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Variation among genotypes in responses to increasing temperature in a marine parasite: evolutionary potential in the face of global warming?

Boris W. Berkhout [1], Melanie M. Lloyd [2], Robert Poulin *, Anja Studer

Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

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ABSTRACT

Climates are changing worldwide, and populations are under selection to adapt to these changes. Changing temperature, in particular, can directly impact ectotherms and their parasites, with potential consequences for whole ecosystems. The potential of parasite populations to adapt to climate change largely depends on the amount of genetic variation they possess in their responses to environmental fluctuations. This study is, to our knowledge, the first to look at differences among parasite genotypes in response to temperature, with the goal of quantifying the extent of variation among conspecifics in their responses to increasing temperature. Snails infected with single genotypes of the trematode *Maritrema novaezealandensis* were sequentially acclimatised to two different temperatures, 'current' (15 °C) and 'elevated' (20 °C), over long periods. These temperatures are based on current average field conditions in the natural habitat and those predicted to occur during the next few decades. The output and activity of cercariae (free-swimming infective stages emerging from snails) were assessed for each genotype at each temperature. The results indicate that, on average, both cercarial output and activity are higher at the elevated acclimation temperature. More importantly, the output and activity of cercariae are strongly influenced by a genotype-by-temperature interaction, such that different genotypes show different responses to increasing temperature. Both the magnitude and direction (increase or decrease) of responses to temperature varied widely among genotypes. Therefore, there is much potential for natural selection to act on this variation, and predicting how the trematode *M. novaezealandensis* will respond to the climate changes predicted for the next century will prove challenging.

1. Introduction

With rising temperatures globally over the past decades (Levitus et al., 2000) and consequently changes in local climates (Karl and Trenberth, 2003), an increasing number of species continue to be affected by climatic changes (Vittoz et al., 2013). Within a given ecosystem, climate change is likely to affect all species, directly or indirectly, but not all of them to the same extent (Menendez et al., 2006). There are different ways in which populations can respond to climatic changes: (i) habitat shifts, (ii) plastic phenotypic responses, or (iii) genetic change (Gienapp et al., 2008; Visser, 2008). Habitat shifts are possible when similar habitats with milder climates are available, but their accessibility may be constrained by barriers and competing species (Kokko and López-Sepulcre, 2006). Phenotypic plasticity consists of changes in behaviour, physiology, morphology or development in response to changes in the environment (Donnelly et al., 2012). However, these are constrained by the organism's plastic limits (Gienapp et al., 2008) that may often be exceeded with rapid, large scale climatic changes (Donnelly et al., 2012). Furthermore, plastic responses are not long-term adaptations (Visser, 2008; Donnelly et al., 2012) and species might already be operating at their plastic limits. Only changes in genetic composition can help populations cope with long-term climatic changes through selection. Populations can adapt through selection for genotypes that are either more resistant to, or better able to benefit from, higher temperatures and other related habitat changes (e.g. longer periods of drought, increased salinity, decrease in pH levels; see Marcogliese, 2001 for a review). However, current changes in climate happen at a rapid rate (Karl and Trenberth, 2003); as a consequence not all

* Corresponding author. Tel.: +64 3 479 7983; fax: +64 3 479 7584.
E-mail address: robert.poulin@otago.ac.nz (R. Poulin).
[1] Present addresses: Institute for Biodiversity and Ecosystem Dynamics, Science Park 904, Postbus 94216, 1090 GE Amsterdam, The Netherlands.
[2] Present addresses: Infectious Diseases Division, Department of Internal Medicine, Washington University School of Medicine, St Louis, MO, USA.

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species will be able to adapt (Bell and Collins, 2008) and some are already becoming extinct (Cahill et al., 2013). This is the case not only for free-living, but also parasitic, species.

Small-bodied ectothermic species such as parasites may be particularly sensitive to climatic changes, but their responses have not been well studied (Marcogliese, 2001; Lafferty, 2009). Parasites play a great variety of roles in ecosystems; they can reduce host numbers (Robar et al., 2010), affect population dynamics (Hudson et al., 1998; Krkosek et al., 2013), and play key roles in shaping communities of free-living species (Poulin, 1999; Mouritsen and Poulin, 2002; Fenton and Brockhurst, 2008). At a population level, parasites can affect host numbers in a variety of ways. They can reduce overall health or increase mortality (Koop et al., 2011), increase susceptibility to secondary infections (Cornet et al., 2009) or increase predation risk (Poulin, 2010). All of these can change species interactions and consequently alter the organisation of ecosystems (Mouritsen and Poulin, 2002; Fenton and Brockhurst, 2008). Therefore, understanding how parasites will respond to climate change is essential to forecast changes in communities and ecosystems.

In many parasites of aquatic ectotherm hosts, the rate at which transmission stages are produced may be sensitive to ambient temperatures. This is particularly true for the cercariae of trematodes, i.e. the free-swimming infective stages produced in the first intermediate host (a mollusc) that generally emerge to infect the second intermediate host of the life cycle. Their output generally increases with rising temperature (Poulin, 2006), at least up to some optimal temperature, above which the output of cercariae declines with further temperature rises (Morley and Lewis, 2013). As a consequence, with modest increases in environmental temperatures the number of individual parasites in ecosystems might be expected to rise (Robar et al., 2010; Studer et al., 2010). Yet, the quality of parasite infective stages produced at higher temperatures may not necessarily match their quantity. For example, while the output of cercariae increases with increasing temperature up to an optimum level, their maximum survival time typically decreases linearly (Studer et al., 2010). However, although there can be substantial intraspecific variation in some trematode responses to rising temperature (see Studer and Poulin, 2014), we currently know nothing of the underlying genetic variation in these responses. It may be that the marked average responses of some trematode species to rising temperatures reflect the high sensitivity of a few genotypes, while many genotypes may show no response. To gain deeper insights into the ability of parasite populations to adapt to future climatic changes, it is essential to quantify the genetic variation in their responses to temperature. The present study therefore investigates the genetically determined variation in the output (quantity) and activity levels (quality) of cercariae in an intertidal trematode species in response to temperature.

We used the intertidal microphallid trematode, *Maritrema novaezealandensis*, and its first intermediate gastropod host, *Zeacumantus subcarinatus,* as a model system. *Maritrema novaezealandensis* has a typical complex life cycle comprising three hosts. Adults of *M. novaezealandensis* live in the intestine of red-billed gulls (*Chroicocephalus scopulinus*) and possibly other shore birds (Martorelli et al., 2004). Within the bird host, adult worms reproduce sexually and release eggs via the bird's excrement (Fredensborg and Poulin, 2005). *Zeacumantus subcarinatus* (Batillariidae: Prosobranchia) snails are the first intermediate host of *M. novaezealandensis* (Fredensborg and Poulin, 2005). After a snail ingests an egg, the parasites reproduce asexually within the snail while slowly replacing the host's reproductive tissue. Still, infected snails can live for several years (Fredensborg et al., 2005), and during that time hundreds of sporocysts (i.e. the parasite stage within a snail host), which may originate from a single egg, can, depending on the environmental temperature, produce up to several hundred cercariae per week (Studer et al., 2010). The cercariae emerge from infected snails when the tide is low and snails are exposed to warmer temperatures in tidal pools. After emergence, the free-swimming cercariae locate and infect second intermediate crustacean hosts (Fredensborg and Poulin, 2005; Koehler and Poulin, 2010), in which they develop into metacercariae, grow and encyst (Fredensborg et al., 2004; Martorelli et al., 2004). To complete their life cycle, the encysted parasites then await ingestion of their crustacean host by a shorebird (Fredensborg and Poulin, 2005). The life cycle of *M. novaezealandensis* offers a great opportunity to study differences in response to climatic changes between genotypes. Not only has cercarial sensitivity to temperature been demonstrated in this trematode (Fredensborg and Poulin, 2005; Studer et al., 2010), but also infection levels in snail populations can be high (up to 80%; Martorelli et al., 2004) with a large portion harbouring single genotype infections (>50%; Keeney et al., 2007). Together with the high number of cercariae produced on a weekly basis, infected snails thus supply a steady flow of genetically identical cercariae that can be tested under different conditions.

The two traits investigated here, i.e. number of cercariae produced and cercarial activity, are clearly traits of the parasite and thus under the influence of the parasite's genotype. Nevertheless, they may also be affected by the snail host in which the parasites develop, since individual snails vary in terms of the resources they provide and their immune reactions to infection. However, any parasite-snail combination still represents a unique genetic complex because a parasite genotype can only be associated with a single snail. In the context of responses to climate change, what matters to the severity of infections in the other hosts of the trematode's life cycle is the extent of inter-individual variation in factors such as cercarial output and activity on which selection can act. Whether part of this variation is ascribed to the snail host instead of all of it being attributed to parasite genes does not diminish its potential relevance for evolutionary responses to warmer climates.

The specific objectives of this study were to (i) test for an interaction between temperature and parasite genotype in two responses, i.e. output and activity of *M. novaezealandensis* cercariae in a long term experiment involving a temperature increase, and (ii) test for any trade-off among genotypes between these two responses, allowing an assessment of relative investment in the quantity (output) or quality (activity levels) of infective stages. The first objective quantifies the genetic variation in how this parasite species responds to rising temperature, whereas the second determines whether the two responses considered here are independent of one another. This is the first known study to look at genetically determined differences between individuals of the same species in response to a highly crucial environmental factor, namely temperature.

2. Materials and methods

2.1. Host and parasite collection

Zeacumantus subcarinatus snails were haphazardly collected from the Lower Portobello Bay mudflat (Otago Harbour, South Island, New Zealand, 45° 50′ S, 170° 40′ E) on 26 November 2012 (early Austral summer) at low tide. Snails were acclimatised to laboratory conditions for 1 week before they were screened for *M. novaezealandensis* infections by inducing cercarial emergence. This is achieved by incubating snails for 24 h at 25 °C (see Section 2.2). Subsequently, 30 infected snails with a shell measuring between 12.0 and 14.0 mm from apex to the base of the aperture were