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Research paper

Strong neutral genetic differentiation in a host, but not in its parasite

Anna K. Rahn [a,*], Johannes Krassmann [a], Kostas Tsobanidis [a], Andrew D.C. MacColl [b], Theo C.M. Bakker [a]

[a] Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany
[b] School of Life Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK

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ABSTRACT

The genetic diversity and population structure of a parasite with a complex life cycle generally depends on the dispersal by its most motile host. Given that high gene flow is assumed to hinder local adaptation, this can impose significant constraints on a parasite's potential to adapt to local environmental conditions, intermediate host populations, and ultimately to host-parasite coevolution. Here, we aimed to examine the population genetic basis for local host-parasite interactions between the eye fluke *Diplostomum* lineage 6, a digenean trematode with a multi-host life cycle (including a snail, a fish, and a bird) and its second intermediate host, the three-spined stickleback *Gasterosteus aculeatus* L. We developed the first microsatellite primers for *D.* lineage 6 and used them together with published stickleback markers to analyse host and parasite population structures in 19 freshwater lakes, which differ in their local environmental characteristics regarding water chemistry and *Diplostomum* abundance. Our analyses suggest that one parasite population successfully infects a range of genetically differentiated stickleback populations. The lack of neutral genetic differentiation in *D.* lineage 6, which could be attributed to the motility of the parasite's definitive host as well as its life cycle characteristics, makes local host-parasite co-adaptations seem more likely on a larger geographical scale than among the lakes of our study site. Our study provides a suitable background for future studies in this system and the first microsatellite primers for a widespread fish parasite.

1. Introduction

In host-parasite interactions both parasites and hosts are expected to adapt not only to changes in their respective environments, but also to changes in each other's defence mechanisms. Since the balance between selection and gene flow is considered the strongest determinant of local adaptation (e.g. Tigano and Friesen, 2016), investigating the rate of genetic exchange among host and parasite populations can help to understand the local adaptive potential in a host-parasite system. Generally, it is assumed that high migration rates and gene flow can hinder adaptation to (temporally stable) habitats where selection by environmental factors is weak (Slatkin, 1987; Lenormand, 2002; Kawecki and Ebert, 2004). While limited gene flow reduces the introduction of maladapted alleles and thus favours local adaptation, genetic drift, which can cause the loss of potentially beneficial alleles, is expected to decrease local adaptation (Blanquart et al., 2012). Host-parasite systems add a further dimension of (reciprocal) adaptations because host populations that adapt their defence mechanisms to the parasites present in their habitat constitute an environment that changes not only in space, but also in time. In temporally variable environments, on the other hand, intermediate levels of gene flow can even maximise adaptation by contributing to genetic variation (Blanquart et al., 2013). Interestingly, a recent meta-analysis found a general trend towards stronger genetic differentiation in hosts than in parasites across a wide range of taxa (Mazé-Guilmo et al., 2016). In light of this, identifying the mechanisms which determine dispersal and genetic differentiation in parasites remains a key question in the study of host-parasite interactions.

The distribution and population structure of a parasite (here we refer to macroparasites) depends on a range of different factors. Host dispersal is commonly considered the most obvious determinant of parasite dispersal (Blouin et al., 1995). Although gene flow requires physical movement between populations and dispersal is usually expected to correlate positively with gene flow (Räsänen and Hendry, 2008; but see Edelaar and Bolnick, 2012), dispersal per se is not the only factor determining parasite genetic structure (Mazé-Guilmo et al., 2016). Host-specificity and life-history traits like the mode of reproduction, the existence of free-living stages, or life-cycle complexity also affect parasite population structures and genetic diversity (see e.g. Barrett et al., 2008; Blasco-Costa and Poulin, 2013; Mazé-Guilmo et al., 2016 for a review and meta-analyses). Since different factors (partly with opposed effects) act on different stages in the life cycle, parasites with complex (multi-host) life cycles are particularly interesting, in this regard. By providing additional dispersal opportunities (intermediate/alternate host(s), water current), life-cycle complexity, host specificity, and the presence and number of free-living stages are expected to contribute

* Corresponding author.
E-mail address: arahn@evolution.uni-bonn.de (A.K. Rahn).

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to weaker parasite genetic differentiation compared to each single host (Mazé-Guilmo et al., 2016). Theoretical models indicate that in parasite species with alternating sexual and asexual reproduction self-fertilisation in the sexual phase results in higher inbreeding coefficients whereas variance in reproductive success among different clones decreases inbreeding coefficients (Prugnolle et al., 2005a). In a recent meta-analysis hermaphroditic parasites were less genetically differentiated than their hosts, which was attributed to a homogenising effect of higher dispersal rates in the (mostly bird-infecting) parasites (Mazé-Guilmo et al., 2016). Quite a few theoretical and empirical studies have focussed on genetic diversity in digenean trematodes, a subclass of parasitic flatworms (Platyhelminthes), which exhibit complex life cycles and comprise many human and livestock infecting species. In general, in digenean trematodes the host with the largest geographic range, i.e. usually the definitive host, is assumed to determine dispersal and genetic structure. This has been shown e.g. in salmon and eel infecting trematodes (Criscione and Blouin, 2004; Blasco-Costa et al., 2012), *Schistosoma mansoni* (Prugnolle et al., 2005b; van den Broeck et al., 2015), *Diplostomum pseudospathaceum* (Louhi et al., 2010), and in marine trematodes (Feis et al., 2015). Further, parasites completing their entire life cycle in aquatic habitats tend show more pronounced population structuring than parasites which use birds or (terrestrial) mammals as definitive host since these facilitate dispersal across aquatic habitat boundaries (Criscione and Blouin, 2004; Blasco-Costa and Poulin, 2013; Feis et al., 2015).

Here, we investigate the population structure of the digenean trematode *Diplostomum* lineage 6. Adult *Diplostomum* sexually reproduce in the intestines of piscivorous birds either through self-fertilisation or outcrossing (facultative hermaphrodites). With the bird's faeces, their eggs are released into the water where larvae (miracidia) hatch and infect lymnaeid snails. Inside the snail host, miracidia develop to sporocysts which clonally multiply and develop further into cercariae. These leave the snail, penetrate the skin of fish within eight minutes or less (Williams, 1966) and move within hours to the lens or to the retina. Thus, the parasite is exposed to the immune system of its host only for a short period of time before it reaches the immune-privileged eye. Despite this short time frame, innate resistance of the three-spined stickleback *Gasterosteus aculeatus* L. against *D. pseudospathaceum* is based on genotype-genotype interactions and (indirectly) involves the adaptive immune system of the host (Rauch et al., 2006; Haase et al., 2014; Haase et al., 2015). Research on host-parasite interactions of *Diplostomum* mainly focuses on lens-infecting species, which form cataracts and can have severe consequences for the competitive ability, growth and mortality of their host, particularly in fish farms (Chappell et al., 1994). *Diplostomum* species infecting the non-lens region have rarely been investigated, although recent molecular studies suggest that *Diplostomum* species diversity within the non-lens region might be higher than previously thought (Locke et al., 2010b; Blasco-Costa et al., 2014; Locke et al., 2015). In the only population genetic study on a *Diplostomum* species of which we are aware, Louhi et al. (2010) analysed the population genetic structure of *D. pseudospathaceum* over a geographic range of >300 km between sampling sites and failed to detect evidence for population structure despite the presence of population genetic structuring in the snail host *Lymnaea stagnalis* (Puurtinen et al., 2004).

In this study, we aimed to compare the population genetic structure of *Diplostomum* lineage 6—an eye fluke from the non-lens region in fishes—with that of its second intermediate host, the three-spined stickleback *Gasterosteus aculeatus* L., on the Scottish island of North Uist. The three-spined stickleback has frequently colonised freshwater habitats from the sea and is known to diverge into genetically differentiated populations within relatively short periods of time (e.g. Lescak et al., 2015). Thus, we expected strong population genetic structuring in the fish host, while we expected the parasite's highly motile definitive host to impede the formation of distinct populations in *D.* lineage 6. The three-spined sticklebacks on North Uist have proven interesting models for various research questions in the recent past regarding e.g. morphology (MacColl et al., 2013; Smith et al., 2014), UV-signalling (Hiermes et al., 2015), patterns of macroparasite distribution (de Roij and MacColl, 2012; Rahn et al., 2016), and spatial differences in susceptibility to a monogenean parasite (de Roij et al., 2010). Therefore, we additionally aimed to establish a useful basis for further studies in this system.

2. Methods

2.1. Study site and sampling

North Uist (Outer Hebrides, Scotland) measures about 300 km^2 and is covered with >180 lakes (Giles, 1983). Due to the influence of shell sediment and peat, these lakes comprise habitats ranging from alkaline clear water lakes in the west to lakes with acidic tea-stained water in the central and eastern part of the island (Giles, 1983). The lakes were likely recolonised by sticklebacks from the North Atlantic (Ravinet et al., 2014) during the last deglaciation approximately 15,000 years ago (Giles, 1983; Ballantyne, 2010) and have been isolated from each other ever since. The North Uist sticklebacks are mostly annual with about 10% experiencing a second winter (Abdul Rahman & Andrew MacColl unpublished data). De Roij and MacColl (2012) and Rahn et al. (2016) have examined the distribution of stickleback macroparasites on North Uist and found substantial differences in *Diplostomum* spp. abundances among lakes, which were largely consistent over several years. As these differences could not be explained by general abiotic habitat characteristics such as geographic distance, pH or the amount of dissolved calcium, they were attributed to local host-parasite dynamics. Prevalences (% fish infected) of *Diplostomum* spp. of the non-lens region (present in all lakes sampled in this study, not identified to species level) ranged from 14 to 100% (55, 31.5, 90; median, 1st, 3rd quartiles) (Table 1; see also Rahn et al., 2016).

We caught approximately 21 (median; 20, 25 1st, 3rd quartiles) adult male and female three-spined sticklebacks per sampling location from 19 freshwater lakes and from three coastal lagoons with open access to the sea (see Fig. 1 and Table 1 for sampling locations and sample sizes). Lakes were chosen with the aim of covering a geographically large part of the island as well as a broad spectrum of sampling locations representing the habitat diversity found on North Uist with regard to *Diplostomum* spp. abundance and presumably resistance to parasites (de Roij et al., 2010; de Roij and MacColl, 2012; Rahn et al., 2016), water chemistry, and stickleback morphology. Fish were caught using minnow traps (Jenzi: green nylon mesh (3–4 mm), Gee: galvanized steel mesh, G40 M, G48 M), which were set overnight in shallow water near the shoreline in spring 2010 (April and May) and 2011 (April). This time of the year marks the beginning of the breeding season when marine sticklebacks enter the coastal bays. At the three brackish water sites resident as well as morphologically distinct (significantly larger, fully plated) anadromous sticklebacks were caught. Therefore, we will speak of a total of 25 sampling locations. We additionally collected fish from the freshwater lakes in summer 2012 (August) to obtain sufficient *Diplostomum* spp. sample sizes.

For dissection, fish were killed by decapitation followed immediately by a cut through the brain and placed under a microscope (Novex RZ-Range, 6.5–45× magnification, illuminated by a cold-light source (Schott KL 1500)). The eyes of the sticklebacks were carefully checked for metacercariae within the intact lenses as well as outside the lens. Fins and metacercariae were conserved in 98% EtOH and stored at room temperature.

2.2. Microsatellite genotyping of the sticklebacks

2.2.1. Amplification

Microsatellite analysis was based on 600 fish caught in spring 2010 and 2011 as well as 25 anadromous sticklebacks from one of the three coastal lagoons ('Aileodair') in 2007 some of which had