Fishing in troubled waters: Revealing genomic signatures of local adaptation in response to freshwater pollutants in two macroinvertebrates

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HIGHLIGHTS

- Ore mine and wastewater treatment plant effluents can act as strong stressors, yet stream populations may adapt to these
- We searched for genome-wide signatures of local adaptation due to these two anthropogenic stressors
- Populations of the flatworm *Dugesia gonocephala* and the caddisfly *Glossosoma conformis* were studied
- One flatworm population affected by century-old ore mining effluents showed strong signatures of positive selection
- We identified *arnt* as a candidate gene involved in xenobiotic response and potentially heavy metal detoxification

GRAPHICAL ABSTRACT
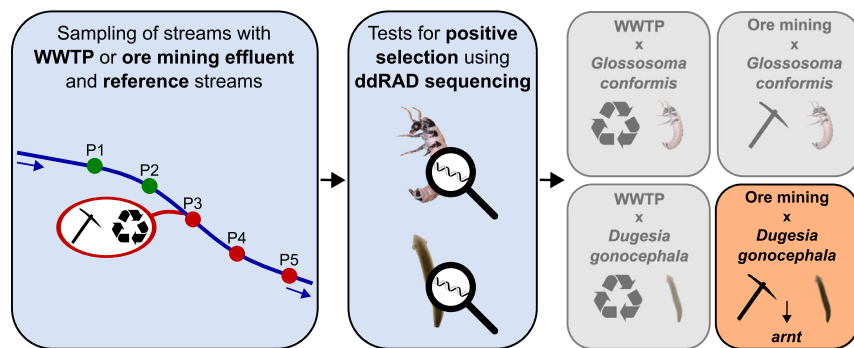
ABSTRACT

Local adaptation is of fundamental importance for populations to cope with fast, human-mediated environmental changes. In the past, analyses of local adaptation were restricted to few model species. Nowadays, due to the increased affordability of high-throughput sequencing, local adaptation can be studied much easier by searching for patterns of positive selection using genomic data. In the present study, we analysed effects of wastewater treatment plant and ore mining effluents on stream invertebrate populations. The two different anthropogenic stressors have impacted on stream ecosystems over different time scales and with different potencies. As target organisms we selected two macroinvertebrate species with different life histories and dispersal capacities: the caddisfly *Glossosoma conformis* and the flatworm *Dugesia gonocephala*. We applied a genome-wide genetic marker technique, termed ddRAD (double digest restriction site associated DNA) sequencing, to identify local adaptation. Ten and 18% of all loci were identified as candidate loci for local adaptation in *D. gonocephala* and *G. conformis*, respectively. However, after stringent re-evaluation of the genomic data, strong evidence for local adaptation remained only for one population of the flatworm *D. gonocephala* affected by high copper concentration from ore mining. One of the corresponding candidate loci is *arnt*, a gene associated with the response to xenobiotics and potentially involved in metal detoxification. Our results support the hypotheses that local adaptation is more likely to play a central role in environments impacted by a stronger stressor for a longer time and that it is more likely to occur in species with lower migration rates. However, these findings have to be interpreted

cautiously, as several confounding factors may have limited the possibility to detect local adaptation. Our study highlights how genomic tools can be used to study the adaptability and thus resistance of natural populations to changing environments and we discuss prospects and limitations of the methods.

1. Introduction

Many ecosystems are severely impacted by human alterations (Haddad et al., 2015; Halpern et al., 2008; McCluney et al., 2014; Newbold et al., 2015). With respect to biological diversity, this results often in reduced population sizes or even in local extinctions. However, genetic adaptation to the new conditions can occur enabling populations with a different, advantageous genetic composition to persist in the altered conditions. Thus, information about the potential of species for local adaptation is an important variable to consider in management strategies as this can help to predict and maximise the resistance and resilience of an ecosystem with its individual biotic components (Chevin et al., 2010; Hoffmann and Sgrò, 2011). However, the potential of species and populations for local adaptation to the altered conditions is influenced by a multitude of factors, such as the effective population size, the migration rate or the demographic history of the population (Sanford and Kelly, 2011). For example, very high migration rates constantly homogenise the gene pool and may thus reduce the potential for local adaptation (Lenormand, 2002). Similarly, a low effective population size, e.g. due to a recent bottleneck event, leads to low genetic diversity with negative effects on the potential for local adaptation, as fewer genetic variants are present which can potentially mediate a fitness benefit (Frankham et al., 2010). Beside such species- and population-specific factors, also stressor-specific characteristics can impact on the possibility for local adaptation (Sanford and Kelly, 2011). For instance, stronger selection pressures in theory accelerate local adaptation (Frankham et al., 2010). However, if a stressor is too strong, a population may go locally extinct before adaptation can take place. Another characteristic impacting on the potential for local adaptation is temporal variation in the strength of the stressor (Kawecki and Ebert, 2004). Here, high variation in stressor intensity results in a lower rate of adaption, as non-adapted genotypes can be at high frequencies in the times of low stressor strength. Hence, a better understanding of the complex interactions of different species-, population- and stressor-specific characteristics is necessary to enable predictions on the ability of populations to adapt to fast, human-mediated environmental changes before they can be incorporated into management strategies. Such an understanding of the complex interactions can be derived empirically by studying ecosystems that are highly impacted by several anthropogenic stressors. In these ecosystems, different species and anthropogenic stressors can be included in comparative studies, potentially allowing conclusions on settings that influence local adaptation most strongly.

One example of a highly impacted ecosystem are streams, which are affected by various anthropogenic changes such as channelisation, the construction of weirs and dams or intense agricultural and urban land-use (Malmqvist and Rundle, 2002). Furthermore, streams are impacted by various chemical contaminants (Pal et al., 2010; Schwarzenbach et al., 2010) that enter the ecosystems sometimes at very specific entry sites. Thus, given the multitude of stressor impacts, streams represent a good starting point for comparative studies on local adaptation. One common and clearly defined entry point of chemical pollutants are wastewater treatment plants (WWTPs). Although it is undisputed that WWTPs have contributed tremendously to the improvement of water quality (Søndergaard and Jeppesen, 2007), they do not filter all chemical compounds from the wastewater. As a result, nutrients and organic matter (Martí et al., 2004, 2009), but also micropollutants such as pharmaceuticals, personal care products or pesticides (Grabicova et al., 2015; Loos et al., 2013; Münze et al., 2017) and microplastics (Eerkes-Medrano et al., 2015), still enter freshwater ecosystems, leading to punctually high concentrations. Another source of chemical contaminants, however restricted to certain regions, is metal mine drainage water influx (reviewed by Byrne et al., 2012). Here, heavy metals, sometimes in combination with very acidic water, enter the streams.

Both sources of chemical contaminants are known to affect the community composition of macroinvertebrates (Burdon et al., 2016; Grantham et al., 2012; Ortiz et al., 2005; Peschke et al., 2014), often resulting in reduced abundances of sensitive taxa such as most Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies; Byrne et al., 2012; Grantham et al., 2012; Ortiz et al., 2005; Peschke et al., 2014; Qu et al., 2010). Hence, strong selection pressures may act on the species in the impacted streams, potentially leading to local adaptation. This is in line with first results from studies on chironomids, where local adaptation to contaminants originating from both sources, heavy metals (Bahrndorff et al., 2006; Groenendijk et al., 1999) and insecticides (Hoffman and Fisher, 1994), was detected.

These reports of local adaptation are typically based on approaches such as transplant or laboratory experiments (Bahrndorff et al., 2006; Groenendijk et al., 1999; Hoffman and Fisher, 1994). However, experimental studies on local adaptation are labour intensive and can only be applied to species fulfilling certain criteria such as the possibility of captive or laboratory breeding (de Villemereuil et al., 2016). An alternative to experimental approaches are genetic studies that apply genome scans (reviewed in Hohenlohe et al., 2010; Nielsen, 2005; Vitti et al., 2013; Weigand and Leese, 2018). These methods, previously unaffordable for species without high economic value or for non-model species, can nowadays be used due to the increased affordability of high-throughput sequencing (HTS). The applicability of the methods was further supported by the development of reduced-representation approaches, i.e. methods in which not the complete genomes of all individuals are sequenced, but only certain fraction that is screened across all individuals (Araneda et al., 2016; Gaither et al., 2015; Swaegers et al., 2015; Van Wyngaarden et al., 2017).

The underlying evolutionary assumption when performing genome scans is that local adaptation arises if selection acts on a beneficial allele, termed positive selection (reviewed in Hohenlohe et al., 2010, Nielsen, 2005, Vitti et al., 2013, Weigand and Leese, 2018). Hence, the frequency of such a beneficial allele increases, ultimately until it approaches fixation in the population. Often populations from locations impacted and unimpacted by certain stressors or environmental conditions are compared in genome scans, focusing on the population differentiation of genetic markers (reviewed in Hohenlohe et al., 2010, Nielsen, 2005, Vitti et al., 2013, Weigand and Leese, 2018). Because positive selection should only act on the impacted populations, the beneficial alleles are assumed to be at high frequencies in these populations, while they should be at low or intermediate frequencies in non-impacted populations, resulting in an increased population differentiation for the beneficial alleles. As allelic variants located on different chromosomes are inherited independently, genomic markers not located on the chromosome containing the alleles mediating local adaptation should not be affected by the shift in allele frequencies. Hence, they typically show a lower population differentiation compared to the beneficial alleles. Likewise, genetic markers at the same chromosomes but in some distance to the beneficial alleles are inherited independently from the beneficial alleles due to recombination events (i.e. crossing-overs).