (s.d. = 1.41), those in trial 2 also harboured a mean of 2.00 (s.d. =1.15).

The difference in number of new hosts infected between the two colonies was significant ($\chi^2 = 4.91$, df = 1, P < 0.05). The difference in mean intensity of infection between hosts exposed to the *L. appendiculatum* colony or the *T. bulhoesi* colony, was not statistically significant.

In the trial to determine the number of females successfully maturing, infected hosts in the *L. appendiculatum* group harboured a mean of 4.55 females (s.d. = 3.78). Those infected in the *T. bulhoesi* colony harboured a mean of 2.77 (s.d. = 3.35). One hundred percent of hosts exposed to the *L. appendiculatum* colony were infected, while 65% (13/20) of those exposed to the *T. bulhoesi* colony were infected. Again, this represents a significant difference in prevalence (Fisher’s Exact Test, P < 0.05), though there is no significant difference in intensity of infection.

There was no significant difference for either species in either prevalence, or mean intensity of infection due to host sex.. There was, however, an apparent difference
in mean intensity. *T. bulhoesi* showed a substantially greater mean intensity of adult worms in female hosts, 4.75 (s.d. = 3.4) compared to 1.2 (s.d. = 0.5) in male hosts, than *L. appendiculatum* at 6.4 (s.d. = 4.6) compared to 3.8 (s.d. = 1.9).

Transmission rates indicated by hosts dissected immediately after 48 hours exposure, calculated by pooling the data from the first and second trials, were 2.23 worms per day for *L. appendiculatum*, compared to 0.30 worm per day for *T. bulhoesi*. During the final trial, during which time was allowed for female worms to mature, *L. appendiculatum*’s transmission rate remained virtually unchanged at 2.6 worms per host per day, while *T. bulhoesi*’s was higher than during the first run at 1.2 worms per host per day. If intensity of new infections only is considered, *L. appendiculatum*, again, shows little difference between the two runs, 3.4 compared to 2.6 worms per host per day. *T. bulhoesi* also shows little difference in the number of worms per infected host between runs, 1.0 worm per host per day compared to 1.7 worms per host per day.
DISCUSSION.

These results indicate that infections with both *T. bulhoesi*, and *L. appendiculatum* can be successfully established after exposure for 48 hours to an infective environment, a result that is quite different than that obtained by Noble (Noble, unpublished data), who did not succeed in establishing any infections; rather, his data show a steady decline in number of larvae per host over a period of approximately two weeks.

The method of exposure I used in this study differs from Noble’s in two ways. First, his allowed only a single exposure to infective stages of the worms, while the current method allowed the possibility of multiple exposures. How significant this difference might have been depends on how frequently cockroaches consume fecal pellets, a factor for which there is currently no data available. Secondly, he attempted to infect cockroaches by placing 30 eggs on a piece of apple, which the cockroach then consumed. Normally, infective eggs are taken in in the course of consuming a fecal pellet, as they were in this study. Is there a factor contained in fecal pellets that contributes to larval establishment? Fecal pellets represent the concentrated end-products of digestion in the colon; perhaps they contain high levels of pre-digested nutrients that larvae may require in their earliest stages of development. Roggen (1971) calculated the minimum body size for nematodes that would be compatible with the requirements of feeding and normal nematode locomotion, and found that the lower limit was approximately 0.3mm, or 300μm. Larvae for all three of the species in this study, when they first emerge from the egg, are well below this size limit (Adamson and Clease, 1989). Below this limit the trade-off between the hydrostatic pressure within the worm...
necessary for locomotion, and the size of the pharynx necessary for it to function properly
as a sealing valve for the intestine, come into conflict. At less than 300\(\mu\)m, maintenance
of sufficient hydrostatic pressure for locomotion requires a pharynx-size that leaves no
room for the intestine. Reduction of the size of the pharynx can only be achieved by
reducing hydrostatic pressure, which will then impair locomotion. Roggen (1971)
predicted that nematodes smaller than 300\(\mu\)m would exhibit abnormal modes of
locomotion, and found, based upon a literature search, that nematodes smaller than this
did display differences in morphology and locomotion from larger nematodes that were in
keeping with these theoretical predictions. Thus, larvae of these pinworms may depend
on stored fat for an initial growth-spurt that takes them beyond the minimum size, or they
may be able to feed, but be limited in their ability to move. In the former case,
insufficiently provisioned larvae may starve to death before reaching an effective feeding
size, and repeated exposure of hosts to infective stages may be necessary for successful
establishment of infection. In the latter case, successful establishment of larvae could be
enhanced through the provision of pre-digested nutrients in the fecal pellet. Given the
slow progress of material through the cockroach hindgut (Snipes and Tauber, 1937), it
may be that the dissolving pellet provides an area of concentrated nutrients for larvae
whose small size makes it difficult to move in search of food.

Thus, infections can be established on demand, but, for the moment there is no
means of precise control of number of hosts infected, or number of worms per new
infection. Further work is required to determine whether the greater success rate in this
experiment was due to multiple exposures of hosts to infective stages, or to some factor
contained in fecal pellets. If the latter proves to be the case, it should be possible to
identify the factor. This knowledge could then be used to create predictable infections, which could, in turn, be useful in creating predictable infracommunities in which possible interspecific competitive interactions could be studied with greater confidence.