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Host manipulation by *Ligula intestinalis*: a cause or consequence of parasite aggregation?

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Abstract

Previous investigations suggest that the infection of the cyprinid roach, *Rutilus rutilus*, with the larval plerocercoid forms of the cestode, *Ligula intestinalis*, creates behavioural and morphological changes in the fish host, potentially of adaptive significance to the parasite in promoting transmission to definitive avian hosts. Here we consider whether these behavioural changes are important in shaping the distribution of parasite individuals across the fish population. An examination of field data illustrates that fish infected with a single parasite were more scarce than expected under the negative binomial distribution, and in many months were more scarce than burdens of two, three or more, leading to a bimodal distribution of worm counts (peaks at 0 and >1). This scarcity of single-larval worm infections could be accounted for a priori by a predominance of multiple infection. However, experimental infections of roach gave no evidence for the establishment of multiple worms, even when the host was challenged with multiple intermediate crustacean hosts, each multiply infected. A second hypothesis assumes that host manipulation following an initial single infection leads to an increased probability of subsequent infection (thus creating a contagious distribution). If manipulated fish are more likely to encounter infected first-intermediate hosts (through microhabitat change, increased ingestion, or both), then host manipulation could act as a powerful cause of aggregation. A number of scenarios based on contagious distribution models of aggregation are explored, contrasted with alternative compound Poisson models, and compared with the empirical data on *L. intestinalis* aggregation in their roach intermediate hosts. Our results indicate that parasite-induced host manipulation in this system can function simultaneously as both a consequence and a cause of parasite aggregation. This mutual interaction between host manipulation and parasite aggregation points to a set of ecological interactions that are easily missed in most experimental studies of either phenomenon.

Keywords: *Ligula intestinalis*; Roach; Macroparasitic aggregation; Host manipulation

1. Introduction

Free-living organisms typically display a combination of migratory and aggregating behaviours (Taylor and Taylor, 1977; Taylor et al., 1983; Tokeshi, 1999), and as a result many organisms show a clumped spatial distribution. Likewise, parasites are not uniformly distributed among their host populations, some hosts contain many more parasites than average while others contain few or none (Dobson and Merenlender, 1991; Grafen and Woolhouse, 1993; Gregory and Woolhouse, 1993; Shaw and Dobson, 1995; Wilson and Grenfell, 1997). Two different theoretical models have been suggested to explain the spatial distribution and local abundance of organisms: first, a behavioural model which involves both migratory and aggregative behaviours in organisms (Taylor et al., 1983; Perry, 1988; Hanski and Gyllenberg, 1993); and second, a demographic model which suggests the existence of a stochastic interplay between demographic population characteristics and environmental heterogeneity (Anderson and Gordon, 1982; Nee et al., 1991; Morand and Guégan, 2000). No consensus exists on the processes shaping clumped distributions in organisms observed in nature, in part due to the simple fact that observations on free organisms consider the importance of individual behaviour, while those on parasites are

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interpreted within the context of stochastic demographic and environmental events.

A number of processes have been implicated in the generation of parasite aggregation. Clumping of infective stages, host heterogeneity (variability in susceptibility due to differences in behaviour, genetics, experience or time) and direct reproduction all tend to increase aggregation, while density-dependent parasite mortality and parasite-induced host mortality act to decrease aggregation (Anderson and Gordon, 1982; Pacala and Dobson, 1988; Rousset et al., 1996; Poulin, 1998).

The parasite species investigated in this study was the Pseudophyllidean cestode, *Ligula intestinalis* (L.), which commonly infects cyprinid fish in European freshwater systems (Bauer and Stolyarov, 1961). *Ligula intestinalis* has a three-host life-cycle (Rosen, 1920). The coracidium larva penetrates the gut wall of a copepod microcrustacean and develops into a procercoid. The infested copepod is ingested by a planktivorous cyprinid fish and the procercoid then develops into a plerocercoid larva located in the host abdominal cavity. The definitive host is an ichthyophagous predatory bird in which the plerocercoid matures. Parasite eggs are then released into the water with bird faeces.

Several studies have shown that plerocercoids have severe effects on fish viability and behaviour (Moisan, P., 1956. La ligulose des poissons d'eau douce. Thèse de doctorat vétérinaire, Maisons-Alfort, France; Arme and Owen, 1968, 1970; Sweeting, 1975, 1976; Taylor and Hoole, 1989; Wyatt and Kennedy, 1989; Loot et al., 2001a,b). Brown et al. (2001) illustrate that behavioural modification is more pronounced in more heavily infected fish. Here we investigate whether these parasite-induced changes in host behaviour are best viewed as a cause or a consequence of parasite aggregation within the host. Specifically, our objectives were (i) to analyse the worm burden distribution within fish in Lavernose-Lacasse gravel-pit, (ii) to experimentally examine the consequences of defined infection conditions on the degree of parasite aggregation, and (iii) to discuss the different processes which are implicated in the regulation of *Ligula* larvae infrapopulations in the second intermediate host, the roach.

2. Materials and methods

2.1. Empirical system

2.1.1. Fish sampling

Samples of roach were collected monthly from April 1998 to March 2000 in Lavernose-Lacasse gravel-pit. Lavernose-Lacasse is situated near the city of Toulouse, on the alluvial plain of the Garonne river, France (see Loot et al., 2001b). Its surface area is 23 ha with a mean depth of around 2 m and the average annual water temperature is around 14 °C (range 5–25 °C). The fish community consists of 12 species, of which only roach (roach comprise 65.75% of the cyprinid fish) are infected with *L. intestinalis*. Consequently, the data analysed in this study are based solely on the roach population.

For this survey, we used gill nets of different mesh sizes (10, 12, 14, 17, 21, 27, 32, 40, and 50 mm measured between adjacent knots) to catch a wide range of fish sizes. Just after their capture, we dissected each individual roach to count the plerocercoid larvae present in the abdominal cavity. In parallel, the fish specimens were aged using scale measurements as detailed by Hartley (1947).

2.1.2. Experimental infestation of zooplankton

A grey heron was fed with an infected fish and parasite eggs were obtained after sifting and centrifuging bird faeces (see Gerdeaux, D., 1986. Ecologie du Gardon (*Rutilus rutilus* L.) et du Sandre (*Lucioperca lucioperca* L.) dans le lac de Créteil de 1977 à 1982. Etude de la ligulose du Gardon. Thèse de doctorat d'état, Paris, France). Planktonic copepods were captured at Lavernose-Lacasse gravel-pit using a plankton net (mesh size 100 μm). We considered that the copepods were parasite-free since in a body of water during severe ligulosis of fish we have collected and examined 1,600 living individuals and only found one crustacean infected with one procercoid. All copepods were kept in an experimental tank ($100 \times 50 \times 50$ cm) in water from Lavernose-Lacasse with a temperature of 18 °C and a natural photoperiod. We introduced approximately 10 *Ligula* eggs per individual copepod present in the experimental tank. Coracidia larvae which swim freely in the water are actively eaten by copepods. Four different copepod species were used during experimental infestation: one diaptomid species, *Eudiaptomus gracilis*, and three cyclopid species, *Cyclops vicinus*, *Acanthocyclops robustus* and *Mesocyclops leuckartii*, all naturally occurring in the Lavernose-Lacasse gravel-pit (Loot et al., 2001b). For each copepod species, 20 specimens were taken per day during 30 days following the addition of the eggs (see Loot, G., 2001. Dynamique de la relation interspécifique Gardon (*Rutilus rutilus* L.)-Ligule (*Ligula intestinalis* L.). Thèse de l'Université Toulouse III, Paul Sabatier, Toulouse, France). We determined, under a microscope, the number of parasitic larval forms in the haemocoel of each copepod individual.

2.1.3. Experimental infestation of roach

Ten uninfected 2-year-old roaches (the most susceptible age-class; see Loot, G., 2001. Dynamique de la relation interspécifique Gardon (*Rutilus rutilus* L.)-Ligule (*Ligula intestinalis* L.). Thèse de l'Université Toulouse III, Paul Sabatier, Toulouse, France) were studied through the course of an experimental infection. Each roach was infected in a single aquarium ($20 \times 20 \times 20$ cm) with a temperature of 18 °C and a natural photoperiod. The roaches were challenged with a saturation dose of 10 infected diaptomid copepods of the species *E. gracilis* (proved to be the only receptive crustacean to infestation; see Section 3.1.2) per fish. Once all the

copepods had been consumed, the fish were placed into separate tanks of 20 × 20 × 20 cm each. The experimentally infected fish were then sacrificed after 6 months (reflecting the maturation period of plerocercoids in roach; see Loot, G., 2001. Dynamique de la relation interspécifique Gardon (*Rutilus rutilus* L.)-Ligule (*Ligula intestinalis* L.). Thèse de l'Université Toulouse III, Paul Sabatier, Toulouse, France), and we determined the number of plerocercoid larval forms in the hosts' abdominal cavity.

2.2. Models of parasite aggregation

When considering the distribution of events across a number of individuals (e.g. parasite counts per fish), the Poisson distribution assumes that each event is independent, and all individuals have the same probability of encountering an event (Hald, 1952). In reality, both of these assumptions are highly likely to fail for parasite count data, and the failure of each assumption leads independently to increased aggregation of parasites, as described by the negative binomial distribution (a popular representation of aggregation in parasite burdens, with the parameter k serving as an inverse index of aggregation). Event-dependence, where one event changes the probability of another event, is explored below using contagious distributions (Pólya, 1930). First, we consider innate individual heterogeneity, using compound Poisson distributions (Greenwood and Yule, 1920).

2.2.1. Compound Poisson distributions (innate host heterogeneity)

Compound Poisson distributions describe the distribution of events across populations of individuals with unequal probabilities of experiencing a given event (Greenwood and Yule, 1920). Thus, for the case of parasitic infections, each host has its own 'infection intensity', ξ, indicating the parameter in a corresponding Poisson distribution, which gives the probability that this host will be subject to x infections. Hence, we can write that the probability that a host chosen at random (with unknown infection-rate intensity) is subject to x infections is (e.g. Hald, 1952, p. 728):

$$p(x) = \int_0^\infty p(x;\xi)p(\xi)d\xi \tag{1}$$

where $p(x;\xi)$ describes a Poisson distribution of x with mean ξ:

$$p(x;\xi) = \mathrm{e}^{-\xi}\frac{\xi^x}{x!}, \quad x = 0,\ 1,\ \ldots \tag{2}$$

The shape of the resulting compound Poisson distribution is defined by the distribution of individual infection means, $p(\xi)$. For instance, Greenwood and Yule (1920) proposed the following form of $p(\xi)$:

$$p(\xi) = \frac{\gamma^\alpha}{(\alpha-1)!}\mathrm{e}^{-\gamma\xi}\xi^{\alpha-1}, \quad \xi = 0 \tag{3}$$

which results in a negative binomial distribution of events across all hosts.

2.2.2. Contagious distributions (event dependence)

Contagious distributions emphasise a second source of heterogeneity, the within-individual heterogeneity in infection intensity ξ resulting from previous events or the passage of time. Rather than specifying a fixed value of ξ for each individual, contagious distributions specify ξ as a function of previous events (x) and the passage of time (t). Pólya (1930) proposed the following function for ξ:

$$\xi = \frac{\alpha + x}{\beta + t}, \quad \alpha \geq 0,\ \xi \geq 0 \tag{4}$$

which is increasing on x (i.e. the intensity of contamination increases with increasing number of previous contaminations) and decreasing on t (i.e. the intensity of contamination decreases with increasing time) and which again leads to a negative binomial distribution of x across the population of hosts.

It is noteworthy that the Greenwood–Yule and Pólya distributions, while converging on the negative binomial distribution, result from two very different hypotheses. Greenwood and Yule (1920) assumed that events are independent, and that intensities vary from individual to individual. Pólya (1930), on the other hand, assumed that events are dependent, the occurrence of an event increasing the probability that further events will occur. Thus, a good fit with a negative binomial distribution can be interpreted in at least two ways, demanding further analysis (Hald, 1952; Anderson and May, 1991).

A number of scenarios based on the contrasting compound Poisson and contagious distribution scenarios were explored, and compared with the empirical data on *L. intestinalis* aggregation in their roach intermediate hosts.

3. Results

3.1. Empirical results

3.1.1. Fish sampling

Fig. 1 presents the distribution of burdens across the fish population, summed across 2 years. Clearly the negative binomial distribution offers a much better representation of the data than the Poissonian distribution, reflecting the use of a second parameter, k. Note that whereas the negative binomial entails a steady decline in frequency with rising x (burden number), the observational data show a clear plateauing of frequencies for values of x between 1 and 5. Thus, it is apparent that the smallest burden counts, particularly the count of solitary worms, are particularly underrepresented. The shortage of solitary parasites becomes particularly evident when the data are broken down into a monthly analysis.

Fig. 2 presents graphically the distribution of non-zero

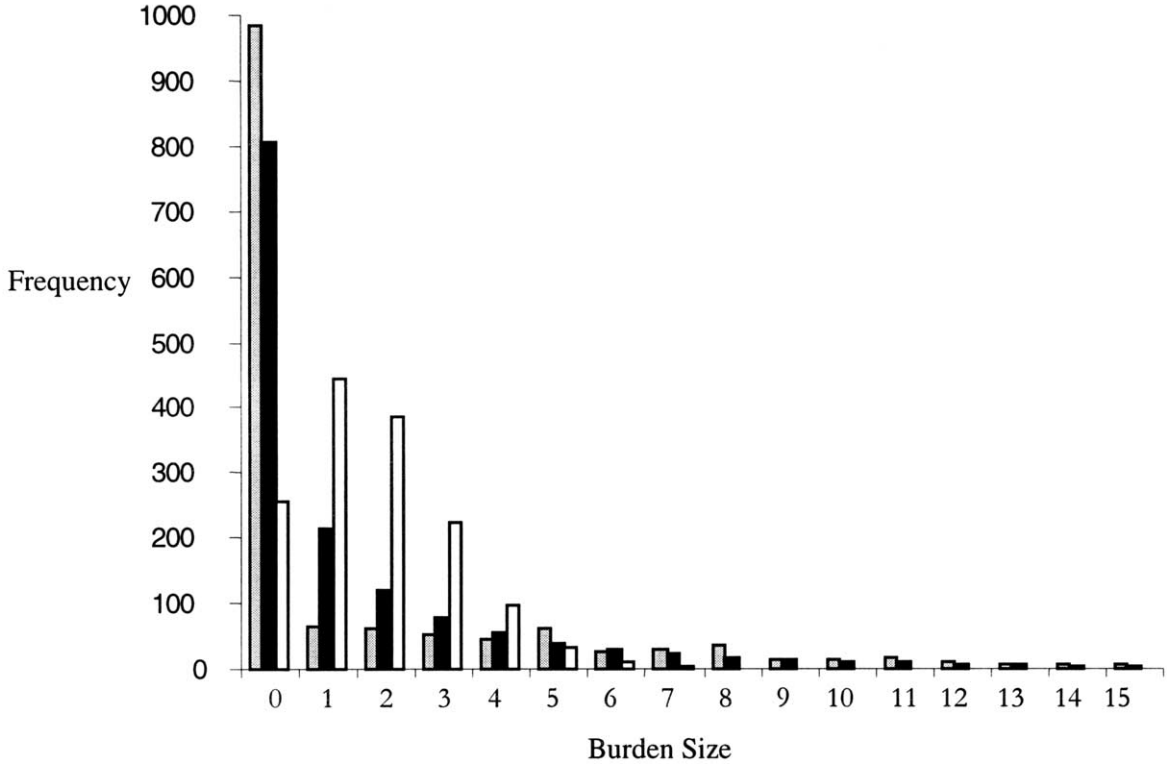

Fig. 1. Distribution of parasite burdens in Lavernose-Lacasse during 1998 and 1999 (mid-grey bars). The expected distributions following the Poissonian (light bars, $\bar{x}= 1.73$) and negative binomial distribution (dark bars, $\bar{x}= 1.73$, $k = 0.31$) are supplied for comparison.

worm burdens month by month. As expected, the modal burden is zero for each sample period, reflecting the combination of strong aggregation and a low mean. More surprisingly, however, a clear majority of sample periods show a subsidiary peak in fish counts for a burden greater than one, suggestive of a bimodal distribution of parasites.

3.1.2. Experimental infestation of zooplankton

After 30 days of experimental infestation, the prevalence of infection reached 99% in the diaptomid species *E. gracilis*, and practically 0% for the three cyclopid copepod species. Invasive procercoids were randomly dispersed among *E. gracilis* hosts ($\bar{x} = 2.69$, $\beta = s^2/\xi = 1.01$) according to a Poisson distribution with an index of aggregation β close to unity (Shaw and Dobson, 1995).

3.1.3. Experimental infestation of roach

Roaches were individually infected with 10 infected *E. gracilis* individuals. After 6 months, only two of the 10 fish were infected, each with only a single plerocercoid. This low success rate in our experimental infections indicates that following multiple challenge (multiple copepods, each multiply infected) not all procercoids are able to establish and mature into plerocercoid larvae in roaches.

3.2. Simulation results

A number of models of host heterogeneity based on the compound Poisson and contagious distributions were explored numerically. Both the Greenwood–Yule and Pólya distributions produce negative binomial distributions, as plotted in Fig. 1 for the values $\bar{x}= 1.73$ and $k = 0.31$. The same values are replotted in Fig. 3a against time, t (the time parameter t is included by making the mean estimate a function of time), illustrating the dynamical approach to the negative binomial distribution of burdens presented in Fig. 1.

In the following two sections, simpler versions of the two contrasting models of aggregation (Section 2.2) are explored, illustrating that bimodalism is possible under both scenarios.

3.2.1. Compound Poisson distributions

Relaxing the continuous distribution of innate susceptibilities – from that underlying the plot in Fig. 3a to a discrete (e.g. Poissonian) distribution – yields a bimodalism when the expected burden distributions are plotted against time (Fig. 3b). Note that a discrete distribution entails a subset of totally resistant hosts (subset with $\xi = 0$). This subset might be reflective of genetically-based resistance. The subset of imperfectly resistant hosts ($\xi > 0$) experiences increasing infection intensities with time (illustrated up to a burden of five in Fig. 3b). As the imperfectly resistant hosts gain further infections, a gap in the burden distribution is established between the perfectly and imperfectly resistant hosts, yielding a bimodal distribution.

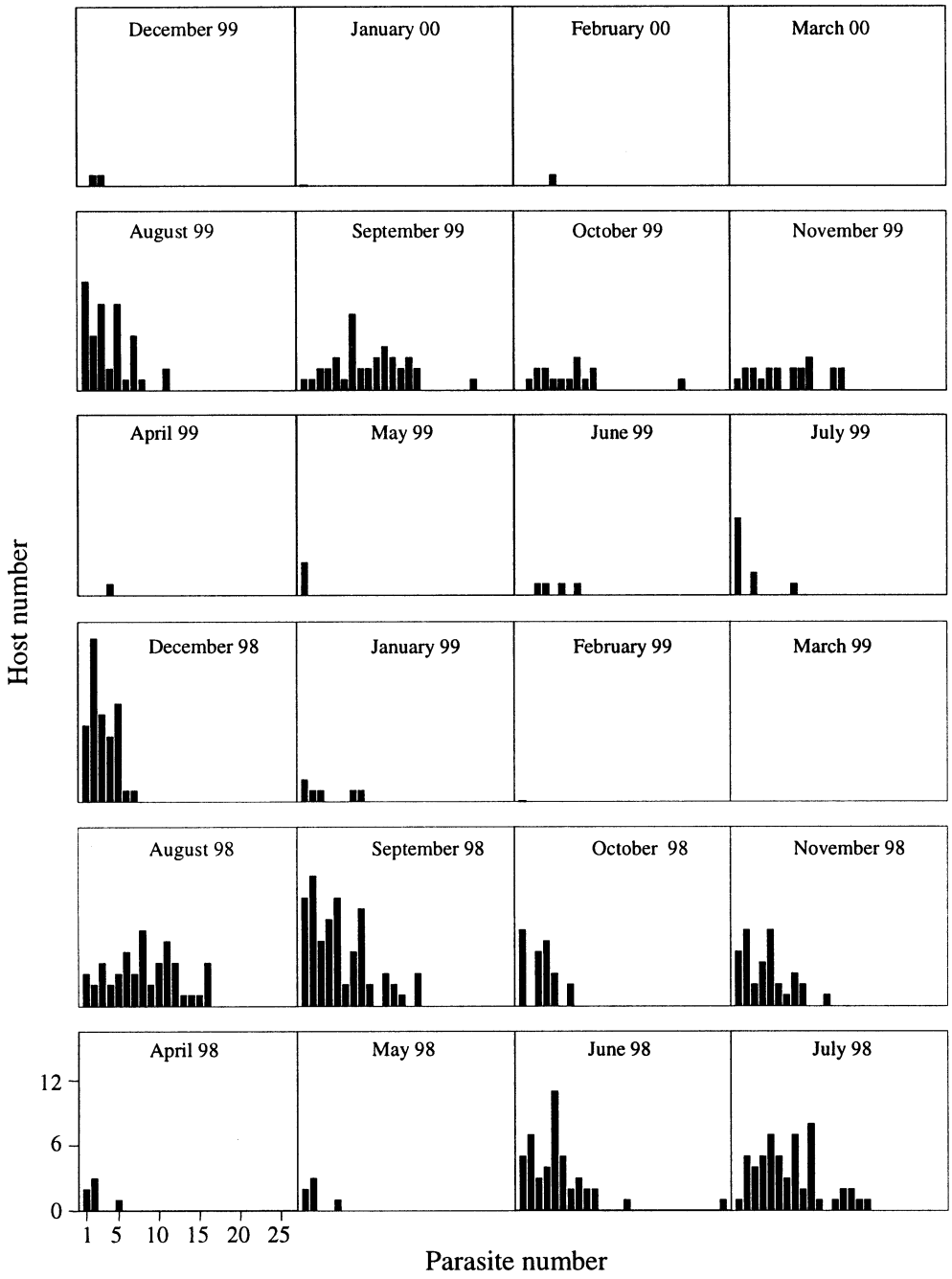

Fig. 2. Distribution of non-zero burdens for Lavernose-Lacasse from April 1998 to March 2000, categorised by month.

3.2.2. Contagious distributions

Relaxing the infection dependence from the function underlying the negative binomial to a simple presence–absence threshold leads to a bimodal distribution (Fig. 4a) (see Loot et al., 2001a for evidence of presence–absence thresholds to manipulative behaviour). The bimodalism is evident by the relative scarcity of single-worm burdens, reflecting the acceleration of the infection rate following an initial infection. Unlike the Pólya function $\xi(x,t)$, here the rate of infection does not continue to increase with increasing burdens, nor does the rate of infection decrease with the passage of time. Note that altering the time-dependence does not influence this qualitative shift from the negative binomial. Evidently, the removal of all dependencies creates a simple Poisson distribution with a single mode (Fig. 4b).

4. Discussion

Anderson and Gordon (1982) used Monte Carlo simulations to explore the interactions among processes affecting

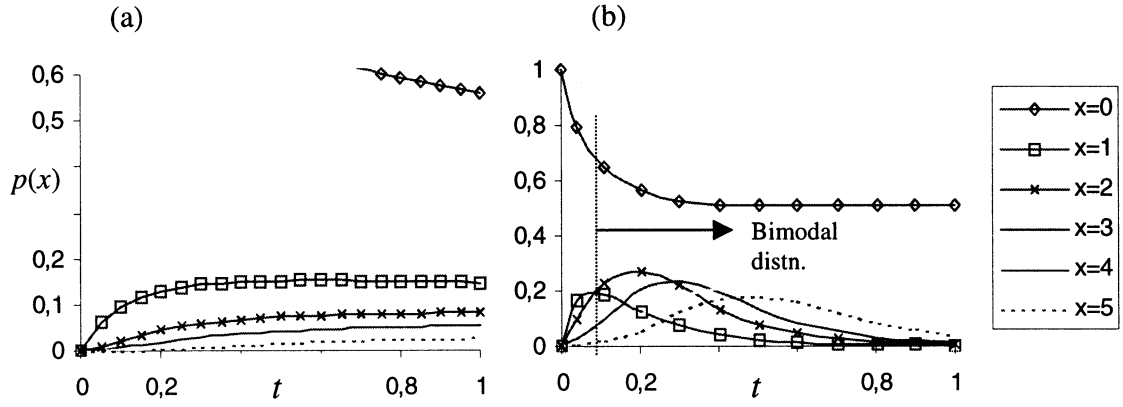

Fig. 3. Distribution of burdens against time, given innate host heterogeneity (compound Poisson distributions). The six lines refer to the probabilities of finding zero, one, two, three, four or five plerocercoid worm burdens, respectively. (a) Innate mean infection rate distributed according to a Greenwood–Yule function, resulting in a negative binomial distribution of parasites (see Section 2.2). The plot ends ($t = 1$) with an identical distribution of burdens to that presented for the negative binomial in Fig. 1. (b) Innate mean infection rate distributed according to a discrete (Poissonian) distribution. The existence of a core of totally unsusceptible hosts (here, 50%) ensures a temporary bimodalism as the wave of increasing infection passes through the susceptible population.

the rates of gain or loss of parasites by host individuals. They demonstrated that heterogeneity in these rates was the major cause of aggregation in the absence of direct reproduction. In this paper, we reviewed two theoretical classes of heterogeneity using two different classes of mathematical models. First, if the rate of parasite acquisition per individual is allocated by a random process, the resulting parasite distributions are aggregated, and can be represented mechanistically by a compound Poisson distribution. Second, if parasite acquisition increases the probability of subsequent parasite acquisition, the resulting parasite distributions are aggregated, and can be represented mechanistically by a contagious distribution.

In addition to the 'compound' and 'contagious' hypotheses (see above), other biological factors need to be considered, for instance the chance of multiple infection when eating a single infected copepod. This is a priori the most parsimonious explanation for the lack of single-worm infections – perhaps fish never pass through the stage of being singly infected, as they always receive infections in packets of two or three. However, during periods of severe fish ligulosis, we failed to find multiply-infected crustaceans in the field, even after the examination of several thousand copepods. Furthermore, our experimental roach infections gave no evidence of the establishment of multiple plerocercoids following multiple procercoid challenge.

Returning to the 'compound' distribution scenario of host heterogeneity, our experimental investigations are indicative of a host heterogeneity independent of the state of infection of the fish, as given identical exposure protocols, only two out of the 10 exposed fish became infected. Evidently these indications require more thorough experimental tests.

Turning to the 'contagious' distribution scenario, we

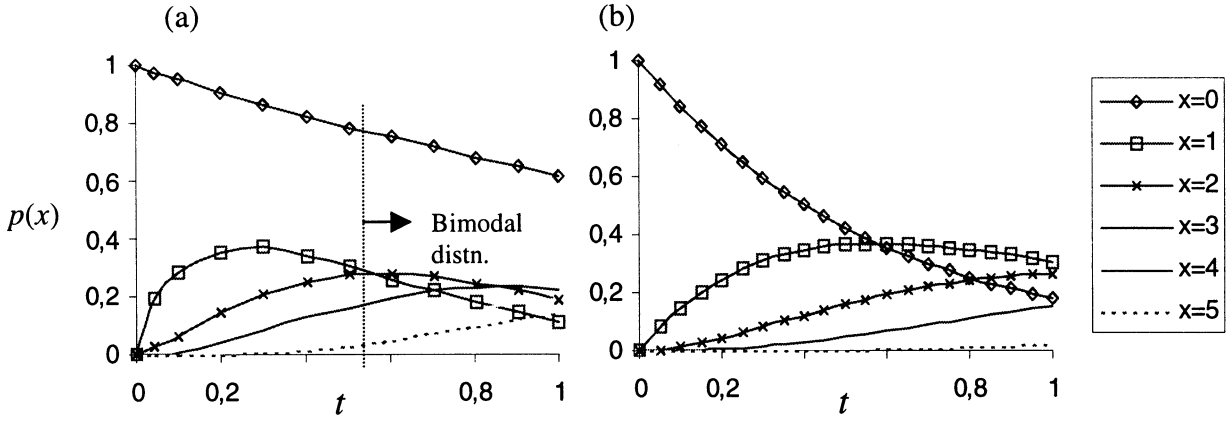

Fig. 4. Distribution of burdens against time, given infection-dependent susceptibility (contagious distribution). The six lines refer to the probabilities of finding zero, one, two, three, four or five plerocercoid worm burdens, respectively. (a) Individual infection rate dependent on the presence of infection; non-parasitised fish have an infection rate of 0.5, whereas infected fish have an infection rate of 3.0. (b) Individual infection rate with both infection state (x) and time (t) dependencies relaxed, producing a Poisson distribution. Note that this plot ends with a distribution of parasite burdens equivalent to that presented for the Poisson distribution with $\bar{x} = 1.73$ in Fig. 1.

considered whether an initial infection with *Ligula* changes the probability of subsequent infection, via effects of the initial infection on host behaviour. Behavioural changes following parasitism have been particularly well documented in a variety of trophically transmitted parasites (see Refs. in Poulin, 2000). For instance, the plerocercoid larvae of the Cestode, *Schistocephalus solidus*, increased foraging behaviour in infested sticklebacks through the effects of an elevated nutrient/energy drain (Walkey and Meakins, 1970; Pascoe and Mattey, 1977; Giles, 1983; Milinski, 1985; Godin and Sproul, 1988). Likewise, *L. intestinalis* grows markedly in the host body cavity (Loot et al., 2002), placing a considerable demand on the energy reserves of its host (Arme and Owen, 1968). Consequently, *L. intestinalis* stimulates host foraging behaviour by increasing feeding motivation (Loot et al., 2001a), as maintained by Pascoe and Mattey (1977), Giles (1983), Milinski (1985) and Godin and Sproul (1988) for the related cestode, *S. solidus*. We have previously discussed the possibility of an adaptive parasite-induced host manipulation in the *L. intestinalis*/roach system, acting to increase parasitic transmission (see Loot et al., 2001a; Brown et al., 2001).

If behaviourally manipulated fish are more likely to encounter infected copepods through increased ingestion due to an elevated energy request, then host manipulation could be a powerful aggregating mechanism, presenting a macroparasitic case of a contagious distribution. In support of the 'contagious' distribution hypothesis, we illustrate the striking scarcity of singly-infected fish, suggesting that following the establishment of a single worm, the probability of subsequent infection is markedly increased (Fig. 3a). However, as discussed in Section 3.2, the same pattern of bimodalism can be accounted for by an innate split between a variably susceptible population and a totally resistant core (Fig. 4a).

The question is a decade old. Is aggregation a cause or a consequence of host manipulation? The causes of host heterogeneity and consequent parasite aggregation are undoubtedly multiple, with both innate heterogeneities (described by compound distributions) and event-dependent heterogeneities (described by contagious distributions) playing a role. In relation to parasite-induced host manipulation, these two theoretical frameworks offer contrasting directions of causality. While the 'contagious' distribution views host manipulation as a cause of aggregation, the 'compound' distribution views host manipulation as a consequence of aggregation (the cause being innate host heterogeneity). We suggest that both directions of causality are liable to exist in tandem. However, experimental studies on host manipulation typically use experimentally manipulated worm burdens, and hence preclude the possibility of feedback between host manipulation and aggregation (see Poulin, 2000 for a review). In the field, this feedback may represent an important cost of host manipulation, due to the typically negative association between parasite worm burden and parasite fitness (e.g. Shostak and Scott, 1993). This cost of aggregation may in turn favour parasite adaptations that act to limit further recruitment by the host (e.g. concomitant immunity; Brown and Grenfell, 2001).

The application of contagious distributions to aggregation in macroparasites allows the formalisation of earlier ideas on acquired (as opposed to innate) heterogeneity in host susceptibility (e.g. Poulin, 1998, p. 100). Contagious distributions are applicable whenever infection causes changes in the host leading to an increased probability of subsequent infection. This chain of events is certainly not limited to cases of adaptive host manipulation, as the increasing energetic demands often associated with parasitism can lead to increased feeding (see e.g. Pascoe and Mattey, 1977, and further references above) and hence increased exposure to parasites.

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