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Gene expression profiling in the aquatic caddisfly larvae *Micropterna lateralis* (Insecta: Trichoptera) in relation to stream drying

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Abstract

Aquatic ecosystems can be either permanently wet or be subject to seasonal or intermittent drying. Insect species inhabiting temporally dry streams have evolved behavioural, morphological and/or life history traits to ensure their persistence. The limnephilid caddisfly *Micropterna lateralis* (Stephens 1837) has a life cycle that is tailored to the temporal dynamics of periodically dry streams where adults usually hatch before drought commences. Their aquatic larvae are, however, also able to survive stream drying, but the mechanisms driving this survival ability have not been studied to date. We conducted an experiment to simulate drying conditions, using samples from a *M. laterialis* population taken from an intermittent stream in the Spessart Mountains of the central German highlands. The experiment simulated three hydrological conditions: wet, moist and dry. In a first test of our system we compared gene expression profiles of three individuals (one per condition) using Illumina-based Massive Analysis of cDNA Ends MACE-Seq. Our first results show large differences in gene expression between the studied phases with many genes exhibiting gradual up- or down-regulation across the three experimental hydrological conditions. Under drought stress, up-regulation was primarily found in genes controlling production and mobilization of desiccation protectants, mainly sugars, whereas down-regulated genes were related to cuticle organisation and lipid metabolism. These preliminary results give valuable insights into genetic and physiological responses of aquatic insects to stream drying.

Keywords: intermittent streams, drought survival, larval stage, Massive Analysis of cDNA Ends

Introduction

Intermittent rivers with periodically ceasing flow are common worldwide and estimated to comprise more than a third of the total discharge and length of the global river network (Tooth 2000). Drying can occur naturally, e.g. due to increased evapotranspiration and lowering of groundwater tables, or anthropogenically due to water abstraction or impoundment (Datry *et al.* 2014). Insect species inhabiting temporally dry streams have evolved behavioural, morphological and life history traits to ensure their persistence under drought (Lytle & Poff 2004). Diapause and aestivation, refuge use, or synchronized emergence and reproduction are some strategies of aquatic insects to persist through natural drought disturbances (Gray & Fischer 1981; Lytle 2002). The limnephilid caddisfly *Micropterna lateralis* (Stephens 1837) has a life cycle that is tailored to the temporal dynamics of periodically dry streams where adults usually hatch before drought commences. Unlike many other limnephilid species inhabiting intermittent rivers *M. lateralis* does not seem to have evolved an ovarian diapause (Hoppeler *et al.* 2017). Seasonality of stream intermittency is highly predictable but the

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onset and length of local drought phenomena is variable and directly linked to weather conditions. In particular the amount of precipitation strongly influences the beginning and ending of drought, and thus directly controls which life stage is exposed to stream drying.

We have observed *Micropterna lateralis* larvae actively moving and foraging on moist and dry stream sediments, and adult emergence from pupal cases in the absence of surface water. The mechanisms driving larval drought survival in caddisflies have not yet been studied. Here, we describe the molecular and physiological basis of responses to water loss in aquatic insects using *M. lateralis* as a model. The onset of drought stress is expected to induce short-term changes in the level of expressed genes, which can be studied using gene expression profiling. With this method, the expression of hundreds of genes is monitored simultaneously, providing a comprehensive picture of the way an organism responds to a changing environment. Molecular responses and candidate genes in desiccation tolerant terrestrial insects have been widely studied (e.g. review by Chown *et al.* 2011). However, the patterns of differential gene expression associated with drought stress in aquatic insects remain unknown. In this study, we investigate the underlying genetic responses and candidate genes for the survival of drying conditions and identify specific physiological pathways regulated by differential gene expression.

Material and Methods

Drought experiment: samples and conditions

Larvae of *M. lateralis* were collected at the Auerbach in the Hessian Spessart (50°16'34.2''N, 9°25'58.7''E) in November 2014, at a summer-dry stream section. Larvae were returned alive to the laboratory on ice and acclimated to climate chamber conditions for three days before use in drought experiments. Conditions in climate chambers were: 14°C with a day/night cycle of L10:D14 with humidity kept at 30 % in order to simulate gradual dry down. Larvae were kept in round 5-litre containers containing two air stones, 900 ml of stream sediment, 800 ml stream water and were initially supplied with 3 g fresh leaf litter taken from the study stream. During the experiment a total of three samples were taken following gradual drying through evaporation. The first sample was taken at day four of the experiment representing wet conditions, where larvae are fully submerged under water. The second sample was taken at day five, representing moist conditions, characterized by the absence of surface water but remaining water films within larval cases. At day eight, the third sample was taken, where the upper sediment layer was dry (i.e. no surface water) and no visible water was left in larval cases. Larvae were removed at each time point and immediately preserved in liquid nitrogen. In this preliminary study there were no control samples taken at the three time points. Thus, the observed gene expression response is believed to be a combination of drought stress, development and stress of the experimental conditions.

RNA extraction and sequencing

Total RNA was isolated in order to measure gene expression in biological samples (n = 3) following the protocol described by Lopez and Bohuski (2007). Frozen larvae were macerated and homogenized in TRIzol Reagent (Invitrogen) and RNA further extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany) The isolation included an on-column digestion with RNase-free DNase (Qiagen, Hilden, Germany).

The Massive Analysis of cDNA Ends MACE-Seq technique was performed as described by Müller *et al.* (2013), using the MACE kit (GenXPro GmbH, Frankfurt, Germany) in combination with Illumina sequencing. In MACE, each transcript is represented by a single, cDNA fragment originating from the 3′ end of each transcript. MACE-Seq libraries were constructed by GenXPro GmbH, Frankfurt am Main, Germany. For preparation of libraries, cDNA was produced by first and second strand synthesis using the provided MACE-Seq Oligos. cDNA products were fragmented randomly to reach an average size of 250 bps and the 3′-biotinylated ends were captured by streptavidin beads. Further, GenXPro's TrueQuant adapters were ligated to label each template molecule with a unique sequence-(barcode) to reduce PCR introduced bias by distinguishing and eliminating PCR copies from original transcripts in the sequence reads.

Sequence annotation and quantification of expression

The reads of each MACE-Seq library were PolyA-trimmed, low-quality reads removed, and mapped to the *Micropterna lateralis* transcriptome (Hoppeler *et al.* 2016) using Novoalign (Novocraft Technologies;

www.novocraft.com). Reads that could not be mapped on the transcriptome were assembled and annotated via BLASTX to the SwissProt and TrEMBL databases. To account for differences in sequencing depth, tag counts were normalized according to Wang *et al.* (2010). Normalization and tests for differential gene expression was performed in R using the DEGSeq2 package V 1.11.1. (Love *et al.* 2014).

Results

We performed transcriptome-wide gene expression analysis with MACE-Seq and identified 204 transcripts that were differentially expressed between the three hydrological conditions wet, moist and dry ($P < 0.001$; logFC ± 2 from wet to dry). Of these transcripts, 104 could be assigned to known genes and thus annotated with protein functions. Overall, 38 genes were up-regulated from wet to dry conditions and 66 were down-regulated. Different patterns of expression levels across all three states were found (Fig.1): gradual increase (A; no. of genes in this category = 25), general increase with highest level under moist conditions (B; n = 5), similar levels for wet and moist and increase during dry (C; n = 9), gradual decrease (D; n = 40), increase from wet to moist but decrease from wet/moist to dry (E; n = 20), and decrease with similar levels during moist and dry (F; n = 5).

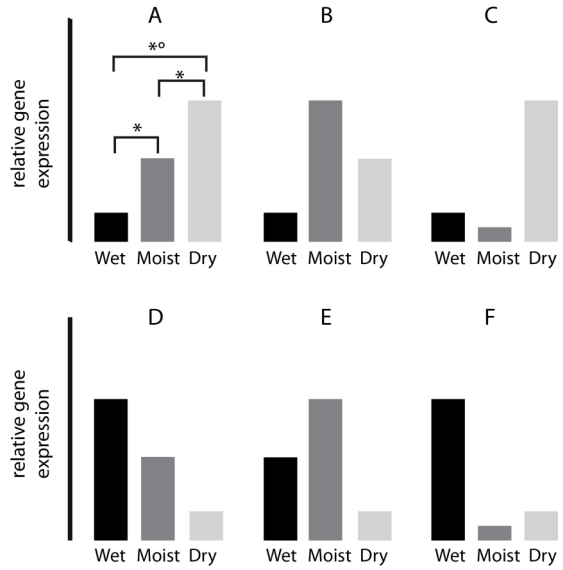

FIGURE 1. Genes that were differentially expressed between wet, moist and dry conditions (*$P < 0.001$; °logFC ± 2) showed six different patterns (A – F) of how relative expression levels changed across these three hydrological conditions. A: gradual increase; B: general increase with highest level under moist conditions; C: similar levels for wet and moist and increase during dry; D: gradual decrease; E: increase from wet to moist but decrease from wet/moist to dry; F: decrease with similar levels during moist and dry.

Up-regulated pathways were primarily part of the carbohydrate metabolism (glycolysis, saccharose, trehalose) and amino acid metabolism (proline, arginine (sugar transporter), glutathione (anti-oxidant)); A, F). In addition, many signalling proteins important for energy mobilization (cAMP), regulation of cellular energy homeostasis (AMPK) and regulation of autophagy (mTOR) showed up-regulation (A). Gradually down-regulated pathways include biosynthesis of lipase, an enzyme for the breakdown of lipids, and digestion and absorption of carbohydrates, lipids and proteins (D). Furthermore, membrane associated signalling and cuticular structure proteins were up-regulated under moist conditions but down-regulated during dry conditions (E). In addition, increased transport activity was observed under moist but not dry conditions.

Discussion

The results provide new insights into processes regulating the loss of free surface water in an aquatic insect adapted to recurring drought phenomena. The gene expression patterns suggest acclimatization to water loss to be largely governed by differential regulation of production and repair mechanisms. Overall, we found larvae to respond through increased production and mobilisation of sugar and amino acids, which act as desiccation protectants and prevent cellular water loss and increase cell stability (Clark *et al.* 2009). Trehalose, the blood sugar of insects, is accumulated in a wide range of insect taxa in response to water stress, and up-regulation of genes related to the biosynthesis of trehalose is assumed critical for desiccation-tolerant arthropods in general (Clark *et al.* 2009; Mitsumasu *et al.* 2010; Teets *et al.* 2012, 2013). Furthermore, Teets *et al.* (2013) observed accumulation of glucose via increased gluconeogenesis and proline synthesis to be a component of dehydration response in the chironomid species *Belgica antarctica* and also present evidence for slowdown of glycogen breakdown as a means of conserving water.

In addition to sugar mobilization, we found expression changes in signalling and cuticular structure proteins that play an important role in the organisation of cellular components. Membrane restructuring has been reported in relation to drought stress in many insects (e.g., Clark *et al.* 2009). Further, many genes down-regulated under drought were associated with digestion. This could potentially imply reduced feeding activity due to a lack of adequate food sources under dry conditions. Reduction in digestive enzymes could allow the larvae to survive without food for extended periods of time (Scriber & Slansky Jr 1981).

Overall, our results suggest aquatic insect larvae to respond in a similar manner as other studied insects exposed to dehydration. However, these results have to be interpreted with caution as only one specimen per condition without controls was sequenced. An increased sample size is necessary to corroborate observed patterns and first results presented here. Nevertheless, this study provides valuable preliminary insights into genetic and physiological responses in an aquatic insect model to stream intermittency crucial to estimate potential effects of changed future environmental conditions on aquatic insect communities.

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