International Journal for Parasitology 40 (2010) 487–497

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Developmental inflexibility of larval tapeworms in response to resource variation

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ARTICLE INFO

Article history:
Received 20 August 2009
Received in revised form 2 October 2009
Accepted 5 October 2009

Keywords:
Calcareous corpuscles
Cercomer
Developmental threshold
Genetic constraint
Larval life history
Macrocyclops albidus
Phenotypic plasticity
Quantitative genetics

ABSTRACT

The timing of habitat switching in organisms with complex life cycles is an important life history characteristic that is often influenced by the larval growth environment. Under starvation, longer developmental times are frequently observed, probably as a consequence of developmental thresholds, but prolonged ontogeny sometimes also occurs under good conditions, as organisms may take advantage of the large potential gains in body size. I investigated whether variation in growth conditions affects the larval development time of a complex life cycle tapeworm (*Schistocephalus solidus*) in its copepod first host. Moreover, I reviewed patterns of developmental plasticity in larval tapeworms to assess the generality of my findings. Copepod starvation weakly retarded parasite growth but did not affect development. Worms grew bigger in larger copepods, but they developed at a similar rate in large and small hosts. Thus, *S. solidus* does not delay ontogeny under good conditions nor does it fail to reach a developmental threshold under poor conditions. Although unusual in comparison to free-living organisms, such inflexibility is common in tapeworms. Plasticity, namely prolonged ontogeny, has been mainly observed at high infection intensities. For *S. solidus*, there were large cross-environment genetic correlations for development, suggesting there may be genetic constraints on the evolution of developmental plasticity.

1. Introduction

Organisms with complex life cycles spend their larval and adult lives in distinct habitats. The timing of habitat switching is an important life history characteristic that is expected to be shaped by a tradeoff between larval size and developmental time (Roff, 1992; Stearns, 1992). Larval size is often positively related to fitness, as it may increase survival and fecundity in the adult habitat (e.g. Scott, 1994; Phillips, 2002; De Block and Stoks, 2005). However, all else being equal, it takes longer to grow to a large size, which increases the risk of dying before moving into the next habitat.

The size and age at which habitat switching occurs is generally dependent on environmental factors, such as temperature (Atkinson, 1994), predation risk (Ball and Baker, 1996; Relyea, 2007), photoperiod (Johansson et al., 2001; Rolff et al., 2004) and growth rate. How variation in growth rate, typically manipulated by altering food availability, might modify the optimal transition time has been particularly well studied, both theoretically (Werner, 1986; Rowe and Ludwig, 1991; Berrigan and Koella, 1994; Abrams et al., 1996; Day and Rowe, 2002) and empirically (e.g. Twombly et al., 1998; Hentschel and Emlet, 2000; Phillips, 2002; De Block and Stoks, 2005; Rudolf and Rödel, 2007). The most commonly observed pattern is that organisms switch habitats later and at a small size with low food availability (i.e. a negative relationship between size and age; reviewed to varying degrees by Hentschel and Emlet, 2000; Morey and Reznick, 2000; Bruce, 2005). This is somewhat counterintuitive, because poor conditions would presumably favour rapid development and an early escape from the larval habitat. Prolonged development in poor conditions, rather than reflecting an optimal transition time, may arise as a consequence of developmental constraints (Wilbur and Collins, 1973; Day and Rowe, 2002; Teder et al., 2008). A minimum size or condition is often necessary for successful transition into the next habitat (e.g. Davidowitz et al., 2003; Plaistow et al., 2004; Lind et al., 2008). The time needed to arrive at such a developmental threshold is longer under poor growth conditions, so an early escape from the larval habitat, although favourable, may be impossible. By contrast, under good conditions the developmental threshold is reached rapidly, and the optimal time spent growing beyond the threshold depends on the balance between size gains and mortality costs.

Numerous parasites have complex life cycles in which they switch hosts multiple times, often via trophic interactions (Parker et al., 2003; Poulin, 2007). Parasite growth and mortality rates in different hosts are thought to influence when host switching should occur (Choisy et al., 2003; Parker et al., 2003; Gandon, 2004; Iwasa and Wada, 2006; Lagrue and Poulin, 2007; Ball et al., 2008; Hammerschmidt et al., 2009; Parker et al., 2009), so like free-living organisms, the optimal developmental time for parasites is expected to vary between environments of different quality. However, how larval parasite development responds to changes in growth conditions is not well-established. A few studies

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0020-7519/$36.00
doi:10.1016/j.ijpara.2009.10.001

have found that parasite development is retarded in starved intermediate hosts compared with well-fed hosts (Voge, 1959; Skorping, 1984; Shostak and Dick, 1986), but others have not (Sandland and Minchella, 2003; Shostak et al., 2008), so it is unclear if the reaction norms typical for free-living organisms also describe developmental plasticity in complex life cycle parasites.

I studied the larval development of a tapeworm with a complex life cycle, *Schistocephalus solidus*. Copepods serve as the first host, and parasites must develop to a certain stage before being able to infect the next host (three-spined sticklebacks) (Clarke, 1954). I tested the effects of several environmental factors on parasite growth and development. Moreover, I partitioned the variation in parasite phenotypes into genetic and environmental components. Previous studies found clear between-sibship variation for larval growth and development in *S. solidus* (Hammerschmidt and Kurtz, 2005; Benesh, 2009), but the extent to which this genetic variation depends on environmental conditions is not known. The expression of genetic variation is often environment-dependent (Hoffmann and Merilä, 1999; Sgrò and Hoffmann, 2004; Charmantier and Grant, 2005), but few studies on larval development in organisms with complex life cycles have estimated genetic parameters under different levels of resource availability (Gebhardt and Stearns, 1988; Newman, 1994; Kause and Morin, 2001; Blanckenhorn and Heyland, 2005). The presence or absence of genotype by environment interactions might hint at genetic constraints on plasticity (Via and Lande, 1985). Finally, I reviewed the literature on developmental plasticity in larval tapeworms to assess the generality of my findings.

2. Materials and methods

2.1. Study system

S. solidus is a simultaneous hermaphrodite with a three-host life cycle. Adult worms live in the intestine of fish-eating birds, where they mate and produce eggs. The eggs are released into the environment with the host faeces. Free-swimming coracidia hatch from the eggs and are eaten by freshwater copepods. Parasites penetrate the copepods' intestine and develop in the body cavity. Transmission to the second intermediate host, three-spined sticklebacks (*Gasterosteus aculeatus*), occurs when an infected copepod is eaten. Parasite sibships that grow larger in copepods induce a lower innate immune response in sticklebacks (Hammerschmidt and Kurtz, 2005), suggesting that a large transitional size is advantageous. Parasites grow for several weeks in sticklebacks before they are able to infect birds.

Infected sticklebacks were collected from Skogseidvatnet, Norway (60°13′N 5°53′E), with minnow traps and seine nets in September 2006. Worms were dissected from fish, weighed and then bred in pairs. All worms used for breeding (and the majority of those collected) were larger than the apparent threshold size for maturation (50 mg; Tierney and Crompton, 1992). Pairs were formed according to size, so as to promote out-crossing (Lüscher and Milinski, 2003), and then placed into an in vitro breeding system that simulates a bird gut (developed by Smyth (1946) and modified by Wedekind (1997)). Each worm pair is hereafter referred to as a parasite sibship. Eggs were collected and stored at 4 °C until needed. To induce hatching, eggs were incubated at 20 °C for 3 weeks in the dark and then exposed to light 1 day before use.

2.2. Measuring parasite development

Benesh (2009) found that larval growth was positively correlated with two developmental traits (cercomer presence and calcareous corpuscle area) in *S. solidus*. Thus, when gauged with these traits, development may simply follow changes in parasite growth. An additional measure of parasite ontogeny that is not necessarily growth-dependent would be useful for examining phenotypic plasticity in development. Clarke (1954) noted that the calcareous corpuscles, conspicuous concretions composed of inorganic and organic components, increase in size and number as worms mature into fully-developed procercoids. If this increase is an isometric function of overall body size, then the relative extent of corpuscle development may be a measure of ontogeny that is not unavoidably dependent on parasite growth. For instance, small worms may be limited in the absolute number of corpuscles they can accumulate, but their corpuscle area relative to body size may approach or exceed levels in larger worms. Thus, I examined the relationship between parasite size, age and corpuscle development.

I experimentally infected copepods (*Macrocyclops albidus*) that were kept in laboratory culture (culture details described by van der Veen and Kurtz (2002)). The laboratory population was started with about 80 individuals collected from the "Kremper Au", a small creek in northern Germany (54°6′N 10°47′E). Adult male copepods were individually isolated in the wells of a microtitre plate (24-well, ~1.5 ml), and starved for 1 day before exposure. Each copepod was exposed to a single coracidium from one of four parasite sibships. Copepods were then kept at 18 °C with a 18:6 h light:dark cycle and were fed 11 freshly hatched *Artemia salina* nauplii per week. At four different time points (11, 13, 15 and 20 days post exposure (PE)), infected copepods (n = 176) were randomly chosen for dissection. Copepods were anesthetized with a drop of carbonated water and worms were teased out using fine needles. Live parasites were photographed twice, worm size (area) was measured using the freeware Image J 1.38x (Rasband, W.S., NIH, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/, 1997–2009), and the two measurements were averaged to give values for individual worms. Worm size was calculated without the cercomer (also in the second infection, Section 2.3). Including it in the calculation might have lead to discontinuous size distributions, because some worms had not developed a cercomer. Nevertheless, all results were unchanged when the cercomer was included in the calculation of worm size. To quantify the area of corpuscles, worms were killed with a drop of 5% formalin and then immediately pressed under a coverslip in 10 µl of water. Killing worms in formalin does not have any obvious impact on their corpuscles (unpublished observation). The flattened worms were photographed and total corpuscle area was estimated using Image J. Corpuscle area was analysed with analysis of covariance (ANCOVA) using parasite size and age (11, 13, 15 or 20 days PE) as predictors. Log transformations, often used in allometric analyses, were not implemented here because the relationship between corpuscle area and size is obviously linear with a non-zero intercept (see Fig. 1A). Transformations can be misleading under these circumstances (see hypothetical example 1 in Packard and Boardman, 2008).

2.3. Phenotypic plasticity in larval growth and development

In this experiment, the resources available for larval parasites were manipulated in two ways: (i) by using different host stages and (ii) by controlling the food intake of the host. To obtain different copepod stages, single, egg-bearing female copepods were placed into small tanks (18 × 12 × 11 cm) and offspring were collected either 2 or 3 weeks later. After 2 weeks, copepods were still juveniles (copepodites) and mostly small copepodites (C1–C3) were collected. After 3 weeks, many copepods matured into adults. Only adult males were taken as they are more susceptible to infection than females (Wedekind and Jakobsen, 1998). I again used laboratory-bred copepods, but to minimise host-related variation I