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# Genetic compatibilities, outcrossing rates and fitness consequences across life stages of the trematode *Diplostomum pseudospathaceum*

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ABSTRACT

Many parasitic helminths exhibit mixed mating systems, and switches between self-fertilization and outcrossing may be influenced by environmental conditions and parasite demography. While inbreeding depression selects against the development of purely self-fertilizing populations, genetic compatibility may contribute to stabilizing mixed strategies. Here we study the effects of inbreeding and genetic compatibility on offspring fitness in the digenean trematode *Diplostomum pseudospathaceum*, a parasite with a three-host life cycle. Hatching rates and infection success in two intermediate hosts, the freshwater snail *Lymnaea stagnalis* and the three-spined stickleback, *Gasterosteus aculeatus*, were used as proxies for parasite fitness. Single trematode clones and combinations of two and three different clones were allowed to reproduce sexually using naïve herring gulls (*Larus argentatus*) as definitive hosts. The hatched larvae were used to assess the proportion of selfed and outcrossed miracidia by means of microsatellite genotyping. These results were matched with hatching rates and infection success of inbred and outcrossed trematodes in both intermediate hosts. Inbreeding effects were obscured by differences in clone performance. In addition, clones outcrossed to a lesser extent than expected in some experimental pairings, indicating the importance of genetic compatibility.



## 1. Introduction

Most parasitic platyhelminths are hermaphrodites and thus have the potential for both self- and cross-fertilization. While selfing provides reproductive assurance, it also represents the most severe case of inbreeding, which can decrease offspring fitness. This is referred to as inbreeding depression and is generally explained by two main genetic mechanisms that are not mutually exclusive. Under the dominance hypothesis, increased homozygosity leads to expression of deleterious recessive alleles under inbreeding. Conversely, under the overdominance model, less heterozygosity across loci entails lower genotype fitness, leading to a general advantage of heterozygotes over homozygotes (Charlesworth and Charlesworth, 1987; Charlesworth and Willis, 2009).

In populations with frequent inbreeding, increasing homozygosity leads to a purging of deleterious, recessive mutations by selection (Byers and Waller, 1999; Crnokrak and Barrett, 2002; Roff, 2002). This has also been observed in parasite populations. Examples include the trematode *Coitocaecum parvum*, which is able to truncate its life cycle by precocious maturation and self-fertilization in the second intermediate host, a process generally referred to as progenesis. In natural populations, up to 60% of individuals of this parasite may be progenetic (Lefebvre and Poulin, 2005a). In experimental studies, *C. parvum* did not appear to suffer fitness loss when selfing (Lefebvre and Poulin, 2005b; Lagrue and Poulin, 2009). In contrast, the cestode *Schistocephalus solidus* was found to experience severe inbreeding effects including lowered hatching rates, reduced growth and infection success, as well as lowered competitive ability (Christen et al., 2002). Even though *S. solidus* clearly benefits from outcrossing, a proportion of its eggs originates from selfing even when mating partners are available (Lüscher and Milinski, 2003). Furthermore, different species of digenean trematodes and cestodes have been reported to both self- and cross-inseminate under experimental conditions (Nollen, 1983).

Parasites' reproductive decisions may be based on the availability of mating partners or on environmental conditions such as host abundance and size (Herrmann and Poulin, 2012). Thus, recurrent selfing may be interpreted as a strategy for reproductive assurance. A worm investing both in selfing and outcrossing may have an advantage when parasite prevalence is low and many individuals do not find a mating partner within the definitive host.

Apart from the heterozygosity conferred to offspring by outcrossing, factors influencing the fitness of progeny include the genetic quality of their parents. Here we can distinguish between

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additive and non-additive genetic variance, corresponding to the notion of 'good genes' and 'compatible genes' (Neff and Pitcher, 2005). In the case of genetic compatibility, offspring fitness may not only be dependent on the quality of their parents' genes but also on interactions between genetic elements (Zeh and Zeh, 1996). In the presence of genetically incompatible mating partners, it may therefore be advantageous to self. Thus, population density fluctuations may also influence the evolution of mating systems. Even though models suggest that mating system evolution should result in populations that are either predominantly selfing or predominantly outcrossing (Lande and Schemske, 1985), both modes of reproduction can coexist within a population (Cheptou and Dieckmann, 2002).

Here, we address the combined effects of genetic compatibility and inbreeding on fitness-related parameters in a digenean trematode. We established four clonal lines of *Diplostomum pseudospathaceum* that were either forced to inbreed or allowed to outcross using herring gulls (*Larus argentatus*) as definitive hosts. Parasite offspring were genotyped to assess outcrossing rates. As in previous studies of the effects of inbreeding in parasites (Christen et al., 2002; Milinski, 2006; Lagrue and Poulin, 2009), performance in two critical life-stage transitions was recorded as a measure of offspring fitness: hatching rates and infection success in its first intermediate host, the snail *Lymnaea stagnalis*, and a typical second intermediate host, the three-spined stickleback, *Gasterosteus aculeatus*. This allowed us to gauge the fitness consequences of inbreeding and relate those to the genetic origin of parasite individuals. While we would expect severe fitness loss to be caused by inbreeding, the outcrossing rates and performance of offspring derived from different experimental pairings would allow us to assess whether there is an overlying effect of genetic compatibility.

## 2. Material and methods

### 2.1. Study system

*Diplostomum pseudospathaceum* is a parasite with a complex, three-stage life cycle and high prevalence in northern Europe, including the study area (Plön, 54°10′ N, 10°25′ E). Mature individuals parasitize gulls or terns as their definitive hosts. Sexual reproduction takes place in the birds' intestines and fertilized eggs are excreted with the hosts' faeces. This is followed by an aquatic stage, where eggs hatch into short-lived miracidia. These miracidia locate and invade their first intermediate hosts, a freshwater snail, *L. stagnalis*. Upon penetrating the snail, the parasite undergoes asexual reproduction (clonal amplification) by producing sporocysts, which in turn produce thousands to tens of thousands of clonal cercariae per day (Chappell et al., 1994). These cercariae move up and down in the water column until they encounter a suitable fish host. Once they have penetrated the fish, the larvae form metacercariae in the host's eye lens, causing cataracts and blindness. The life cycle is completed when the fish is eaten by a bird. Rauch et al. (2005) found infected snails from natural populations to carry between one and nine clones while fish hosts showed a much greater genotypic diversity, ranging from six to 67. Since the number of different parasite clones in the final host's intestine is dependent on the genotypes in the fish vectors, such admixing of genotypes may lower the risk of inbreeding. This may constitute a selective advantage of a complex life cycle.

### 2.2. Establishing the parasite clonal lines

Four clonal lines (I, V, XII and 19) of *D. pseudospathaceum* were created by isolating trematode eggs from gull faeces collected at the lake Großer Plöner See, Germany (GPS). Hatching larvae were used for monomiracidial infection of *L. stagnalis* snails (all snails used in the experiment were laboratory-bred offspring of a pool of snails originating from the GPS). Four snails infected with *D. pseudospathaceum* were identified and laboratory-bred, three-spined sticklebacks (*G. aculeatus*) were exposed to the clonal cercariae. In order to ensure parasite-free definitive hosts, herring gull eggs *(L. argentatus*) that were close to hatching were collected from an island in the GPS. In the wild, these birds are likely to experience infection intensities of tens to hundreds of worms (Karvonen et al., 2006). Therefore, comparable doses were chosen for the infection treatment. The chicks were fed with infected sticklebacks containing either 150 metacercariae of a single genotype, or 75 metacercariae of each of two genotypes. Thus, we obtained four birds infected with a single genotype, forcing the parasites to inbreed, and six birds infected with one of the possible combinations of two of these genotypes, (I + V, I + XII, I + 19, V + XII, V + 19, XII + 19), allowing the parasites to outcross.

### 2.3. Sampling and measuring fitness-related parameters

Survival of parasite stages was recorded during three stages of the life cycle. Stool samples from the gulls were collected every 2–3 days from 4 to 16 days p.i. The samples were washed with tap water and filtered through 100 μm gauze. The isolated parasite eggs were stored at 18 °C in culture bottles in tap water and regularly checked for hatching miracidia.

In order to assess hatching rates, the hatched and non-hatched eggs in the samples were counted under a stereomicroscope 15 weeks after sample collection and the data were compiled in a binomial data table. Monoclonal line 19 produced no eggs. A second naïve gull was infected with this line, which did not excrete any eggs either.

Secondly, miracidia from all sample dates were collected in order to assess snail infection rates. Small individuals of laboratory-bred *L. stagnalis* were placed in 12-well culture plates in water. Miracidia were obtained from the gull faecal samples and added individually to the wells with pipettes. Since miracidia hatched haphazardly and hatching success varied greatly between the different clonal lines, varying numbers of snails were exposed to each genotype combination (between four and 140 snails per clonal line). The snails were kept at 18 °C overnight. After 24 h, they were transferred to 16 L aquaria, each aquarium containing up to 48 snails. After 10 weeks, single snails were placed in plastic cups filled with well water and subjected to light stress. The water was checked for released cercariae after 3 h.

Finally, infection rates in the second intermediate host were assessed. Uninfected laboratory-bred sticklebacks from four sibships (i.e. the offspring of four breeding pairs) were used as fish hosts. Fish of comparable size were chosen for infection. Since clonal line 19 failed to produce eggs and multiclonal lines including clone 19 generally performed poorly, these lines were eliminated from the fish infection trial. Thus, only lines I, V, XII and combinations thereof were included. Cercariae originating from snails infected with double and triple clonal lines were genotyped as described in Section 2.4 to ensure that only outbred parasites were used for fish infections. The cercariae were isolated by placing infected snails under light for 3 h and the cercariae of the same clone or clonal combination from all surviving snails were pooled and gently mixed. A total of 10 fish from each sibship was exposed to each parasite clone and clone combination. Fish were placed individually into 1 L tanks and subjected to doses of 150 cercariae. After at least 1 week, the fish were dissected under a stereomicroscope and the numbers of metacercariae in the eye lenses were recorded.