Host manipulation by *Ligula intestinalis*: accident or adaptation?

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(*Received 3 February 2001; revised 19 April and 4 June 2001; accepted 4 June 2001*)

SUMMARY

Numerous studies have demonstrated that parasites with complex life-cycles can cause phenotypic modifications in their hosts that lead to an increased rate of transmission, and suggest that these modifications are the result of parasitic adaptations to manipulate the host. Little attention is paid, however, to separating the possibility of adaptive host manipulation from incidental (if fortuitous) side-effects of infection. In this study we combine statistical and analytical tools to interpret the impact of the macroparasite *Ligula intestinalis* L. (Cestoda, Pseudophyllidea) on the behaviour of its intermediate fish host (the roach, *Rutilus rutilus* L.), using field data on a natural system. Two distinct sets of generalized linear models agree that both the presence and the intensity of infection contribute to a modified behavioural response in the host. This was illustrated by a preference for the lake-edge in infected fish during autumn. Furthermore, the effect of parasites upon their host is heterogeneous with respect to parasite size, with larger parasite individuals having a disproportionate impact. A series of game-theoretic models of adaptive host manipulation illustrate a potential rationale for a size-dependent manipulation strategy in parasites. These findings illustrate the potential complexity and functionality of the impact of *L. intestinalis* upon its fish host, which together reduce the parsimony of the alternative 'incidental effect' hypothesis.

Key words: *Ligula intestinalis*, roach, host manipulation, adaptation, trade-off.

INTRODUCTION

Parasite-induced alterations in host phenotype have been reported for a wide range of macroparasites, particularly in those with complex life-cycles (Poulin 1994*a*, 1998; Thomas, Renaud & Poulin, 1998 and references therein). The simplest explanation is that these changes are non-adaptive coincidental side-effects of parasite infection. As Williams (1992) warned, complex biological systems can create numerous incidental effects which are not necessarily adaptive. Nonetheless, the complex, functional nature of certain parasite-induced changes is suggestive of adaptive modification (see Dawkins, 1986), designed by selection to benefit either the parasite (adaptive host manipulation) or the host (adaptive host defence). According to the manipulation hypothesis, a parasite may alter the behaviour or physiology of its host in order to promote its own transmission (see reviews by Dawkins, 1990; Keymer & Read, 1991; Poulin 1994*a*, *b*, 1995, 2000).

Demonstrating that the expression of a trait correlates with the fitness of its originator can provide evidence of adaptation (Williams, 1992). With reference to the focal system in this study, Loot and co-workers have demonstrated both by experiments and field observation differential microhabitat use in parasitized fish (Loot *et al.* 2001*a*; Loot *et al.* unpublished observations), while Van Dobben (1952) found that 30% of the roach eaten by cormorants in the Netherlands were infected by *L. intestinalis*, whereas only 6·5% of the total roach population were infected by the parasite. Whether biological amplification of parasites in the food chain is good evidence of adaptive host manipulation is unclear, as parasites may increase transmission through no more than a side-effect of infection (Dawkins, 1990). Conversely, adaptive traits may not reveal an association between trait level and fitness, due to the brevity of experimental tests relative to the time-scale of natural selection (Williams, 1992; Ridley, 1993). Thus, positive associations between observed phenotypic alterations and parasite transmission do not guarantee the presence of adaptation. Given the limitations of fitness tests of adaptive hypotheses, it is important to

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Parasitology (2001), **123**, 519–529.
DOI: 10.1017/S0031182001008678 Printed in the United Kingdom

scrutinize the biological details of a proposed adaptation. In the absence of evolutionary experiments, the most general argument in favour of adaptation by natural selection is the conformity between *a priori* design specification and observed phenotype (Mayr, 1983; Williams, 1992).

In this paper, we address the problem of adaptive host manipulation from both an empirical and theoretical perspective. Our empirical study is based on the parasite *L. intestinalis*. This pseudophyllidean cestode has a complex life-cycle, consisting of 2 aquatic intermediate hosts and a definitive avian host. On contact with water, parasite eggs release ciliated coracidia larvae, which are ingested by the copepod first intermediate host. Infected copepods may then be ingested by cyprinid fish, the second intermediate host, where the parasite develops into plerocercoid larvae, the longest-lived parasite stage. Plerocercoid larvae can live within the fishes' abdominal cavity for up to 2 years, growing in this period from microscopic procercoids to a plerocercoid larvae that can exceed the weight of its host (Arme & Owen, 1968). Piscivorous birds complete the parasite life-cycle, being the definitive host. The adult parasite stage is brief, consisting of approximately 1 week of egg production, with eggs being distributed in the bird's faeces.

We present a statistical analysis of observational data on the distribution of *L. intestinalis* among a population of roach in a natural setting. Firstly, following the work of Loot and co-workers (2001*a*), we explore the hypothesis that *L. intestinalis* causes a behavioural change in roach. Secondly we consider the hypothesis that *L. intestinalis* has a size-differential impact on host behaviour, by analysing fish position in relation to individual parasite size. Finally, we present a game theoretical analysis of adaptive host manipulation as a function of individual parasite size, under varying assumptions of behavioural complexity.

MATERIALS AND METHODS

Data collection

The data analysed in this study concern a population of *L. intestinalis* from Lake Pareloup (Garonne basin, south-west France). Monthly overnight roach samples were made from January to December 1998, using 30 m × 1·7 m clear nylon monofilament gill nets, placed at right-angles to the shore. A total of 18 gill nets was placed each month according to the protocol described by Loot *et al.* (2001*a*). The dataset consists of 611 roach. One of 3 distinct capture positions was recorded for each fish, corresponding to differing sections of the net. Following capture, each fish was dissected in order to determine its age (by counting the number of scale rings) and the number of *L. intestinalis* present, together with their individual weight. The following fish variables are used in this study: MONTH (integer covariate), time of capture, coded 1 for January to 12 for December; SEASON (factor), time of capture, coded 1 for January–June and 2 for July–December; AGE (integer covariate), in years; BIOMASS (continuous covariate), total parasite fresh weight in g; SBIOMASS (continuous covariate), total fresh weight in grams of small parasite individuals (individual parasites weighing less than 1 g. The use of this particular threshold value is justified in the Statistical Results section b); LBIOMASS (continuous covariate), total fresh weight in g of large parasite individuals ($> = 1$ g); NUMBER (integer covariate), number of parasites per fish; SNUMBER (integer covariate), number of small parasites (< 1 g) per fish; LNUMBER (integer covariate), number of large parasites ($> = 1$ g) per fish; PRESENCE (factor), coded 1 for parasitized, 0 for non-parasitized; POSITION (integer covariate), position of fish capture: 0 for within 10 m of the shore, 10 for between 10 and 20 m from the bank, and 20 for between 20 and 30 m from the shore. Note that SBIOMASS + LBIOMASS = BIOMASS, and SNUMBER + LNUMBER = NUMBER. As elsewhere in this paper, 'parasite' refers to *L. intestinalis*.

Statistical analysis

In order to explore the relationships between POSITION (indicating fish behaviour) and the various explanatory variables, a series of Generalised Linear Models or GLIM (McCullagh & Nelder, 1989; Crawley, 1993; Wilson, Grenfell & Shaw, 1996; Wilson & Grenfell, 1997) were developed to assess simultaneously which explanatory variables and/or their interactions best explain differences in fish capture position. The introduction of different explanatory variables into models allows certain parameters to be kept constant when interpreting the effect of another variable under investigation. The models were developed using the Splus statistical package (MathSoft Inc. 1999, Seatle WA; Venables & Ripley, 1994).

The response variable POSITION was found to be significantly under-dispersed, having 3 closely matched levels ($n = 204$, 191 and 216 for the near, intermediate and far positions respectively). A series of analyses revealed that a gamma error structure combined with a log-link function provided the most appropriate model, giving a ratio of residual deviance to degrees of freedom close to 1. The use of the raw data allowed the simultaneous comparison of the roles of PRESENCE, BIOMASS and NUMBER in determining POSITION. The variables NUMBER and BIOMASS were nested within PRESENCE = 1, to reduce interdependence among explanatory variables. In addition, a separate analysis was performed on a contingency-table derived from the

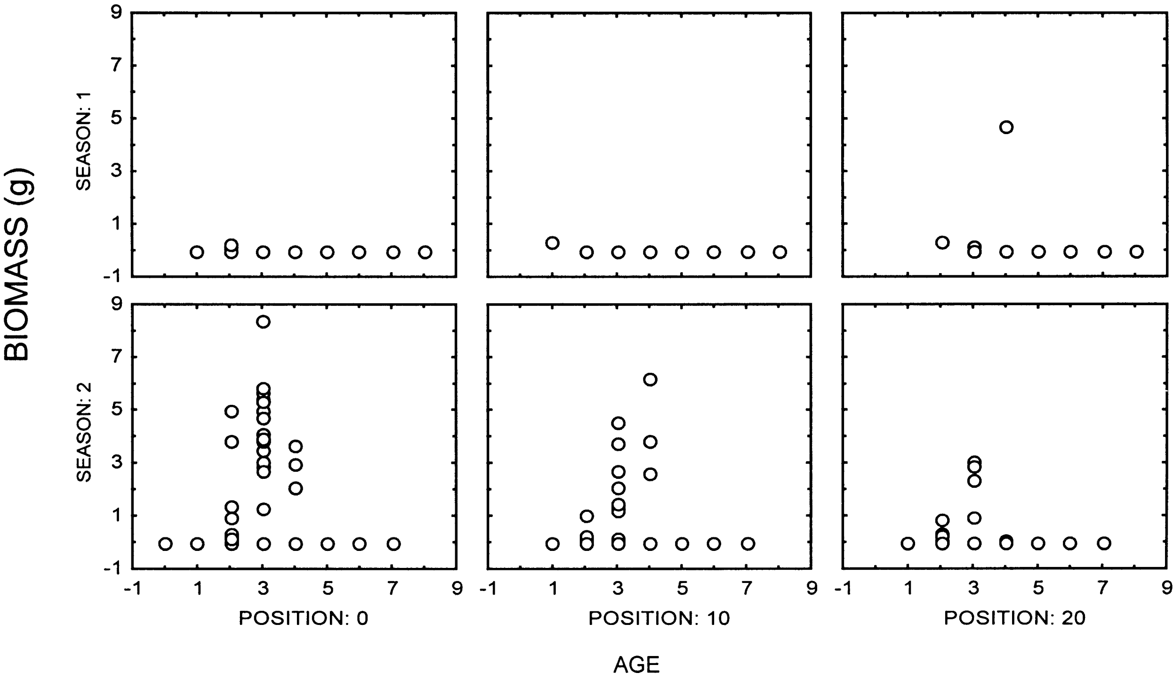

Fig. 1. Scatter-plots of individual fish values of BIOMASS (fresh weight of parasites per fish) on AGE (age of fish in years), for differing combinations of POSITION (fish capture position; columns) and SEASON (fish capture time; rows).

raw data (Crawley, 1993). For clarity, we do not present results from the contingency-table analyses, since they yielded similar results. Materials and methods can be obtained from the senior author on request.

For both the raw data and contingency-table analyses, the same principles of model simplification were followed. Following the use of a maximal model including all terms and their interactions (in some cases the level of interactions was limited by the complexity of the model, though in all cases 2-way interactions were included), a series of minimal models were selected using a backwards stepwise elimination procedure (Crawley, 1993). The significance of GLIM terms were tested by comparing the model deviance (analogous to mean-squares in classical linear models) with and without each term in turn. The use of empirically estimated dispersion parameters determined that the model deviances were compared using F-tests, with the F-values equal to the difference in model deviance with and without each term, divided by the dispersion parameter and by the degrees of freedom gained (McCullagh & Nelder, 1989). Model validity was verified by plotting the residual deviances against the expected values.

STATISTICAL RESULTS

The collective role of parasites in defining capture position

A number of key variables are graphically introduced in Figs 1 and 2, as a prelude to the statistical analyses. Fig. 1 illustrates in a number of scatterplots the distribution of raw BIOMASS data for differing combinations of SEASON, POSITION and AGE. A number of relationships are immediately clear, most notably that the first half of the year (SEASON = 1, upper row) is largely parasite-free. The marked outlier (SEASON = 1, POSITION = 20, AGE = 4) is a rare parasitized host, potentially maintaining the parasite population from one year to the next (larval parasites can live in fish for up to 2 years). Moving to the second season (SEASON = 2, lower row), the overall level of parasitism is higher for practically all combinations of AGE and POSITION, though markedly more so for POSITION = 0 (the near-bank position), and for AGE = 3.

Looking at the role of MONTH in dictating parasite charge, we again see that the first 6 months are virtually parasite-free (Fig. 2A and B). The arrival of new parasites is clearly documented as a change in prevalence (Fig. 2A) between June and July. Note that the mean mass of parasites scarcely changes between these months (Fig. 2B), reflecting the small size of the newly arrived parasite individuals. From July through to November a marked accumulation of parasites is observed in the near-bank position, leading to a prevalence of over 90 % in October and November, coinciding with the arrival of seabird definitive hosts (Fig. 2A). In many sites of *Ligula*–roach interaction, a Great Crested Grebe functions as the definitive host, exerting maximal predation in the spring and summer. However, Great Crested Grebes are unknown at Pareloup, whereas a large and regular overwintering population

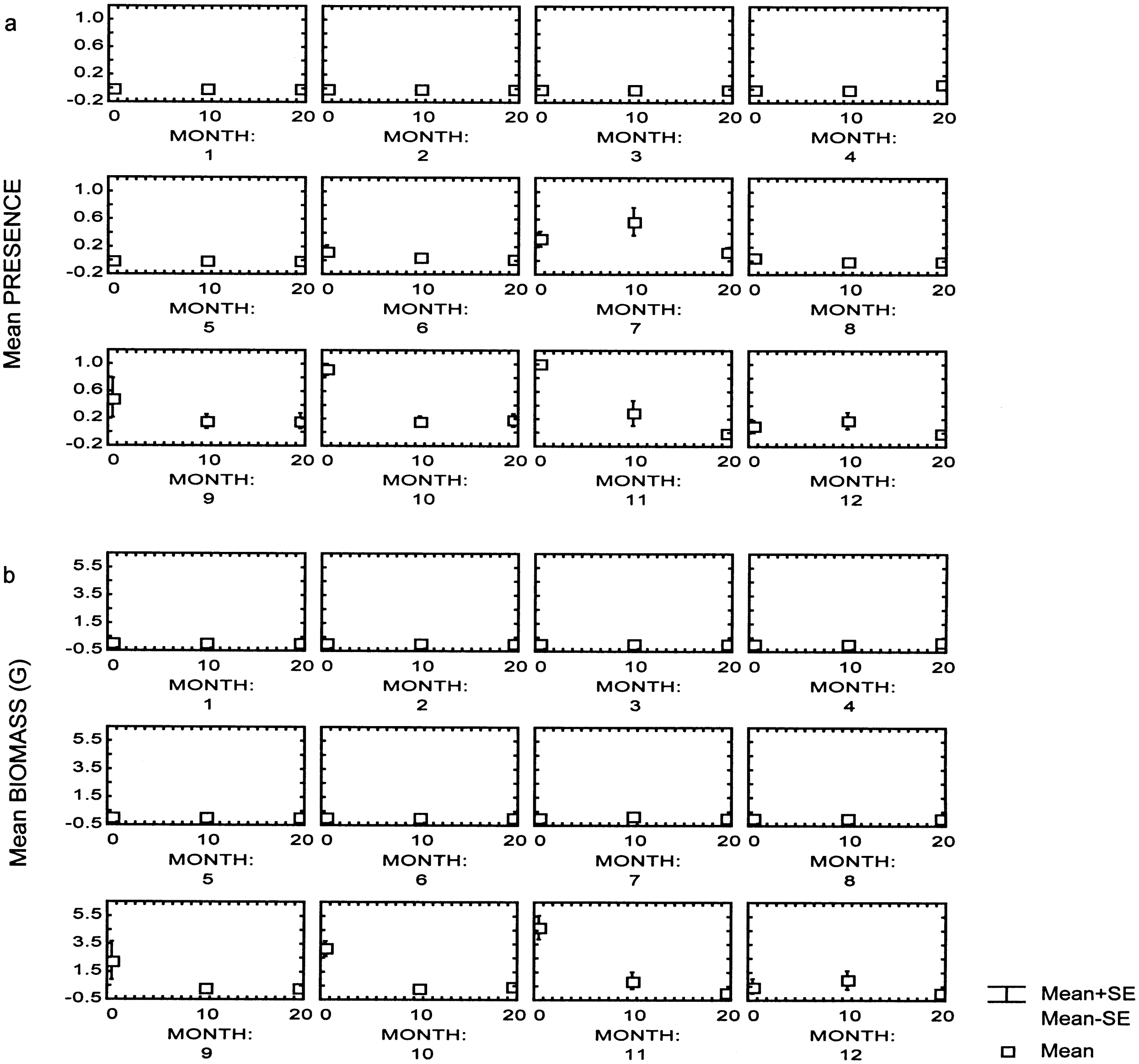

Fig. 2. Mean PRESENCE (parasitized fish coded as 1) per fish ±S.E. (A); mean BIOMASS (fresh weight of parasites per fish) per fish ±S.E. (B); as a function of POSITION (fish capture position) and MONTH (fish capture time).

of Common Cormorants is well-established as the local definitive host (Loot *et al.* 2001*b*). During the heavily parasitized months of September, October and November, the preference of parasitized fish for the near bank position is most clearly seen (Fig. 2A and B). The increase in parasitism during these 3 months is most notable for the BIOMASS scores (Fig. 2B) reflecting both the increasing recruitment of parasite individuals in already parasitized hosts, and the increasing growth of the parasites already present. The arrival of the definitive hosts in late autumn is followed by a marked loss in parasite prevalence and intensity between November and December (Fig. 2A and B).

The minimal adequate GLIM model (Table 1) indicates a significant influence of both parasite presence and intensity of infection (measured both in terms of number of parasites, and total parasite biomass) in determining the capture position of fish. PRESENCE, NUMBER and BIOMASS all have a significantly negative impact on POSITION, indicating that parasitized fish tend to be found closer to the shore, with the intensity of parasitism (as measured by both number and mass of parasites) correlating with proximity to the shore (Fig. 1). The influence of fish age on capture position is found to be marginally significant as a main effect, though strongly significant in interaction with the number of parasite individuals. A further significant interaction term was found between the number of parasite individuals and the sum of parasite biomass. AGE has a marginally significant positive impact on POSITION, indicating that older fish are found further from the shore. The two interaction terms involving NUMBER are strongly significant, suggesting that both AGE and BIOMASS modify the

Table 1. Summary of a Generalised Linear Model for POSITION

(The parameter estimate, residual deviance (r.d.), residual degree of freedom, F-statistic and associated probability are presented below for each model term. The dispersion parameter for the Gamma distribution of errors was estimated at 0·889.)

	Parameter	r.d.	res.d.f.	F-value	$P(>\|F\|)$
Null model		663·8	610		
Intercept	1·976				
AGE	0·040	660·4	609	3·79	0·052
PRESENCE	−0·240	651·7	608	9·78	0·002
NUMBER	−0·881	644·9	607	7·61	0·006
BIOMASS	−0·077	639·4	606	6·28	0·012
AGE * NUMBER	0·530	616·9	605	25·21	0·000
NUMBER * BIOMASS	−0·220	601·3	604	17·55	0·000

role of NUMBER in determining POSITION when NUMBER is kept constant in the analysis.

In agreement with both the graphical and statistical analyses above (Figs 1 and 2 and Table 1), the contingency-table analyses indicated that both parasite biomass and host age play a role in determining fish capture position. In addition, the time of capture was illustrated to have an indirect effect on capture position, as capture time is significantly associated with host age and parasite biomass (results not shown).

The individual role of parasites in defining capture position

Optimality models suggest that individual parasites might only contribute to host manipulation when above a threshold size (see Game-Theoretical Results section). To distinguish the effects of small against large parasite individuals, parasites were first categorized into 6 size bands, based on individual parasite weight. To examine the prediction that only the large parasite individuals cause behavioural changes in the host, we begin with a graphical examination of the distribution of parasite biomass and parasite numbers among the 3 fish capture positions, for 6 categories of individual parasite size (Fig. 3). From this crude analysis, it is clear that regardless of individual parasite size category, a greater number and biomass of parasite are found in the near-bank position, in agreement with Figs 1 and 2, and the GLIM model above (Table 1). It is possible to note, however, that the trend is more marked among the larger parasite individuals, with a threshold existing at approximately 1 g (between parasite categories 3 and 4 in Fig. 3). As noted above, the distribution of 'small' parasites (less than 1 g), is still biased in favour of the near-bank position. This may be, however, a consequence of sharing the host with larger, more manipulative individuals. To attempt to separate these possibilities, we present below a number of GLMs exploring the relative explanatory power of small parasite individuals (<1 g) and large parasite individuals ($>=1$ g) in accounting for host capture position (see Table 2).

To begin to investigate the claim that large parasite individuals are disproportionately significant, 4 simple GLIMs are of assistance. The first 2 explain POSITION in terms of AGE and either NUMBER or BIOMASS (no longer nested in PRESENCE) plus interactions, thus presenting more simple versions of the GLIM presented in Table 1 above (data not shown). The second 2 models (Table 2) are identical to the first pair, except that the variables NUMBER and BIOMASS are replaced with their constituent SNUMBER/LNUMBER and SBIOMASS/LBIOMASS variables plus interactions, in order to gauge the relative importance of small and large parasite individuals in a simultaneous test. Maximal models were used including all interactions, to ensure consistency in model comparisons.

The relative importance of large parasite individuals is first revealed through the much greater explanation of deviance compared to small parasite individuals, which is in turn reflected in the relative F-values of the split parameters. Looking first at parasite mass (Table 2B), large parasites (LBIOMASS) account for 5·2 % of the total deviance (F = 17·08), while small parasites (SBIOMASS) account for 2·0 % (F = 6·66). Likewise for parasite numbers (Table 2A), large parasites (LNUMBER) account for 6·0 % of the total deviance (F = 18·66), while small parasites (SNUMBER) account for 2·3 % (F = 7·11).

The claims of a special role for large parasites remain ambiguous, as the relative importance of large parasite mass can also be accounted for by the simple fact that large parasites make up 75 % of the total parasite mass (but only 37 % by number).

Nonetheless, the disproportionate significance of large parasites is further hinted at by comparison with the 2 simpler models lacking the small–large parasite distinction. The total deviance explained by NUMBER is 20·6, whereas LNUMBER plus

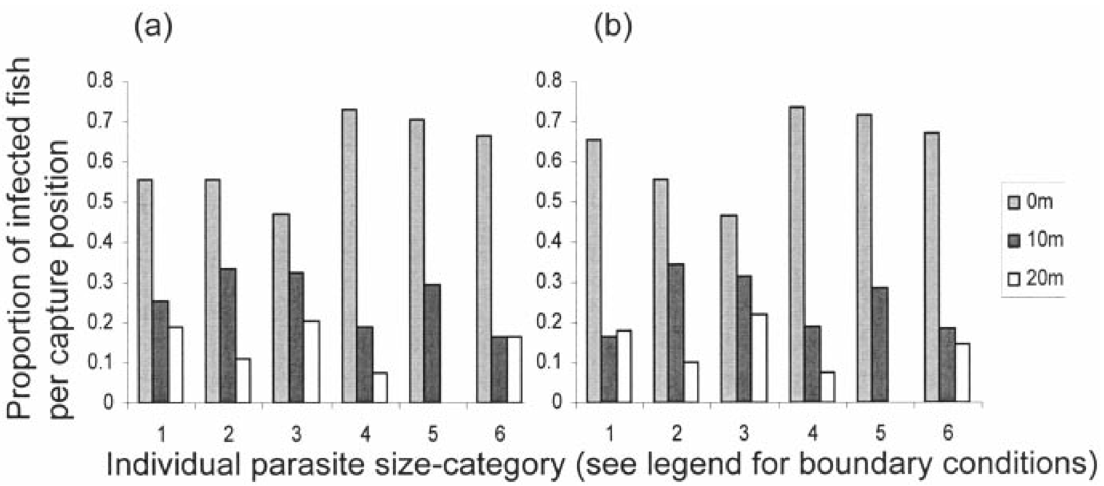

Fig. 3. Distribution of parasite numbers (A) and parasite biomass (B) among fish capture positions, for 6 categories of individual parasite size. The individual parasite size categories are as follows: 1 = 0–0·25 g; 2 = 0·25–0·5 g; 3 = 0·5–1 g; 4 = 1–1·5 g; 5 = 1·5–2 g; 6 = 2 g$^+$ (inclusive of lower boundaries).

Table 2. Two separate GLIMs of POSITION on (A) AGE, SNUMBER, LNUMBER plus all interactions and (B) AGE, SBIOMASS, LBIOMASS plus all interactions

(The models use gamma errors and log-link functions.)

	Parameter	r.d.	res.d.f.	F-value	*P*(> |F|)
(A) Age of host, numbers of small and large parasites. Explained deviance = 19·5 %					
Null model		384·9	324		
Intercept	2·033				
AGE	0·100	379·7	323	4·19	0·014
SNUMBER	−2·618	370·8	322	7·11	0·008
LNUMBER	−15·960	347·5	321	18·66	0·000
AGE * SNUM	1·232	346·2	320	1·08	0·299
AGE * LNUM	5·121	342·4	319	2·99	0·085
SNUM * LNUM	8·391	332·1	318	8·29	0·004
AGE * SNUM * LNUM	−3·031	310·0	317	17·70	0·000
(B) Age of host, masses of small and large parasites. Explained deviance = 18·2 %					
Null model		384·9	324		
Intercept	2·009				
AGE	0·111	379·7	323	4·45	0·036
SBIOMASS	−8·713	371·8	322	6·66	0·010
LBIOMASS	−3·293	351·8	321	17·08	0·000
AGE * SBIOMASS	4·241	351·2	320	0·48	0·488
AGE * LBIOMASS	0·934	336·3	319	12·73	0·000
SBIOMASS * LBIOM.	8·451	328·4	318	6·75	0·010
AGE * SBIOM. * LBIOM.	−3·374	314·9	317	11·50	0·001

SNUMBER explain a combined 32·2, easily justifying the addition of an additional parameter. LNUMBER alone explains 23·3, more than NUMBER alone. The same contrast between BIOMASS and its constituent SBIOMASS plus LBIOMASS reveals a deviance of 27·7 for BIOMASS, and the less-impressive total of 27·9 for its constituent parts. The addition of highly significant interaction terms, however, ensures that for both parasite mass and parasite intensity measures, the introduction of 2 parasite-size subgroups leads to an overall doubling or tripling of model performance (from 11·3 to 18·2 % explained deviance in the mass models; from 6·8 to 19·5 % explained deviance in the number models).

This doubling of model performance following the division of the parasite parameters NUMBER and BIOMASS into large-individual and small-individual subsets is suggestive that parasite individuals are not homogeneous in their effects on host behaviour. The existence of a subset of active players would provide support for the host manipulation hypothesis, as behavioural diversification of this kind is less likely to be an incidental effect (Williams, 1992).

GAME-THEORETICAL RESULTS

This section is based on the assumption that contributing to manipulation carries a cost, in order to create testable predictions separating adaptive from 'incidental effect' hypotheses. If manipulation was simply a costless (and un-alterable) side-effect of parasite growth, then clearly game-theoretical models are not necessary to make predictions on how host manipulation changes with the properties of the parasite infrapopulation. However, it is also possible that the parasitic contribution to host manipulation is an evolutionary variable for parasites, shaped by the marginal value of increased transmission against increased costs of host manipulation. In order to investigate this second possibility, we here examine theoretically the consequences of host manipulation being in part an evolutionary variable for parasites.

The results of the previous section suggest that strategic differentiation exists at the level of the individual parasite, with larger parasites contributing disproportionately to manipulation. Thus we must incorporate a parasite-size dynamic into the analysis. Two contrasting scenarios are developed. The first assumes that parasites only have information on their own size, whereas the second assumes that individual parasites have information on both their own size, and the total parasite biomass within the host.

A simple model of mass-dependent manipulation fitness

Manipulation of the roach by *L. intestinalis* occurs in a social context; up to 30 individual worms may share in the benefits of manipulation even if not all worms contribute to the costs (Loot, unpublished data). Assuming that contributing to host manipulation has a negative fitness impact on the individual, and a positive fitness impact on the group, parasite fitness, w, can be represented as a product of an individual (I) and group (G) component

$$w(m_1 m_2) = I(m_1)G(n\bar{m}). \tag{1}$$

Here m is the individual contribution to host manipulation, and $w(m_1 m_2)$ is the fitness of an m_1 strategist in a group of m_2 strategists. $I(m_1)$ equals the individual fitness function, a declining function of m_1, and $G(n\bar{m})$ equals the group fitness function, typically a rising function of summed host manipulation. Under the assumption of static parasite size, a simple example of a manipulation fitness function (presented earlier by Brown, 1999) is $w(m_1, m_2) = (1-cm_1)(p+n\bar{m})$. Here c represents the cost of manipulation, and p represents passive fitness, the fitness of a non-manipulating parasite in a group of non-manipulators. $n\bar{m}$ is the summed group manipulative effort, $n\bar{m} = m_1+(n-1)[rm_1+(1-r)m_2]$, where r is the pairwise coefficient of relatedness.

To translate this logic to the case of variable parasite size, we can view manipulation strategy m as a rate term, thus making the manipulative effort of an individual parasite $m_s M_s$. The subscript s indicates the focal parasite, thus m_s is the manipulation rate of the focal parasite s, and M_s is the mass of parasite s. So, by analogy with the previous static size equation, we have

$$w(m_1, m_2) = M_s(1-cm_1)(p+M\bar{m}). \tag{2}$$

Here, M is the total parasite biomass within the host, and $M\bar{m}$ is the summed group manipulative effort, $M\bar{m} = M_s m_1+(M-M_s)[rm_1+(1-r)m_2]$. $M\bar{m}$ can be understood as the manipulative effort of the focal parasite biomass, M_s, plus the manipulative effort of the remaining parasite biomas, $M-M_s$, modulated by the pairwise relatedness parameter, r. Following a standard game-theoretic procedure (Maynard-Smith, 1982; Brown, 1999), an ESS of manipulation, dependent on individual size, is found to be

$$m^*(M_s) = \frac{cp-M_s-r(M+M_s)}{c(M_s(r-1)-M(1+r))}. \tag{3}$$

Plotting for arbitrary values of c, p and r (c and p are assumed to be positive, while r is assumed to be low), a threshold function dependent on the size of the focal parasite (M_s) emerges (Fig. 4A). Below this threshold, individual parasites are too small for manipulation to pay, whereas above this size-threshold manipulation becomes a worthwhile investment of individual resources.

Given the naive assumption of a fixed total parasite mass M (plotted here for $M = 5$ g), we find an ESS of manipulation dependent on individual size, showing in Fig. 4A a threshold size close to $M_s = 1$ g, descriptively mirroring the threshold size discussed in relation to the empirical results in Fig. 3 and Table 2. Two potential scenarios follow from this optimization model, dependent on the access individual worms have to information on the total mass of parasites with which they share the host. If individuals do have access to information on the total mass M (perhaps by sensing the density of parasite excretory/secretory products, forming an analogy with bacterial quorum-sensing; see Brown & Johnstone, 2001), then individual parasites could optimize their strategy on both total (M) and personal (M_s) parasite biomass. If not, parasite individuals could at most optimize their strategy on a knowledge of M_s plus an expectation of M.

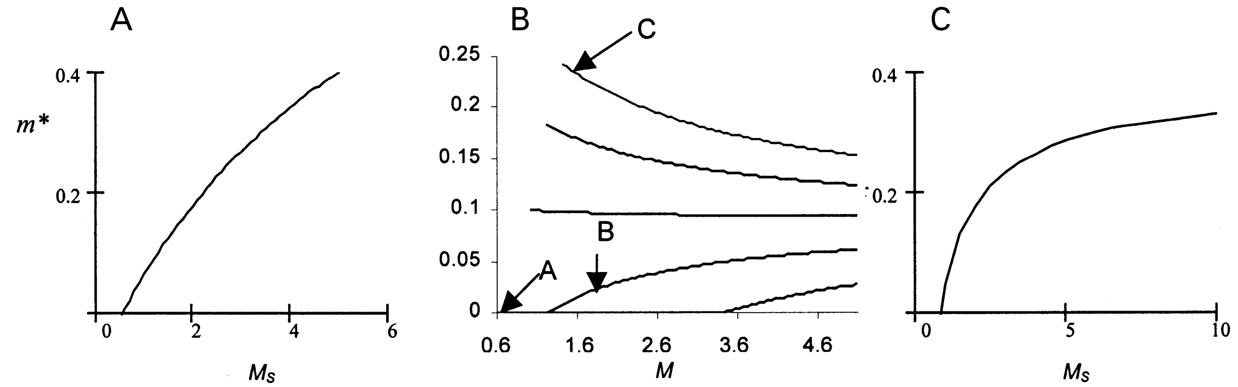

Fig. 4. ESS of host manipulation m^*, as a function of (A) individual parasite mass, M_s. $M = 5$ g, $c = 1$, $p = 1$, $r = 0{\cdot}1$. Drawn from Equation 3; (B) total parasite mass, M, plotted for 5 levels of individual parasite mass, M_s. M_s values for the lowermost to the uppermost lines are 0·6 g, 0·8 g, 1·0 g, 1·2 g and 1·4 g respectively. Other parameters; $c = 1$, $p = 1$, $r = 0{\cdot}1$. For arrows, see text. Drawn from Equation 3; (C) individual parasite mass, M_s. $c = 1$, $p = 1$, $r = 0{\cdot}1$. μ_M and σ_M were estimated from the current dataset to be 0·70 and 1·33 respectively. Drawn from Equation 5.

Complete information (M and M_s)

Fig. 4A illustrates strategic sensitivity to individual parasite mass, M_s. Using the same formula for m^* (Equation 3), we illustrate in Fig. 4B the predictions for a strategic sensitivity on total parasite biomass per host (M), for different levels of individual parasite mass.

When a small parasite is alone (e.g. $M_s = M = 0{\cdot}6$ g, point A, Fig. 4B), manipulation is not an ESS (due to positive passive fitness, see Brown, 1999). As other parasites are recruited ($M \gg M_s$, e.g. point B, Fig. 4B), cooperative manipulation may become worthwhile, with m^* rising with increasing M – above a threshold value. In contrast, a lone large parasite (e.g. $M_s = M = 1{\cdot}4$ g, point C, Fig. 4B) has sufficient resources to make manipulation worthwhile. Here, being joined by others has a dilutory effect on manipulation, due to the decreasing unity within the total parasite biomass, brought about by low r. This dilutory effect is illustrated in Fig. 4B by the decay in m^* for large M_s with increasing M. Note that M is necessarily constrained to be greater than M_s, explaining the lower cut-off points evident in the topmost curves in Fig. 4B.

The threshold between 'small' and 'large' parasites is clearly defined by the middle line in Fig. 4B ($M_s = 1$ g). Below this threshold, manipulation is contingent on group size M. Small individuals can only manipulate as part of larger collective. In contrast, above the threshold, large parasites always manipulate. Furthermore, the threshold value of individual size ($M_s = 1$ g) defines the effect of being joined by additional parasites. Below $M_s = 1$ g, additional parasite recruitment leads to a rise in individual manipulative rate, as the cooperative gains of a small level of investment outweigh the within-host competitive costs. Conversely, above $M_s = 1$ g, parasite recruitment leads to a decline in individual manipulative rate, as the strategic integrity of the total parasite biomass is undermined by the arrival of competing genotypes.

Incomplete information: knowledge of own size (M_s) only

Fig. 4A presents a function $m^*(M_s)$ under the simplest assumption of a static total mass M. In order to find a function $m^*(M_s)$ given a known distribution of M values, the fitness function $m^*(M_s)$ must be integrated over this distribution. The distribution of total parasite biomass will itself be dependent on individual size M_s, as total mass M must always be greater than M_s. A simple probability density function for M given M_s, based on the assumption of a log-normal distribution of total parasite biomass (M) is as follows:

$$P(M) = \mathrm{dlnorm}(M, \mu_M, \sigma_M) / (1 - \mathrm{clnorm}(M_s, \mu_M, \sigma_M)). \tag{4}$$

Here μ_M and σ_M are the mean and standard deviation of the total parasite biomass M per fish, across the host population. dlnorm(x, μ, σ) is an inbuilt log-normal probability density function of the MathCad computer package (MathSoft, Inc. 1995; Seattle WA), returning the probability of x given the population parameters μ and σ. clnorm(x, μ, σ) is a similar inbuilt function returning the cumulative probability of x. Note that the function $P(M)$ has a range from M_s to infinity. As the focal parasite size M_s increases from zero, the probability density function $P(M)$ departs further from the log-normal defined by dlnorm(M, μ_M, σ_M).

Given a distribution function $P(M)$ for M given M_s (Equation 4), we can derive an expression for m^* dependent solely on M_s, μ_M and σ_M, by integrating Equation 3 over $P(M)$, between M_s and infinity.

$$m^* = \int_{M_s}^{\infty} P(M) \frac{cp - M_s - r(M + M_s)}{c(M_s(r-1) - M(1+r))} \, \mathrm{d}M. \tag{5}$$

Equation 5 is plotted in Fig. 4C, using estimates of μ_M and σ_M derived from the current data set. Once more a threshold function emerges against individual size. In contrast, however, to the 'complete information' scenario, m^* here does not change in response to M (the unknown), only in response to the population parameters governing M, namely μ_M and σ_M.

As in Fig. 4B, an M_s of 1 g emerges as a key watershed in parasite behaviour in this system. Above 1 g, manipulation is stable, while below 1 g, manipulative effort is constrained to zero, regardless of total parasite biomass, M. To conclude this modelling section, a number of game-theoretical models can produce results consistent with the suggested threshold size to parasite manipulative effort (see Statistical Results section b). In the Discussion section, we consider how these models can be empirically tested.

DISCUSSION

Accident or adaptation? Testing the hypotheses

The null hypothesis facing this study is that *L. intestinalis* has no effect on the behaviour of its roach intermediate host. The results presented in the Statistical Results section a can be used to reject this null hypothesis, though alternately one can reverse the causality and argue that the statistical association between behaviour (capture position) and parasitism is driven by fish behaviour, not parasites. However, independent experimental results favour the parasite-driven interpretation, as only infected fish show a behavioural preference for surface waters (Loot *et al.* unpublished observations). Furthermore, direct observation of naturally infected fish illustrates differential microhabitat use (Loot *et al.* 2001*a*) and altered morphology (Loot *et al.* 2001*c*). Thus one can conclude in favour of host modification governed by parasite presence, and the intensity of infection as measured both by parasite number and total biomass, modulated by age. The existence of host modification allows, in turn, a range of potential explanations, ranging from the purely accidental, to a variety of adaptive scenarios.

Firstly, we have the incidental modification hypotheses. Behavioural and physiological changes in the host may follow incidentally from the presence of sufficient parasite biomass, number or simply presence itself, for instance due to the increased oxygen demand in parasitized fish (Giles, 1983, 1987). In contrast with the size-dependent behavioural diversification discussed in the Statistical Results section b and Game-Theoretical Results section, the simplest non-adaptive hypothesis predicts that parasites act homogeneously on host behaviour, with no specialist subsets of manipulators (e.g. larger parasites contribute more to manipulation, but only in proportion to their mass). Admittedly, a simple constitutive and homogeneous relationship between parasitism and host phenotypic response could be adaptive, but would at present be more parsimoniously explained by a simple non-adaptive direct effect on host phenotype of parasite presence or activity within the host.

At present, the adaptive manipulation hypotheses are supported only indirectly, with reference to the apparent functionality and complexity of the host modification. The potential complexity of host modification by *L. intestinalis* has been discussed extensively in this paper with reference to size thresholds to manipulation. The apparent functionality is reflected in the probable shift in predation on fish brought about by the change in fish position. The pattern of parasite-induced migration from deep locations to shallow peripheral waters is likely to cause the balance of predation to shift from fish to birds. Unparasitized fish preferentially occupy deeper waters, preferentially encountering fish predators like pike, zander and perch which are not definitive hosts for *L. intestinalis* (but see Sweeting (1976) for a demonstration that pike do feed on infected roach). On the contrary, by heading towards shallow surface waters in autumn, parasitized fish (and hence parasites) increase their encounter rate with avian predators, definitive hosts in the parasite's life-cycle (but see results of Wyatt & Kennedy (1988) showing that there is overwinter mortality of infected fish in the absence of predation).

The empirical suggestion of a greater role for larger parasite individuals, independent of their inevitably larger mass, adds credence to the adaptive host manipulation hypothesis. A number of adaptive scenarios can account for such a threshold phenomenon. Two scenarios based on the costs and benefits of cooperation in socially living parasites were explored in the Game-Theoretical Results section, the incomplete information hypothesis and the complete information hypothesis. How can these various hypotheses be tested? Behavioural assays under controlled infection conditions would allow comparisons between the behaviour of fish infected with single or multiple parasites of varying individual and total sizes.

The two adaptive hypotheses (or more exactly, behavioural diversification hypotheses) are distinguishable from the homogenous effect hypotheses by the prediction that a solitary large worm can produce a greater effect on host behaviour than multiple small parasites of a greater total mass, thus offering a potential rejection of the homogenous effect hypotheses. The complete information hypothesis (Game-Theoretical Results section) predicts that individual parasite behaviour will change in response to both individual and total parasite biomass, so we can distinguish between the 2 adaptive hypotheses by contrasting the behaviours of hosts singly and multiply infected with small parasite individuals. While it is unlikely that anything like the exact parasite size distributions illustrated above can be generated experimentally, by controlling the timing and extent of exposure to infected copepods by naïve fish it may be possible to generate statistically distinct parasite infrapopulations reflecting the distinctions captured in Fig. 4. Note that should the behavioural impact of *L. intestinalis* be found to be heterogeneous with respect to size, further explanations exist beyond the 'social evolution' scenarios developed. Following the work of Poulin, Curtis & Rau (1992) and Tierney, Huntingford & Crompton (1993) on the developmental timetable of host manipulation, it is important to consider the potentially changing nature of manipulation trade-offs through the course of parasite maturation.

To conclude, in this paper we have emphasized the importance of close biological scrutiny in the assessment of adaptive hypotheses. In the case of the host manipulation hypothesis, we argue that, in addition to the standard bench-mark of transmission tests, equal attention should be paid to assessing the complexity and functionality of parasite-induced host manipulation. For *L. intestinalis*, statistical and game-theoretical analyses suggest that individual parasites act heterogeneously to create a purposeful shift in host microhabitat. These findings can serve as a starting point for future experimental studies on the behavioural, mechanical and physiological complexities of the roach/*L. intestinalis* interaction, and other parasite–host systems in general.

We thank Anatoly Teriokhin for help with the statistical analysis. S.P.B. is grateful to the European Science Foundation for financial support to visit the C.E.P.M. Lab, Montpellier, France. G.L. was supported by a junior fellow grant from the French Ministry of Research and Higher Education.

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