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Alternative development in *Polystoma gallieni* (Platyhelminthes, Monogenea) and life cycle evolution ☆

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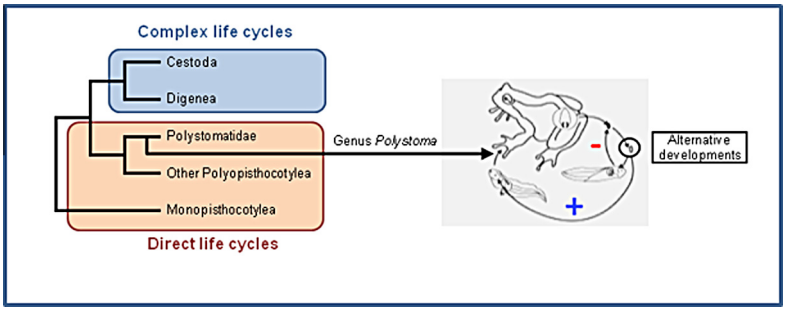
HIGHLIGHTS

- Developmental plasticity is investigated within a monogenean species.
- Infestation success depends on the parental origins of infective larvae.
- Evolution of parasitic life cycles is constrained by developmental features.

GRAPHICAL ABSTRACT



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ABSTRACT

Considering the addition of intermediate transmission steps during life cycle evolution, developmental plasticity, canalization forces and inherited parental effect must be invoked to explain new host colonization. Unfortunately, there is a lack of experimental procedures and relevant models to explore the adaptive value of alternative developmental phenotypes during life cycle evolution. However, within the monogeneans that are characterized by a direct life cycle, an extension of the transmission strategy of amphibian parasites has been reported within species of *Polystoma* and *Metapolystoma* (Polyopisthocotylea; Polystomatidae). In this study, we tested whether the infection success of *Polystoma gallieni* within tadpoles of its specific host, the Stripeless Tree Frog *Hyla meridionalis*, differs depending on the parental origin of the oncomiracidium. An increase in the infection success of the parasitic larvae when exposed to the same experimental conditions as their parents was expected as an adaptive pattern of non-genetic inherited information. Twice as many parasites were actually recorded from tadpoles infected with oncomiracidia hatching from eggs of the bladder parental phenotype (1.63 ± 0.82 parasites per host) than from tadpoles infected with oncomiracidia hatching from eggs of the branchial parental phenotype (0.83 ± 0.64 parasites per host). Because in natural environments the alternation of the two phenotypes is likely to occur due to the ecology of its host, the differential infection success within young tadpoles could have an adaptive value that favors the parasite transmission over time.

1. Introduction

Processes involved in the origin and diversification of complex life cycles and developmental stages among parasitic platyhelminthes have been debated for more than two decades but still remain a source of interest and controversy (Rohde, 1994; Littlewood et al., 1999; Boeger and Kritsky, 2001; Olson and Littlewood,

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2002; Lockyer et al., 2003; Parker et al., 2003; Park et al., 2007; Perkins et al., 2010). According to the most comprehensive phylogeny of the Neodermata, the Monogenea (Monopisthocotylea and Polyopisthocotylea) is considered as paraphyletic and the Polyopisthocotylea is regarded as the sister group of the Digenea – Cestoda clade (Perkins et al., 2010). This suggests that a diet change from epithelium to blood feeding would have precluded the diversification of within-host habitats and the sophistication of transmission patterns (Perkins et al., 2010). Furthermore complex life cycles would have arisen independently following addition of invertebrates as intermediate hosts, mollusks and crustaceans, respectively within the Digenea and Cestoda (Park et al., 2007; Perkins et al., 2010). Therefore if we consider the addition of intermediate transmission steps during life cycle evolution, developmental plasticity and developmental canalization forces must be invoked to explain new host colonization and maintenance of life cycles (see Badets and Verneau, 2009). This conflicting scenario relies on a possible parental effect whether plastic traits are directly inherited or not. Indeed, the offspring performance of the phenotype that invades new hosts has to be strong to be selected, and the arising phenotype must also produce infective larvae that are able to reenact the former phenotype. So far, the importance of parental effect models and their role in adaptive development have been depicted to mostly favor the selection of plastic traits (see Badyaev and Uller, 2009). Unfortunately, the lack of experimental procedures and relevant models to explore the adaptive value of alternative developmental phenotypes during host colonization rendered this hypothesis untestable.

Whether addition and deletion of intermediate hosts have been quite common events during evolution of parasitic platyhelminthes (Poulin and Cribb, 2002; Cribb et al., 2003; Lefebvre and Poulin, 2005; Lagrue and Poulin, 2007; Benesh, 2010), only the Digenea with abbreviated life cycles have been investigated to question the role of developmental plasticity during life cycle evolution (see Lagrue and Poulin, 2009). On the other hand, within the monogeneans that are characterized by a direct life cycle, an extension of the transmission strategy of amphibian parasites has been reported within species of *Polystoma* and *Metapolystoma* (Polyopisthocotylea; Polystomatidae) (Combes, 1967, 1968; Du Preez and Kok, 1992; Maeder, 1973; Murith et al., 1977, 1978; Murith, 1979, 1981; Kok and Du Preez, 1989, 1998; Kok, 1990). In these two genera, an additional step of transmission may be considered because the parasite shows two alternative phenotypes depending of the ecological stage of amphibians. It is actually the sole case reported so far where developmental plasticity allows reproduction in distinct developmental stages of the host, which extends the life cycle of the monogenean species. The bladder phenotype, which occurs in the urinary bladder of the adult frog, releases eggs that are flushed out with the urine of its host. The resulting infective stage, *i.e.* the oncomiracidium, is able to trigger alternative developmental strategies depending on the developmental stage of the frog tadpole at the time of infection (Badets et al., 2010). If one oncomiracidium infects a tadpole in pro-metamorphosis, it develops slowly towards the bladder phenotype within the branchial cavity and migrates to the urinary bladder of the froglet during host metamorphosis, where it reaches maturity (Gallien, 1935; Williams, 1961; Combes, 1968). This strategy reflects actually a classic instance of a direct life cycle. However, if an oncomiracidium attaches to the gills of a tadpole in pre-metamorphosis, it develops quickly towards the branchial phenotype, reaching sexual maturity between 16 and 30 days. This phenotype reproduces on gills and dies when gills are resorbed during host metamorphosis. The offspring of the branchial phenotype thus provides an indirect and significant supply of infective stages in the course of parasite transmission as long as the host tadpole does not reach metamorphosis (Badets et al., 2010). Because the branchial phenotype of *Polystoma* and *Metapolystoma* species acts as an intermediate step, it gathers the two fundamental requirements behind the extension of direct life cycles: the conquest of an unusual habitat and a significant derived role in the course of the parasitic transmission. Although the addition of this intermediate step within the life cycle of *Polystoma* does not perfectly match to the evolution of complex life cycles, which usually involves invertebrates as intermediate host species, it is nonetheless appealing to measure the relative performance of both derived phenotypes as they represent the closest relatives of such evolution.

In this study, we tested under experimental condition favoring the development of one or the other phenotype whether the infection success of *Polystoma gallieni* within tadpoles of its specific host, the Stripeless Tree Frog *Hyla meridionalis*, differs depending on the parental origin of the oncomiracidium. Among young tadpole, we expected that the infection rate of larvae hatching from eggs released by the branchial phenotype would be higher than the infection rate of larvae hatching from eggs released by the bladder phenotype. In that specific case the addition of an intermediate step within the direct life cycle of certain polystomatids could reflect a constraint on the transmission strategy rather than a self-sustainable adaptation.

2. Material and methods

2.1. Host – parasite sampling and development

Amplecting pairs and calling males of the Stripeless Tree Frog *H. meridionalis* were collected at night all around and in non-permanent ponds of the vicinity of Opoul-Périllos (Pyrénées Orientales – Southern France) (see Badets et al., 2010). Egg clutches were incubated at 23 °C in 6 L of aerated water and resulting tadpoles were reared in groups of 30 following the same conditions, but water was renewed twice a week. To identify frogs infected with bladder parasites, calling males were isolated in small containers containing 20 ml of water that was screened daily for polystome eggs released with the urine flow. Infected frogs were kept three weeks in order to collect large amounts of eggs that were subsequently incubated in the dark at 23 °C in Petri dishes with bottled still water until hatching (see Badets et al., 2009). More than 20 positive hosts infected by 1–4 parasites were daily screened to ensure a wide genetic diversity of larvae (see more details about the natural host population in Badets et al. (2010)).

2.2. Experimental design

Preliminary infection experiments were performed with young tadpoles to get mature branchial phenotypes. One hundred, five days old tadpoles were individually exposed to three oncomiracidia for 6 h (see Badets et al., 2009) and maintained in bigger tanks at a density of 5 tadpoles per liter. According to Badets et al. (2009, 2010), these larvae should develop towards the branchial phenotype and reach maturity about three weeks after infection. Eggs produced by branchial parasites are first released in the branchial cavity of tadpoles and then expulsed with the continuous water flow via the sinistral spiracle. The water of the tanks was screened daily for polystome eggs that were then incubated following the same conditions as for eggs collected from the bladder phenotypes (see above).

Two series of experimental infections were conducted to investigate the developmental plasticity of the infective larval stage depending on its parental origin, *i.e.* branchial versus bladder phenotype. In the first batch experiment, two groups of 30 tadpoles aged five days were individually exposed to three oncomiracidia for 6 h, the first group with oncomiracidia hatching from eggs released by

branchial parasites (obtained from tadpoles experimentally infected, see above) and the second one with oncomiracidia hatching from eggs released by bladder parasites (obtained from wild infected frogs). Because these larvae must develop towards the branchial phenotype, a greater infection success for the first experimental group would reveal the suitability of a permanent branchial transmission. In the second batch experiment, two groups of 30 tadpoles aged 30 days were individually exposed to five oncomiracidia, the first group with oncomiracidia hatching from eggs of branchial parasites and the second one with oncomiracidia hatching from eggs of bladder parasites. Conversely, because these larvae should develop towards the bladder phenotype, a greater infection success was expected for the second experimental group. To test for the reproducibility of the results, each batch experiment was performed twice in the same conditions but with genetically distinct groups of tadpoles and parasites. Finally, each group of 30 tadpoles used for experimental infections was reared apart in tanks filled with 6 L of water. Four days post infection, tadpoles were anesthetized with MS 222, sacrificed and dissected in order to record the total number of developing parasites. Although some tadpoles were found uninfected, they were still retained for statistical analyses.

To test whether or not there is an effect of the parental origin of larvae, branchial or bladder, on the infestation success of their offspring, data were compared by performing GLM (Poisson error and log link function). Because the number of infective larvae was different in the two experimental procedures, two independent analyses were run using the number of developing larvae per tadpole as a dependent variable while replicates and parental origins were hold as categorical factors.

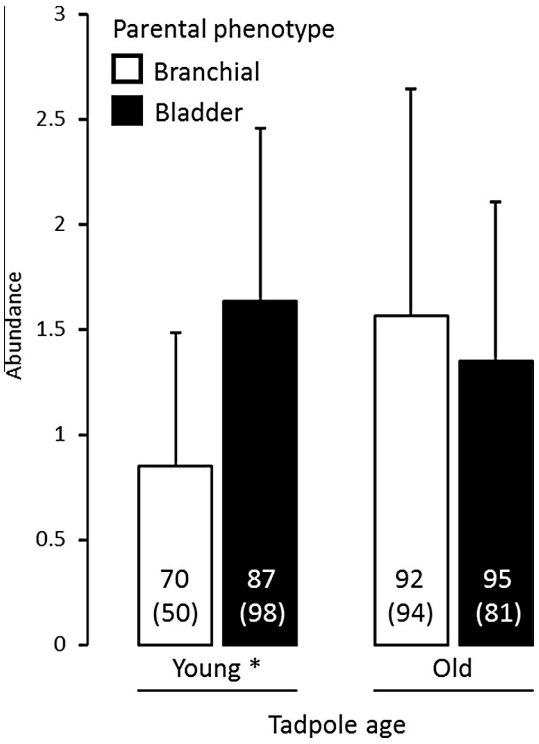

Fig. 1. Mean abundance of parasites recovered from young and old tadpoles following infestation with oncomiracidia hatching from eggs released by branchial and bladder parasites. Numbers in the boxes represents the prevalence of infected host and the total number of developing larvae between brackets.

3. Results

In the first batch experiment, all parasites developed towards the branchial phenotype, which was expected according to Badets et al. (2009, 2010). No significant difference for the infection success was found between both replicates (GLM-poisson; $p = 0.511$). Twice as many parasites were recorded from tadpoles infected with oncomiracidia hatching from eggs of the bladder parental phenotype (1.63 ± 0.82 parasites per host) than from tadpoles infected with oncomiracidia hatching from eggs of the branchial parental phenotype (0.83 ± 0.64 parasites per host) (GLM-poisson; $p < 0.001$) (Fig. 1). In the second batch experiment, all parasites developed towards the bladder phenotype, which was expected according to Badets et al. (2009, 2010). No significant difference for the infection success was found between both replicates (GLM-poisson; $p = 0.538$). Contrary to the first batch experiment, no difference was fund between tadpoles infected with oncomiracidia hatching respectively from eggs of the bladder parental phenotype (1.35 ± 0.75 parasites per host) and from eggs of the branchial parental phenotype (1.56 ± 1.07 parasites per host) (GLM-poisson; $p = 0.326$).

4. Discussion

Experimental studies revealed that infection success of young tadpoles is higher for oncomiracidia that originated from the bladder phenotypes than for branchial phenotypes. In older tadpoles no difference in infection success was noted for the two phenotypes. Three nonexclusive hypotheses could explain these results: (i) asymmetric resources, (ii) selection of better competitors or (iii) alternative program of development. (i) The difference in the diet and living conditions of branchial and bladder phenotypes may cause variations in the physiological response of the offspring and consequently in the parasite infection success towards young tadpoles. Because branchial phenotypes develop rapidly in a temporary environment, the cost associated in eggs production might be higher than for bladder phenotypes. This would result in differences in the composition of the egg itself and then a reduction in the infection rate under repeated stress conditions. However, and according to Gallien (1935), eggs that are released by each phenotype are morphologically similar and the infection success is the same within old tadpoles, regardless origin of oncomiracidia. (ii) Competitive interaction among branchial developing phenotypes could favor the selection of better competitors. Badets et al. (2010) showed from experimental infestations that polystome larvae developing into the branchial phenotype within a young tadpole compete with each other until a single parasite survives per tadpole, regardless the total amount of oncomiracidia that were used during infection experiments. Therefore, the offspring of the branchial phenotype could be the result of a selective pressure in the initial polystome population toward the selection of highly competitive individuals. The expression of these competitive traits would then intensify the level of negative interactions during the second round of infestation among their offspring. As a consequence, the infestation success of the offspring from branchial phenotypes is more susceptible to decrease as the selection of better competitors increases. (iii) The differential infection success within young tadpoles could have an adaptive value that favors the parasite transmission over time. In natural environments, the alternation of the bladder and branchial phenotypes within *P. gallieni* is likely to occur due to the ecology of its host (Badets et al., 2010). Because most of the Stripeless Tree Frogs spawn early in the breeding season, March – April, oncomiracidia that hatch from eggs that are released by the bladder parasites at this stage are more likely to infect young tadpoles. The branchial phenotypes that arise generate a second generation of infective oncomiracidia, which are on the contrary more likely to infect older tadpoles in pro-metamorphosis and develop towards the bladder phenotype (Badets et al., 2010). An oncomiracidium that would encounter a young tadpole and develop towards the branchial

phenotype by the end of the frog breeding season in June would indeed have a lower probability to reach maturity and reproduce since tadpoles develop faster when temperature increases and the pond dries. In this context, the predictability of abiotic and host environment variations could directly influence developmental programming and transmission strategies of the parasite. Indeed, the differential infection success within young tadpoles could result from a fine developmental and plastic strategy of polystomes to favor the alternation of both life cycles and transmission through host generations. A physiological constraint may occur within the branchial phenotype to canalize their potential for developmental plasticity towards the bladder phenotypes. This third hypothesis differs from published parental effect models and their role in adaptive developmental plasticity (see Badyaev and Uller, 2009). An increase in the infection success of the parasitic larvae when exposed to the same experimental conditions as their parents was expected as an adaptive pattern of non-genetic inherited information. This would have linked directly this phenomenon of developmental plasticity with a simple adaptive framework and should have corroborated phylogenetic scenario described for the family of the Polystomatidae (see Tinsley, 1990; Verneau et al., 2002; Badets and Verneau, 2009).

The developmental plasticity and parental effects are poorly documented among platyhelminthes making it difficult to interpret the ecological constraints and adaptive processes behind their success. For instance, the timing required to develop and grow within an additional host may be highly dependent of the relative development and growth within definitive host (Parker et al., 2009). A preliminary period of development for parasites was also shown to be essential before the activation of two manipulative strategies to ensure efficient establishment within the definitive host (Hammerschmidt et al., 2009; Perrot-Minnot et al., 2011). In regards to abbreviated life cycles within platyhelminthes, such a ticking clock has also been postulated to explain the importance of developmental plasticity in the course of parasite transmission (Lefebvre and Poulin, 2005; Lagrue and Poulin, 2009). However, our knowledge on the diversity and developmental plasticity within the Monogenea is based mainly on morphological description reports (see Kearn, 1994; Cribb et al., 2002). Therefore, the occurrence within mostly *Polystoma* species of a plastic developmental strategy involving the addition of an intermediate step where time constraints are clearly defined should be considered as an opportunity to gain insight in the origin and diversification of complex life cycles within the Monogenea.

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