Genetic differences in the interactions of a microsporidian parasite and four clones of its cyclically parthenogenetic host

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(*Received 29 March 1993; revised 24 May 1993; accepted 27 May 1993*)

SUMMARY

Host–parasite interactions were studied for the microsporidium *Pleistophora intestinalis* and its host, *Daphnia magna*. Two host clones were established from the same population from which the parasites were taken (home–1 and 2), and two clones from two other ponds (neighbour and Munich clone). With increasing clutch number infected females from home–1 clone produced relatively smaller clutches than uninfected females. Age and body length at maturity were not affected by the infection, but body length of the sixth adult instar was reduced. In an experiment including all four host clones, the parasite reproduced well in the two home clones and in the neighbour clone, but poorly in the Munich clone. Juvenile growth and age at maturity was not affected in the two home clones, but for the neighbour and the Munich clone age was delayed by 2·2 days and 4·1 days, and juvenile growth reduced by 16 and 23%, respectively. Significant host–clone × parasite-treatment interactions were also found for size at maturity and clutch size. This pattern of host–parasite interactions suggests that there is no general positive relation between disease severity and parasite multiplication rate.

Key words: host–parasite interactions, *Daphnia*, Microsporidia, genetics, disease severity, life-history.

INTRODUCTION

Host–parasite co-evolution is believed to be driven by the reciprocal evolution of host resistance and parasite virulence. On the basis of a gene-for-gene type host–parasite interaction (see Thompson & Burdon (1992) and Frank (1992) for recent reviews) only two possible outcomes for any host–parasite interactions are expected: susceptibility, which combines high disease severity with high parasite success, and resistance, which indicates parasite failure and no or little reduction in host fitness. This type of positive relationship between disease severity and parasite reproduction has been used successfully in graphical and mathematical models of host–parasite interactions (e.g. Ewald, 1983, 1988; Frank, 1991). However, the general validity of a positive relationship between parasite success and disease severity is questioned by some studies in which multiplication rate of the parasite and disease severity were measured simultaneously (e.g. Barbosa, 1975; De Nooij & Van der Aa, 1987; De Nooij & van Damme, 1988). For example, De Nooij & van Damme (1988) found no correlation between host mortality and parasite growth in a fungus–plant pathosystem. In the myxoma virus–rabbit system disease-induced host mortality decreases with virus strain I to V, but virus titre in the host is the same for virus strains I, III and IV, and is only lower for strain V (Fenner, Day & Woodroofe, 1956; Dwyer, Levin & Buttel, 1990). Barbosa (1975) studied several strains of two *Biomphalaria* snail species which were infected with *Schistosoma mansoni*. Across strains he found negative correlations between host mortality and number of cercariae shed per day. In summary, from the few studies in which parasite multiplication rate and disease severity have been measured simultaneously, there is no evidence that a positive relation between these two traits exists. Therefore, the quantification of both host fitness reduction and parasite reproduction is needed to gain insight into the evolutionary genetics of host–parasite interactions.

The microparasites of the cyclic parthenogenic crustacean *Daphnia magna* provide an ideal system to study the relation between parasite reproduction and host life-history. Since, so far, nothing is known about genetic interactions of microsporidia and their hosts, *Daphnia* clones with very different genetic background were chosen to cover a wide range of possible genetic interactions with the parasite strain.

MATERIALS AND METHODS

The four *Daphnia* clones used in this study were identified as *Daphnia magna* Straus using the identification keys from Flössner (1972) and Margaritora (1985). Since *Daphnia magna* is easily distinguished from other species of the subgenus *Ctenodaphnia* and no sibling species are reported, it seems unlikely that the four clones belong to different species.

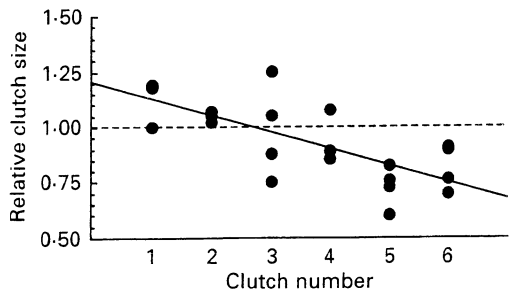

Fig. 1. The effect of microsporidiosis on clutch size in *Daphnia magna*. The relative clutch size is the ratio of the mean clutch size (family mean) of the parasitized to the non-parasitized group. For 4 families this ratio was calculated for each clutch. Parasites and host were from the same pond (home).

Two clones (home–1 and home–2) of *D. magna* were established from a pond in southern Oxfordshire, England, which was designated the 'home pond', since the parasite strain and the clones from this pond were sympatric. Another clone of *D. magna* was established from the 'neighbour pond' 1 km distant from 'home pond'. The three clones were established by collecting individual egg-bearing females in August 1992. A fourth clone of *D. magna* ('Munich clone') was raised from a sexual egg (ephippia) collected from a pond located close to Munich, Germany (about 1000 km south-east of the two other ponds, see description of 'carp pond' by Ebert (1991)). All four clones were kept for several generations in the laboratory before the beginning of the experiments. As clones home–1 and home–2 did not differ in any life-history trait (see below), the possibility cannot be excluded that these clones are genetically identical.

The parasite

The microsporidium present in *D. magna* was identified as *Pleistophora intestinalis* Chatton (Green, 1974; Larsson, 1981, but see Canning & Nicholas (1980) and Larsson (1988) for doubt about the generic affiliation of this species). It is a cytoplasmic parasite of the gut epithelium of *Daphnia magna* and *D. pulex* (Green, 1974). From July to December 1992, this parasite was found in high prevalences (70–100% of all adults) in the home and the neighbour pond. The parasite was identified by its sporophorus vesicles (= SV), of 8–15 μm diameter, found in the epithelial cells of the host gut. The SVs contain variable numbers of pyriform spores measuring 2×3 μm (Green, 1974; Larsson, 1981). Parasite infestation was quantified by counting SVs in the dissected host gut under a microscope at 400 × magnification. Spores expelled from the host with the faeces are infectious for other daphnids when ingested.

Several hundred infected *D. magna* females were collected from the home pond and kept at high density in the laboratory ($\approx$ 200 females/l). Under these conditions infestation of *P. intestinalis* increased rapidly. After 10 days the daphnids were starved to death. At 20 °C the remains decomposed within a week and were then sieved (mesh width 0·1 mm) to remove animal carapaces. The resulting suspension was left for 3 days to allow sedimentation of spores and debris. The water was decanted and the sediments were suspended in 500 ml of fresh medium and kept for less than a month at 4 °C. Half of this suspension was boiled in a waterbath for 1 h to kill spores. A pilot experiment had shown that daphnids treated with the non-boiled suspension became infected, while the heated suspension was not infectious. Suspensions were prepared from two batches of field-collected *D. magna*. The first, prepared in August 1992, was used in the experiment with home–1 clone and the second, prepared in October 1992, was used in the four-clone experiment.

Experiments

Experiments were performed under constant light/dark cycle (16/8 h) at 20 °C. *D. magna* were grown in water collected from a pond in Oxford University park, boiled and filtered through an 8 μm mesh filter. A 1:1 mixture of the monocellular algae *Scenedesmus* sp. and *Chlamydomonas reinhardii* was used as food at a rate of 10^5 cells/ml of culture medium daily.

To minimize the impact of variation in size at birth on host life-history (Ebert, 1991), newborn daphnids obtained from the same clutch (here called 'families') were used for treatment and control. These females from the same clutch were treated as repeated measurements in analysis of variance.

Parasite effect on the home–1 clone

To minimize environmental and maternal effects (Ebert, Yampolosky & Stearns, 1993), 4 replicate lines of the home–1 clone were kept for 2 generations under standardized conditions in beakers containing 120 ml of medium. When the females of the second generation of these 4 lines had their third clutch, 6 newborn daphnids were isolated and each was held alone in beakers with 25 ml of medium. During the first 3 days of their life 1 ml of spore suspension was added to each beaker daily. Three newborn of each family received heated and 3 received non-heated suspension. All daphnids (2 treatments × 4 families × 3 replicates = 24) were fed daily. At day 5, and subsequently after every adult moult, animals were moved to beakers with fresh medium. Body length at the first adult instar (= length at maturity) and the sixth adult instar, the sizes of clutches 1–6 and age at maturity were recorded. All females

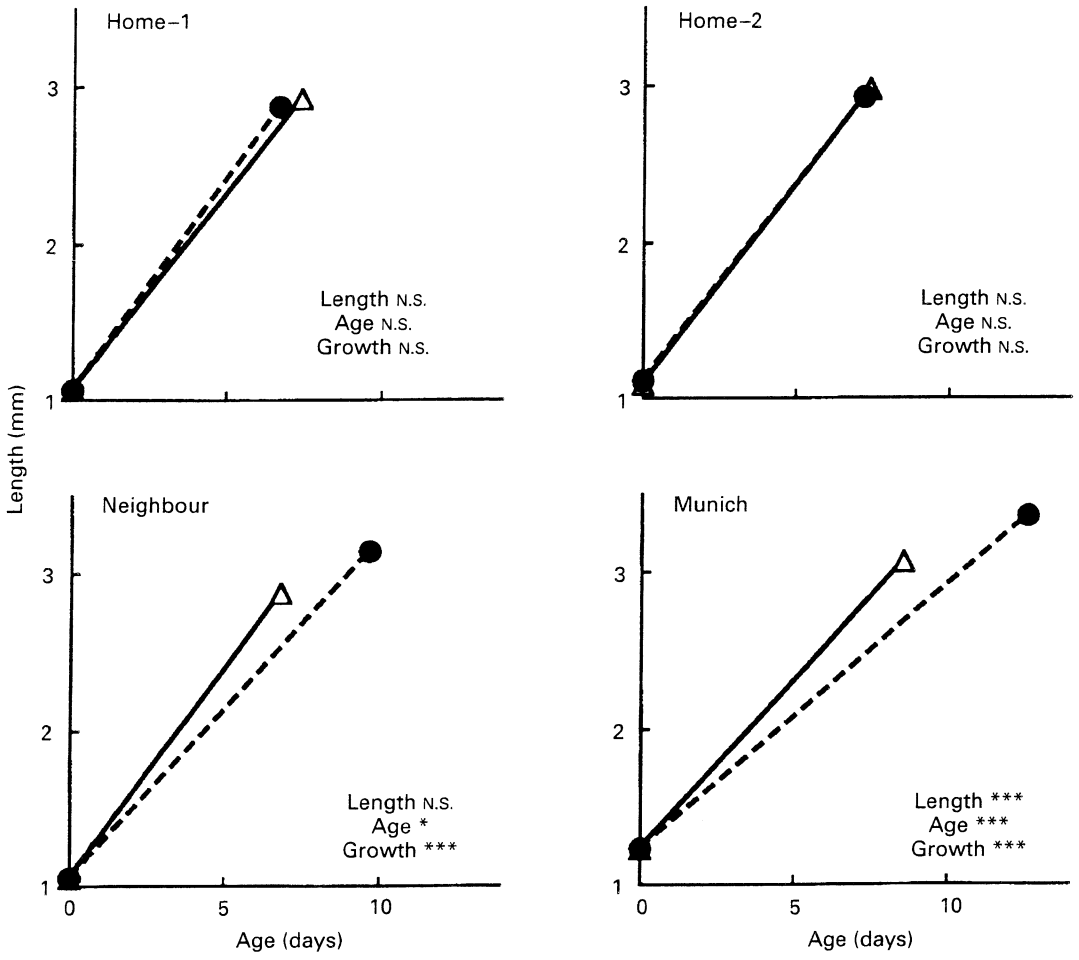

Fig. 2. Growth from birth to maturity for parasitized (--●--) and uninfected females (—△—) from 4 clones of *Daphnia magna*. Clonal means for length at birth and for length and age at maturity are used. For each clone the significance of pair-wise *t*-tests is given, testing for differences in length at maturity (length), age at maturity (age) and juvenile growth (growth). ***$P < 0{\cdot}001$; *$P < 0{\cdot}05$; N.S., not significant.

survived until the sixth adult instar. They were subsequently dissected and examined for parasites by counting the number of SVs.

Four-clone experiment

Ten replicate lines of each of the 2 home clones and 15 lines of the neighbour and Munich clones were kept for 2 generations as described above. When individually kept females of the second generation of these 50 lines produced their third clutch, 3 newborn daphnids from the same clutch of each line were collected. These 150 daphnids were transferred each in a beaker with 25 ml of medium and their body length was measured within 12 h of birth. Two of each set of 3 daphnids received 1 ml of non-heated and the third 1 ml of heated suspension during the first 3 days of life. Beakers were inspected at 12 h intervals. From each of these families one of the two daphnids, which had received the infective suspension, was dissected at day 7 and SVs were counted. The other animals were transferred to beakers with fresh medium. Age and body length at maturity, and the number of eggs in the first clutch, were recorded. All daphnids were dissected at age 13 days and the number of SVs in the gut counted. Juvenile growth rate was calculated as (length at maturity − length at birth)/age at maturity.

RESULTS

One-clone experiment

In all but one of the females treated with the infective spore suspension, large numbers of parasite sporophorous vesicles were found at the end of the experiment (1527 ± 412 S.D.). The uninfected female was excluded from the subsequent analysis. All females that received the heated spore suspension in this and in the four-clone experiment remained parasite free.

Body length at maturity did not differ between the parasitized ($2{\cdot}835 \pm 0{\cdot}123$ mm) and the non-parasitized females ($2{\cdot}842 \pm 0{\cdot}128$ mm, $F_{1,6} = 0{\cdot}01$, $P > 0{\cdot}9$). The same was the case for age at maturity (parasitized $8{\cdot}42 \pm 0{\cdot}333$ days; non-parasitized $8{\cdot}04 \pm 0{\cdot}83$ days, $F_{1,6} = 3{\cdot}74$, $P > 0{\cdot}10$). However, body length of the sixth adult instar was significantly,

Table 1. Two-way analysis of variance for the effects of host clone (with families nested within clones) and parasite treatment

(Dependent variables were body length and age at maturity, juvenile growth rate and the size of the first clutch.)

Source	D.F.	Type III SS	F	P
Body-length at maturity				
Clone	3	1·357	10·55	0·0001
Family (clone)	45	1·929	1·60	0·06
Treatment	1	0·184	6·89	0·01
Clone × treatment	3	0·610	7·61	0·0004
Error	41	1·095		
r-square = 0·81				
Age at maturity				
Clone	3	201·60	25·32	0·0001
Family (clone)	45	119·42	1·31	0·19
Treatment	1	43·53	21·43	0·0001
Clone × treatment	3	82·81	13·59	0·0001
Error	41	83·26		
r-square = 0·86				
Juvenile growth				
Clone	3	0·0779	17·39	0·0001
Family (clone)	45	0·0672	1·91	0·02
Treatment	1	0·0101	13·00	0·0008
Clone × treatment	3	0·0169	7·21	0·0001
Error	41	0·0320		
r-square = 0·85				
Size of first clutch				
Clone	3	19·503	2·35	0·08
Family (clone)	45	124·521	1·48	0·10
Treatment	1	6·877	3·67	0·06
Clone × treatment	3	19·269	3·43	0·025
Error	41	76·812		
r-square = 0·70				

though only slightly, reduced in the parasitized group (3·715 ± 0·087 versus 3·840 ± 0·039 mm, $F_{1,6} = 6·46$, $P < 0·05$). The relative clutch size (parasitized/non-parasitized) significantly decreased over the first six clutches (Fig. 1; $r^2 = 0·56$; corrected for family effect $r^2 = 0·77$, $F_{4,16} = 10·91$, $P = 0·0002$). There was a tendency for early clutches of parasitized daphnids to produce more eggs than did non-parasitized daphnids.

Four-clone experiment

Infections with *Pleistophora intestinalis* had no significant effects on the early life-history of females from the home clones (Fig. 2). In contrast the neighbour clone showed a significantly reduced juvenile growth rate (16 % reduction) and delay in age at first reproduction (2·2 days). The Munich clone showed the strongest response with highly significant effects on age (4·1 days delay) and body length at maturity as well as juvenile growth rate (23 % reduction, Fig. 2). Two-way analysis of variance with the 4 clones and the 2 treatments as main effects revealed significant clone, treatment and also clone × treatment interaction effects for age and length at maturity, as well as for juvenile growth rate

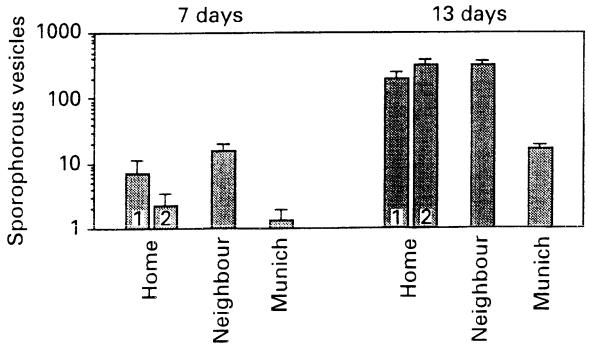

Fig. 3. Number of sporophorous vesicles (±S.E.) of *Pleistophora intestinalis* found in females of four clones at age 7 and 13 days. Note that parasite load is shown on a $\log_{10}$-scale.

(Table 1). The size of the first clutch showed neither a significant clone, nor treatment, effect but a significant clone × treatment interaction.

The number of sporophorous vesicles increased from host age 7 to age 13 by more than one order of magnitude (Fig. 3, $F = 184·3$, $P < 0·0001$). Clone effects were highly significant, with the Munich clone having considerably lower parasite infestation than the three other clones ($F = 25·83$, $P < 0·0001$).

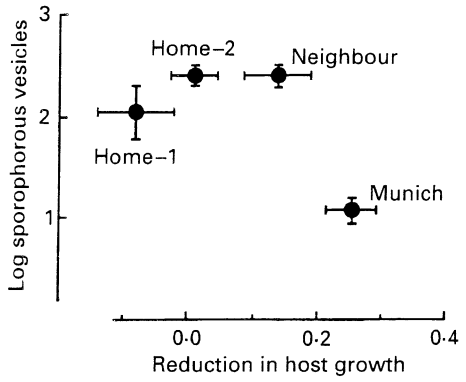

Fig. 4. Relation between number of sporophorous vesicles (age 13, $\log_{10}$-transformed) and reduction in host juvenile growth. Means ± S.E. are given. Reduction in growth was calculated relative to the growth of the parasite-free females, e.g. the Munich clone grew 23 % (≈ 0·23) slower when parasitized. Note that a negative reduction indicates that the parasitized females grew better than the parasite-free females (home–1 clone: +9 %).

To visualize the relation between disease severity and parasite multiplication rate, the number of SVs at age 13 and the relative reduction in juvenile growth rate of the four clones were plotted against each other (Fig. 4). The Pearson correlation coefficient was $r = -0{\cdot}58$ ($P = 0{\cdot}4$, $n = 4$). After correcting the data for clonal effects, no correlation was found among the residuals ($r = 0{\cdot}10$, $P = 0{\cdot}9$).

DISCUSSION

The four host clones differ remarkably in their interactions with the microsporidian parasite *P. intestinalis* in both host response and parasite reproduction. This is the first time that host genotype-specific microsporidiosis has been demonstrated, which underlines for another group of hosts and parasites the importance of genetics in studying host–parasite ecology and evolution (e.g. Toft, Aeschlimann & Bolis, 1991; Ladle, 1992). Genetic differences among clones and populations of *D. magna* have been reported previously for allozyme markers and for various morphological and life-history traits (e.g. Hebert, 1974; Carvalho, 1988; Ebert, 1991), but not for disease resistance.

P. intestinalis reproduction led to an exponential increase over the time in the number of sporophorous vesicles in the host gut epithelium. When being infected with such a parasite, it seems beneficial for the host to mature as early as possible to ensure reproduction before the potential reproductive death of the host (Minchella, 1985). Infected daphnids of the two clones sympatric with the parasite strain showed neither reduced growth nor delayed maturity compared with the uninfected daphnids, while the two allopatric clones did so. Such a delay in age at maturity bears high costs in terms of a reduced intrinsic rate of increase (Stearns, 1992). The parasite, on the other hand, grew well in its home clones and in the neighbour clone, but not in the Munich clone. Although this pattern might indicate local co-adaptation of the sympatric host and parasite, one should not forget that only 4 clones (or possibly just 3) were used in this study. Any conclusion about local adaptation would require the study of more than one clone from each population.

In many host–parasite studies hosts are classified as being 'susceptible' or 'resistant' by considering the performance of the host or the parasite. For example, hosts are classified as susceptible when parasites establish successfully in the host (e.g. Flies & Cram, 1949; Lively, 1989; Burdon & Jarosz, 1991; Shykoff & Schmid-Hempel, 1991). This type of assessment only allows conclusions to be drawn about the evolution of host resistance, when parasite success and host fitness reduction through the disease are positively correlated with each other, as would be the case for a gene-for-gene type interaction (see Introduction section). Classifying the four host clones according to disease severity, one could classify the two home clones as resistant, the two foreign clones as susceptible. On the other hand, using parasite reproduction as an indicator for host–parasite interaction, the Munich clone would be classified as resistant, while the three other clones appear to be susceptible. These two ways of classifying the hosts lead clearly to contrary results for some of the clones, indicating that evolutionary implications on the basis of parasite success or host performance alone does not help to understand co-evolutionary processes. Pathogens which perform well in their home population without severe damage to their hosts, might be the key to understanding why 'resistance' (viewed from the parasite success) to locally occurring pathogens appears to be rare (e.g. Parker, 1985; Clarke, Campbell & Bevan, 1990; Burdon & Jarosz, 1991). Also, see Frank (1992) for a discussion of this problem.

The low parasite fitness found in the host clone with the strongest disease-induced reduction in growth could be the result of an unspecific defence mechanism with high costs for the hosts, e.g. the killing of infected cells (Clem, Fechheimer & Miller, 1991). If defence bears costs for the host (e.g. tissue damage) which are higher than the costs of parasitism, selection would favour host genotypes with a reduced defence. This would result in a fitness increase for the host, but also for the parasite. It could be that the home clones suffered the least from the disease because they reduced the costs of their parasite defence.

I am grateful to J. Green for help with the identification of the parasite, and to W. D. Hamilton, K. Mangin, R. Ladle, S. Krackow, J. Shykoff and two anonymous reviewers for helpful comments on the manuscript. The author was supported by the Deutsche Forschungsgemeinschaft.

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