Received: 5 July 2018 | Revised: 15 November 2018 | Accepted: 27 November 2018

DOI: 10.1111/mec.15001

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

The genetic architecture underlying diapause termination in a planktonic crustacean

Till Czypionka[1] | Peter D. Fields[2] | Jarkko Routtu[2,3] | Edwin van den Berg[1] | Dieter Ebert[2] | Luc De Meester[1]

[1]Laboratory of Aquatic Ecology and Evolutionary Biology, KU Leuven, Leuven, Belgium

[2]Department of Environmental Sciences, Zoology, University of Basel, Basel, Switzerland

[3]Molecular Ecology, Martin-Luther-Universität, Halle-Wittenberg, Germany

Correspondence
Till Czypionka, Laboratory of Aquatic Ecology and Evolutionary Biology, KU Leuven, Leuven, Belgium.
Email: till.czypionka@kuleuven.be

Funding information
KU Leuven, Grant/Award Number: C16/2017/002 and PF/2010/07; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung; Deutsche Forschungsgemeinschaft, Grant/Award Number: CZ 230 1/1 and CZ 230 2/1; Universität Basel; Fonds Wetenschappelijk Onderzoek, Grant/Award Number: G0C3818

Abstract

Diapause is a feature of the life cycle of many invertebrates by which unfavourable environmental conditions can be outlived. The seasonal timing of diapause allows organisms to adapt to seasonal changes in habitat suitability and thus is key to their fitness. In the planktonic crustacean *Daphnia*, various cues can induce the production of diapause stages that are resistant to heat, drought or freezing and contain one to two embryos in developmental arrest. *Daphnia* is a keystone species of many freshwater ecosystems, where it acts as the main link between phytoplankton and higher trophic levels. The correct seasonal timing of diapause termination is essential to maintain trophic interactions and is achieved via a genetically based interpretation of environmental cues like photoperiod and temperature. Field monitoring and modelling studies raised concerns on whether populations can advance their seasonal release from diapause to advances in spring phenology under global change, or if a failure to adapt will cause trophic mismatches negatively affecting ecosystem functioning. Our capacity to understand and predict the evolution of diapause timing requires information about the genetic architecture underlying this trait. In this study, we identified eight quantitative trait loci (QTLs) and four epistatic interactions that together explained 66.5% of the variation in diapause termination in *Daphnia magna* using QTL mapping. Our results suggest that the most significant QTL is modulating diapause termination dependent on photoperiod and is involved in three of the four detected epistatic interactions. Candidate genes at this QTL could be identified through the integration with genome data and included the presynaptic active zone protein *bruchpilot*. Our findings contribute to understanding the genomic control of seasonal diapause timing in an ecological relevant species.

KEYWORDS
bruchpilot, dormancy, ephippium, phenology, plankton resting stage, trophic mismatch

1 | INTRODUCTION

The life history of many organisms includes the avoidance of unfavourable conditions through migration in space (e.g., migratory birds) or in time, i.e., through some sort dormancy response (e.g., diapause; Varpe, 2017). Diapause stages are often resistant to environmental stressors like cold, heat or drought, but on the other hand cannot exploit beneficial habitat conditions for growth or reproduction. The exact timing of diapause induction and termination are thus fitness relevant life-history traits through which

these organisms integrate in the overall phenology of the habitat (Forrest & Miller-Rushing, 2010; Williams et al., 2017). Phenology, the timing of biological events, here refers to the seasonally elevated availability of resources like nutrients or food items, but also of predators, parasites or detrimental abiotic factors (e.g., winter cold), whose fluctuations over the course of the year are recurrent and predictable (Williams et al., 2017). Diapause timing is achieved through the interpretation of environmental cues, which can be abiotic cues like photoperiod or temperature, or biotic cues like predator kairomones or a reduction in food availability or quality (Walsh, 2013). Genetic differentiation in the response to these cues has been quantified over latitude as well as on a micro-geographic scale (De Meester & De Jager, 1993; Roulin et al., 2015; Schmidt, Matzkin, Ippolito, & Eanes, 2005) indicating that this trait can evolve.

Currently, many ecosystems are subject to environmental changes causing a modification of phenology (Parmesan, 2006). Phenology shifts of many species, in particular the advances of spring abundance peaks, have been interpreted as a plasticity response towards increased temperatures due to current climate change (Donnelly et al., 2012; Gienapp, Teplitsky, Alho, Mills, & Merilä, 2008). Also changes in biotic interactions (e.g., predation and parasitism) can cause a change in phenology (Hairston & Walton, 1986). The correct timing of diapause termination is crucial for the individual fitness, as failure to resume development at the right time is potentially lethal when it occurs under detrimental conditions (e.g., a harsh winter or during a period of high predation pressure). Even individuals terminating diapause under still beneficial conditions, but outside their optimal temporal niche, will likely be outcompeted by individuals with a diapause termination timing optimally adapted to habitat phenology (Vanoverbeke & De Meester, 2009). In particular species timing their diapause in response to environmental cues like photoperiod (which do not change due to climate change or following species invasions) will therefore need to adapt their diapause response in order to cope with the new situation (Bradshaw & Holzapfel, 2008; Stoks, Geerts, & De Meester, 2014). Indeed, such genetic changes in photoperiod dependent diapause induction behaviour associated with recent climate change have been observed for the North American pitcher plant mosquito *Wyeomyia smithii* (Bradshaw & Holzapfel, 2001) and the Asian tiger mosquito *Aedes albopictus* (Urbanski et al., 2012). However, in the water flea *Daphnia*, failure of a population from temperate Lake Washington to sufficiently advance their release from diapause eggs to compensate for a climate driven advance of spring time led not only to a decline of a local *Daphnia* population, but also entailed severe changes in ecosystem functioning through the modification of trophic interactions (Winder & Schindler, 2004a, 2004b). An increase in the break-up of extant trophic interactions due to climate change is predicted for the future (Stenseth & Mysterud, 2002), and there are concerns if local populations have the potential to adapt their diapause timing to these anticipated changes (Dupuis & Hann, 2009; Edwards & Richardson, 2004). Hence, an understanding of how the timing of diapause can evolve is highly warranted.

Our capability to predict the evolution of life history phenotypes requires, however, information on its physiological underpinnings and the genetic architecture controlling these traits, as the number of genes and their effect, genetic linkage and epistatic interactions can impose evolutionary constraints or facilitate adaptation (Flatt & Heyland, 2011). This knowledge gap has been emphasized specifically for the seasonal timing of diapause (Williams et al., 2017), which mostly occurs in response to photoperiod (Bradshaw & Holzapfel, 2007). For invertebrate diapause, most progress has been made in identifying genes underlying local adaptation in diapause induction (reviewed in Meuti & Denlinger, 2013), which were repeatedly found to be associated with the circadian clock (Denlinger, Hahn, Merlin, Holzapfel, & Bradshaw, 2017). For instance, the circadian clock gene *timeless* was found to be in control of diapause incidence (Sandrelli et al., 2007) and allele frequencies of *timeless* alleles differentially affecting diapause induction behaviour were shown to covary over latitude (Tauber et al., 2007) in *Drosophila*. This pattern, a functional role of one or several circadian clock genes in the control of diapause induction and associated cline in allele frequencies for these genes in natural populations is recurrently observed, e.g., for the parasitic wasp *Nasonia vitripennis* (*period*, *cycle* and *cryptochrome*; Paolucci, Salis, Vermeulen, Beukeboom, & Zande, 2016) or more recently for the speckled wood butterfly (*period* & *timeless*; Pruisscher, Nylin, Gotthard, & Wheat, 2018). However, Emerson, Dake, Bradshaw, and Holzapfel (2009) found that the photoperiodic seasonal clock in control of diapause can evolve independently from the circadian clock. Indeed, Roulin, Bourgeois, Stiefel, Walser, and Ebert (2016) identified a rhodopsin photoreceptor instead of a circadian gene as the main gene responsible for variation diapause induction in the water flea *Daphnia*. There is ongoing debate regarding if, and to which extent, the circadian clock and the (seasonal) photoperiodic timer are distinct or overlapping (Bradshaw & Holzapfel, 2010; Denlinger et al., 2017).

In contrast to this discussion, which is fuelled by a rich literature on the genetics of diapause induction, insights on genes in control of diapause termination are still scarce (Hand, Denlinger, Podrabsky, & Roy, 2016) and limited to terrestrial insects: Mathias, Jacky, Bradshaw, and Holzapfel (2007) mapped QTLs photoperiod dependent diapause termination in the pitcher-plant mosquito, *W. smithii*, and identified polygenic architecture involving epistatic interactions. While the circadian clock gene *timeless* was not located in any of the detected QTLs, it was shown to epistatically interact with one of the QTLs detected by Mathias et al. (2007). In contrast, differences in the seasonal diapause termination between two North American strains of the European Corn Borer *Ostrinia nubilalis* are controlled by a single, sex-linked QTL pointing to a large genomic inversion harbouring multiple genes, including circadian clock genes (Dopman, Pérez, Bogdanowicz, & Harrison, 2005; Wadsworth & Dopman, 2015). A similar sex-chromosome located locus is also associated diapause termination timing in swallowtail butterflies (Kunte et al., 2011). For *Rhagoletis* flies, Ragland et al. (2017) showed that the genetic architecture in control of diapause induction and termination are largely independent genomic modules, with diapause

termination timing being controlled by multiple linked blocks of loci. Thus, there is already large variation in the genetic architecture in control of diapause termination potentially affecting evolutionary trajectories between different species of terrestrial insects.

In this study, we investigated the genetic architecture in control of diapause termination in the water flea *Daphnia magna*. *Daphnia* is a crustacean zooplankter that is a pivotal herbivore in ponds and lakes (Miner, De Meester, Pfrender, Lampert, & Hairston, 2012). It produces environmental resistant diapause structures consisting of a protective capsule, called ephippium, which in the case of *D. magna* can contain two diapausing embryos in an early developmental stage. Diapause termination in *Daphnia* is known to be triggered by environmental factors such as temperature and photoperiod (Vandekerkhove et al., 2005), the latter being a relevant cue for inferring seasonality in natural populations of cladocerans (Jones & Gilbert, 2016). We therefore quantified the fraction of embryos terminating diapause under different photoperiods for clones of an established *quantitative trait locus* (QTL) mapping panel (Routtu et al., 2014). We used QTL mapping to identify genomic loci for diapause termination and to test three hypotheses regarding aspects of the genetic architecture of relevance for trait evolution. (i) Diapause termination is controlled by multiple loci of small to moderate effect (e.g., Bradshaw, Emerson, Catchen, Cresko, & Holzapfel, 2012) instead of a single major effect locus (Wadsworth, Li, & Dopman, 2015). (ii) There are epistatic interactions between these loci (e.g., Mathias et al., 2007), as opposed to the occurrence of many independent loci of additive effect, which is of relevance for adaptive capacity (Hansen, 2013). (iii) There are allele specific effects of photoperiod on diapause termination. Given the relevance of the cue photoperiod for seasonal timing in cladocerans (Jones & Gilbert, 2016), this would identify genes and alleles on which selection on seasonal timing could act, and hence would be of particular relevance for the capacity of populations to locally adapt diapause termination timing in relation to seasonal habitat phenology.

2 | MATERIALS AND METHODS

2.1 | Mapping panel

In order to map QTLs for diapause termination in *D. magna*, we used the genetic map and QTL mapping panel of Routtu et al. (2014). This panel had been built by crossing two inbred *D. magna* clones: one inbred clone originating from a fish-breeding pond near Munich, Germany (latitude: 48°12') and another inbred clone from an ephemeral rock pool in Tvärminne, South-Western Finland (latitude: 59°49'). F2 clones had been produced via clonal selfing of a single F1 clone resulting from sexual reproduction between the two inbreed clones and were genotyped at 1,324 genomic markers (average marker density 1.13 cM). The genotyped F2 clones were maintained in asexual parthenogenetic reproduction mode (i.e., clonally) since the development of the panel (Routtu et al., 2014) under standard laboratory conditions, before their use in this experiment. More details on the panel can be found in Routtu et al. (2014).

2.2 | Diapause stage production

In order to assess genotype specific diapause termination, we produced diapause stages from a random subset of 212 clones of the mapping panel. Monoclonal lineages were cultured in dechlorinated tap water at 20°C under a 16 hr photoperiod. In a first phase, we provided optimal culture conditions with an ad libitum food supply of algae (*Acutodesmus obliquus*), regular changes of culture medium a and if necessary the repeated expansion of monoclonal cultures over multiple jars (1 L) to avoid population crowding. In a second phase, we induced ephippia production by growing monoclonal cultures to initiate population crowding, a switch to short (8 hr) photoperiod and a reduction of food level and feeding interval (see Supporting Information). The short photoperiod treatment was interrupted every five days by two days with a long (16 hr) photoperiod, so that repeated changes in photoperiod were given, which has been shown to result in a more continuous production of ephippia (De Meester & De Jager, 1993). The position of jars containing the different clonal populations was randomized during the entire process. As ephippia production levelled off (typically after 3 to 4 weeks), ephippia were collected, counted and stored at 4°C in the dark for a refractory period of at least 3 months.

2.3 | Diapause termination assessment

The first hatchlings of the year in the source habitats of the two clones used to construct the mapping panel are typically observed in April for the Munich clone (7°C–8°C; 13 hr daylight) and in May for the Finnish clone (7°C–8°C; 17 hr daylight). Given the diapause termination at different photoperiod in the natural context as well as the general relevance of photoperiod as a cue for seasonal phenology integration (Bradshaw & Holzapfel, 2007), we assessed diapause termination under a short (8 hr) and long (16 hr) photoperiod in incubators set to a constant temperature of 17°C. Ephippia were taken from the cold storage and distributed over flat-bottomed 96-well laboratory plates, with each well receiving a single ephippium. Sets of ephippia from the same clone were split between plates to randomize positional or plate effects. Wells were filled with dechlorinated tap water, covered with a lid and placed in the incubators. For clones with a limited number of ephippia, we preferentially tested diapause termination under the short photoperiod treatment and only added analysis of the long photoperiod treatment if a sufficient number (~50) of ephippia were available. Plates were positioned on racks within incubators (Supporting Information Figure S2) and their position was changed daily during the hatching monitoring step to randomize positional effects.

Plates were monitored daily for the occurrence of new hatchlings for a duration of 21 days to account for the temporal spread of hatching in *Daphnia* (Vanoverbeke & De Meester, 2009). Given the large number of plates, we used a photographic monitoring technique (Czypionka, Reeves, Vanhamel, & De Meester, 2016) to allow a short assessment span relative to the 24 hr monitoring interval. Briefly, photographic monitoring consisted in the detection of

hatchlings due to their position change between photographs taken in 10 s intervals using the software Cellprofiler (Jones et al., 2008). As some ephippia only contained a single embryo, we modified the original method (Czypionka et al., 2016) to detect the number of hatchlings per well (see supporting information). The number of hatchlings was integrated over the full 21 days assessment period. At the end of the monitoring period, ephippia in the 96-well plates were bleached using with 2.12% hypochlorite solution (Sorgeloos, Bossuyt, Laviña, Baeza-Mesa, & Persoone, 1977) to check for unhatched embryos under a binocular. If our analysis indicated the sum of hatchlings and unhatched embryos per ephippium to be >2 (occurring for 89 of 27,774 wells), we resolved these inconsistencies by reviewing the photographs from the monitoring and corrected our data accordingly. For each clone, diapause termination fraction (DTF) was calculated as the number of observed hatchlings divided by the number of diapausing embryos entering the experiment (i.e., $n_{hatchlings}/(n_{hatchlings}+n_{unhatched\ embryos})$). DTFs were calculated separately for the long and short photoperiod treatment.

2.4 | QTL mapping

QTL mapping was performed in R v3.4.0 (R Core Team, 2017) with the package R/QTL v1.41-6 (Broman, Wu, Sen, & Churchill, 2003). Diapause termination characteristics in *Daphnia* have been shown to be largely determined by the genotype of the mother producing the ephippium (De Meester & De Jager, 1993). We therefore mapped DTF based on the genomic marker information of the clones that had produced the ephippia and not on the genotypes of the embryos or hatchlings themselves. As some clones of the panel had produced very few diapausing embryos we only used DTF estimates based on a minimum of 40 embryos for QTL mapping. This filtering was done to avoid introducing artefacts resulting from DTF estimates that were based on a low number of observations and thus prone to stochastic error.

In a first step, we performed single-QTL scans for DTF including photoperiod as a covariate to explore the locus specific interactions with this diapause termination cue. In the current version v1.41-6 of R/QTL, this is only implemented under a model assuming that the trait follows a normal distribution. As this was not the case for our data, we performed an additional single-QTL scan using a nonparametric model not including a covariate to validate our findings (see Supporting Information). We integrated our findings in a multiple QTL model and screened for additional QTLs after accounting for their effects.

In a second step, we performed a two-dimensional QTL scan to detect QTLs whose effect had been masked due to epistatic or additive compensatory interactions. Newly identified QTLs and interactions were integrated in the multiple QTL model and the positions of all QTLs in the model were refined using a maximum likelihood approach implemented in the R/QTL package. Confidence intervals for the resulting QTLs were calculated as 95% Bayes credible intervals. Scripts and data for QTL mapping are provided as supplementary material to this publication.

2.5 | Mapping QTLs to the genome

Marker sequences for the detected QTLs were mapped against the *D. magna* draft genome v2.4 (http://arthropods.eugenes.org/genes2/daphnia/daphnia_magna/BLAST/) using BLASTn (E-value cut-off 0.01) (Altschul, Gish, Miller, Myers, & Lipman, 1990). The fragmentation of the draft genome did not allow us to define genomic regions of interest for the identification of candidate genes. We therefore mapped (E-value cutoff = 0.01; all significant hits verified by eye) QTL markers confining the confidence intervals of selected QTLs to a new, less fragmented, PacBio based genome assembly of the same genotype of *D. magna* (P. D. Fields, in preparation). The package GVIZ v 1.22.3 (Hahne & Ivanek, 2016) was used to visualize the gene content in these genomic regions.

3 | RESULTS

3.1 | Diapause stage production

A large number of ephippia (>31,000) was produced through clonal selfing by 209 of the 212 F2 clones of the *D. magna* QTL panel that were included in the experiment. The number of ephippia per clone varied widely, ranging from 3 to 910 (see Supporting Information Figure S1). In addition, we noticed a large variability among clones in the fraction of ephippia that contained no or only a single diapausing embryo. The average filling of the ephippia ranged from 0% to 83.3% per clone (with 100% indicating two embryos in all ephippia). The number of produced ephippia per clone was not used as a trait for QTL mapping as this is beyond the scope of this study and was done previously (Roulin et al., 2013).

3.2 | Diapause termination fraction

DTF data were available for 198 clones, of which 119 were assessed at both photoperiods. DTF estimates and the number of diapausing embryos on which this estimate was based ($n_{hatchlings} + n_{unhatched\ embryos}$) are provided in the QTL analysis input file in the supplementary. Figure 1a illustrates the distribution of DTF under long (16 hr) and short (8 hr) photoperiod. The majority (62%) of the 70 clones assessed at both photoperiod had either a high (>80%; top right in Figure 1a, 25 clones) or low (<20%; bottom left in Figure 1a, 19 clones) DTF at both photoperiods. This divide was independent of photoperiod with the average difference in DTF between photoperiods for individual clones being much smaller (average increase 4.7% DTF for high DTF clones, 1.4% DTF for low DTF clones) when compared to the difference between them (>80% DTF vs <20% DTF).In contrast, the 26 clones not belonging to the groups with a high (>80%) or low (<20%) DTF at both photoperiods showed an average DTF increase of 11.2% DTF, which was much more pronounced than for the entire dataset (70 clones, average increase 6.2% DTF). This agreed with the overall trend for an DTF increase at long photoperiod (49 of 70 clones (70%) falling in the top left half of Figure 1a). While the opposite (higher

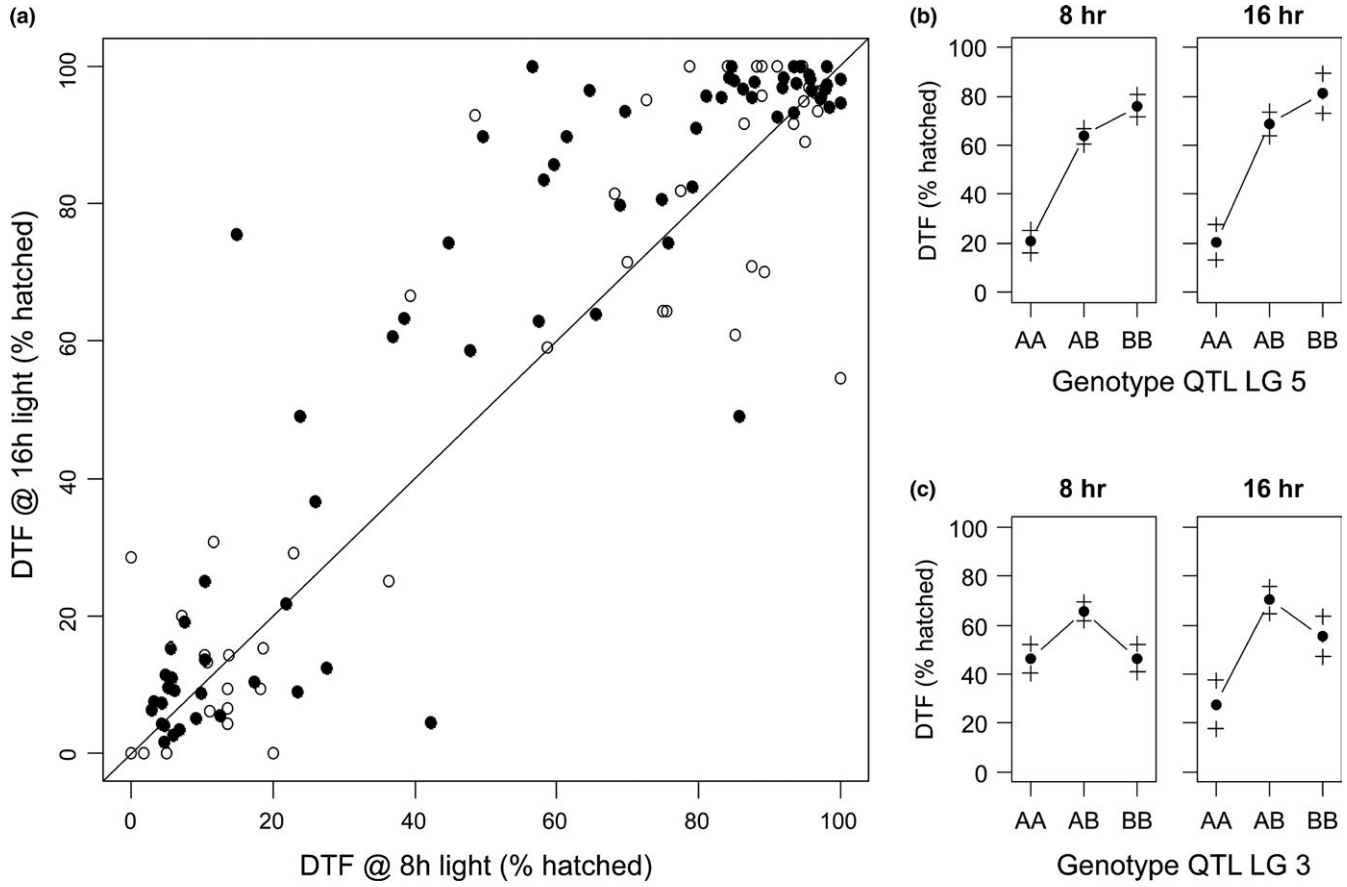

FIGURE 1 Diapause termination fractions (DTF) for different clones or for specific QTLs. (a) Scatterplot of DTF for different clones at short (8 hr light; x-axis) and long (16 hr light; y-axis) photoperiod. The diagonal line indicates the isocline where DTFs at both photoperiods are identical. Black dots: clones with each DTF estimate based on>= 40 diapausing embryos; black circles: clones with sample sizes of <40 diapause for one or both photoperiods. Note that DTFs of clones that could only be assessed at one photoperiod are not included. The overall distribution is characterized by: (i) a separation in clones with an either high (>80%) or low (<20%) DTF, and (ii) a tendency for a higher DTF at long photoperiod (i.e., circles above the isocline). (b) DTFs of the different genotypes of the QTL at LG 5 at short (left panel) or long (right panel) photoperiod. A clear effect of genotype is observed at both photoperiods, with genotype AA (German homozygous) characterized by a lower DTF than the other two genotypes at both photoperiods. Midpoints indicate average DTF, error bars indicate 1 standard error. (c) DTF of the different genotypes of the QTL at LG 3 at short (left panel) or long (right panel) photoperiod. An effect of genotype as well as of photoperiod is observed

DTF at short photoperiod) was also observed for some clones, this mainly related to DTF estimates based on less than 40 embryos (empty circles in Figure 1a), or to members of the group of clones with a generally low DTF. The relatively low number of diapausing embryos for some clones mainly reflects the large number of ephippia not containing embryos and the high variability in the number of produced ephippia between clones and was, to a lesser extent, reduced further by incubator fails during DTF assessment.

3.3 | Mapping results

QTL mapping analysis was performed based on 147 clones, of which 70 could be assessed at both photoperiods, after excluding DTF estimates based on less than 40 diapausing embryos as well as an outlier clone that showed an increase from 14.8% to 75.6% DTF between short and long photoperiod.

3.3.1 | Single-QTL scan

The single-QTL scan identified significant ($p < 0.05$) QTLs on linkage groups 3 and 5 that were confirmed using a nonparametric model (see Supporting Information Figures S4 and S5). Inclusion of photoperiod as an additive and interacting factor provided evidence for the QTL on LG 3 modulating DTF in response to photoperiod, as the inclusion of photoperiod as an interacting factor raised the LOD score of the QTL on LG 3 from 4.63 to 5.46 (Supporting Information Figure S4). The effect of the QTL on LG 5 mirrored the photoperiod independent divide in the groups of clones with a low or high DTF (bottom left vs top right in Figure 1a), as the homozygous German genotype (AA) had an average DTF of approximately 20% at both photoperiods, while homozygous Finnish (BB; 75.9% & 81.1%) and the heterozygous clones (AB; 63.9% & 68.7%) had higher DTFs (Figure 1b). In turn, photoperiod treatment dependent differences

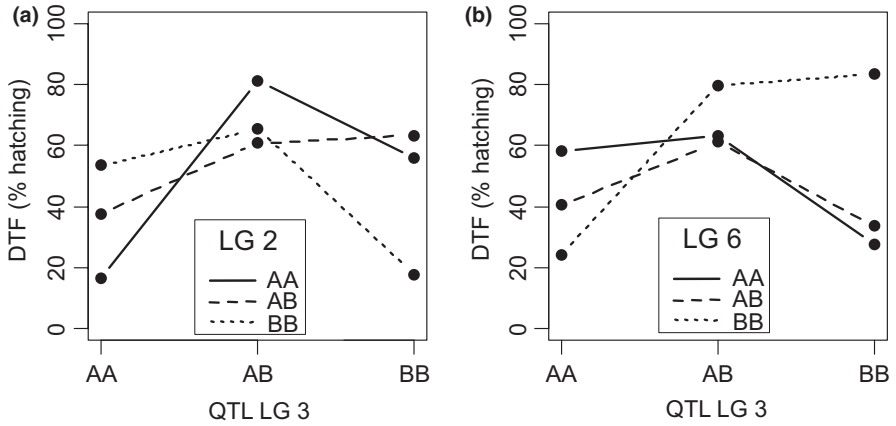

FIGURE 2 Effect of epistatic interactions on average diapause termination fraction (DTF). (a) Effect of epistatic interactions between the QTLs on LG 3 (genotypes on x-axis) and LG2 (genotypes represented by different lines) on average diapause termination fraction (DTF; y-axis). Lowest average DTFs are observed in the original combination of homozygous alleles (i.e., AA-AA & BB-BB). (b) Effect of epistatic interactions between the QTLs on LG 3 and LG 6 on average DTF. Highest average DTFs are observed in the original combination of homozygous alleles. Effect plots for the remaining epistatic interaction are provided in the supplementary material as Figure S6. Alleles: AA, German homozyogous; BB, Finnish homozygous; AB, heterozygous

in average DTF were observed for the different genotypes for the QTL at LG 3 (Figure 1c), where clones with German and Finnish homozygous alleles were comparable at a short photoperiod (both 46.2%), but with a tendency for a difference in average DTF (27.5% [AA] vs 55.3% [BB]) being observed at a long photoperiod. As some clones were only assessed at a single photoperiod, differences between the two panels in Figure 1c cannot be exclusively attributed to the effect of photoperiod, but subsequent analyses confirmed a role for the QTL on LG 3 for photoperiod dependent diapause termination (see Supporting Information). The heterozygous genotype (AB) had a higher average DTF than both homozygous genotypes at both photoperiods (65.8% at short photoperiod and 70.2% at long photoperiod).

3.3.2 | Two-dimensional QTL scan

The two-dimensional QTL scan identified five new significant QTLs ($p_{fv1} < 0.05$ & $p_{interaction} < 0.05$; in one case $p_{interaction} = 0.052$; see Supporting Information Table S1). These consisted of three loci (on LGs 2, 6 and 10) that were all interacting with the QTL on LG 3 and a pair of interacting QTLs on LGs 1 and 8.

The epistatic interaction between the QTLs at LG 3 and 2 was associated with low average DTFs of <20% for the original parental combinations of homozygous alleles (AA-AA 16.3%, BB-BB 17.5%) but with an average DTF of >50% for clones combining homozygous alleles of both backgrounds (i.e., AA-BB 53.5%, BB-AA 62%;Figure 2a). This was inversed for the interaction between the QTLs on LGs 3 and 6, where the original combinations of homozygous alleles had higher average DTFs (AA-AA: 58.1% and BB-BB: 83.4%) than the German-Finnish combinations (AA-BB: 22.5% and BB-AA: 27.5%; Figure 2b). A heterozygous genotype for the QTL at LG 3 did on average contribute to an increase in DTF (average DTF 67.4%), as was already found in the single QTL analysis. Corresponding information for the epistatic interactions between the QTLs on LGs 1 and 8 and the QTLS on LGs 3 and 10 are provided in the supplementary.

3.3.3 | Multiple QTL model

The identified QTLs and epistatic interactions were integrated in a multiple QTL model. One additional QTL on LG 4 was detected by scanning for additional QTLs after accounting for the effects of the two QTLs at LG 3 and 5 derived through the single-QTL scan. Refining of QTL positions via a maximum likelihood approach in R/qtl led to the relocation of the QTL on LG 2 from 168.9 cM to 28.4 cM (Figure 3). Overall, the final model included eight QTLs and four interactions and explained 66.5% of the variation in DTF ($LOD_{model} = 51.52$; see Supporting Information Table S2 for individual effects). The QTL on LG 3 explained the highest amount of variation in DTF of all identified QTLs by its own effect (12.9%) as well through epistatic interactions (Supporting Information Table S2).

3.4 | Candidate genes

Confidence intervals for all QTLs (Supporting Information Table S3) are provided together with the corresponding genomic scaffolds of the *D. magna* genome draft v2.4 in the supplementary. The confidence intervals of the two most significant QTLs on LG 3 and 5 converged to the central marker position (Supporting Information Table S3). As this corresponds to an unrealistic confidence interval of 0 cM, we defined our genomic regions of interest by mapping the two adjacent markers against an improved version of the *D. magna* genome (P. D. Fields, in preparation). Figure 4 shows the genes in the candidate region of the QTL on LG 3. The central marker position was located in three gene model annotations (*putative ELKS/Rab6-interacting/CAST family member 1*, *ERC protein*, and *putative ELKS/*

Rab6-interacting/CAST family member), which are synonyms for the same protein. Further genes near the marker position were: *Mediator of RNA polymerase II transcription subunit 20*, *Facilitated trehalose transporter Tret1–2* and an uncharacterized protein.

The definition of a genomic region of interest for the QTL at LG 5 was challenging as the marker order between the genetic map (Routtu et al., 2014) and the newly assembled genomic contig did not agree. As the two adjacent markers mapped about 90 and 110 kb downstream of the central marker position of the QTL, we extended the associated genomic region of interest also 90 kb upstream of this position. Genes in the immediate genomic vicinity of the central QTL marker included: *putative DNA-directed RNA polymerase*, several genes for *actin* proteins, several uncharacterized proteins, *DNA damage binding protein 1*, and *Methyltransferase 9-like protein* (see Supporting Information Figure S11 for more details).

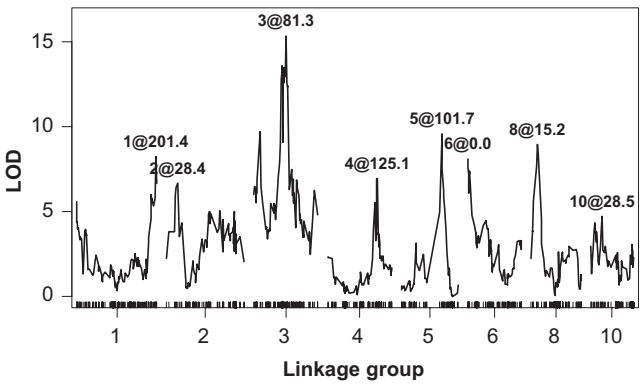

FIGURE 3 LOD score profile of the multiple QTL model. LOD score (y-axis) profile are provided at the genetic map positions (x-axis) at those eight linkage groups holding a QTL for DTF. The LOD score of the full multiple QTL model with 8 QTLS and four epistatic interaction is 51.52. Note that the QTL on LG 2 was relocated from 168.9 cM to 28.4 cM during maximum likelihood remapping and thus does not correspond to the QTL described for the results of the two dimensional QTL scan in Figure 2a. Ticks at the x-axis corresponds to SNP markers of the genetic map by (Routtu et al., 2014) which was used for QTL mapping

4 | DISCUSSION

In this study, we investigated the genetic architecture for diapause termination in the water flea *Daphnia magna* using a QTL mapping approach. There was ample genetic variation in the diapause termination response between the clones of the mapping panel. The largely bimodal separation in clones with a very high or low diapause determination fraction (Figure 1a) and its association with a QTL on LG 5 (Figure 1b) suggests the contribution of a major effect locus with a single on-off switch for diapause termination. However, our final results, which also account for the results from the two-dimensional QTL scan, revealed a genetic architecture involving eight loci and four epistatic interactions. This model explained 66.5% of the observed variation in diapause termination fraction, with the individual QTL accounting for 3.5% to 12.9% of the variation (Supporting Information Table S2). This supports our first hypothesis of multiple loci with small to moderate effects controlling diapause termination.

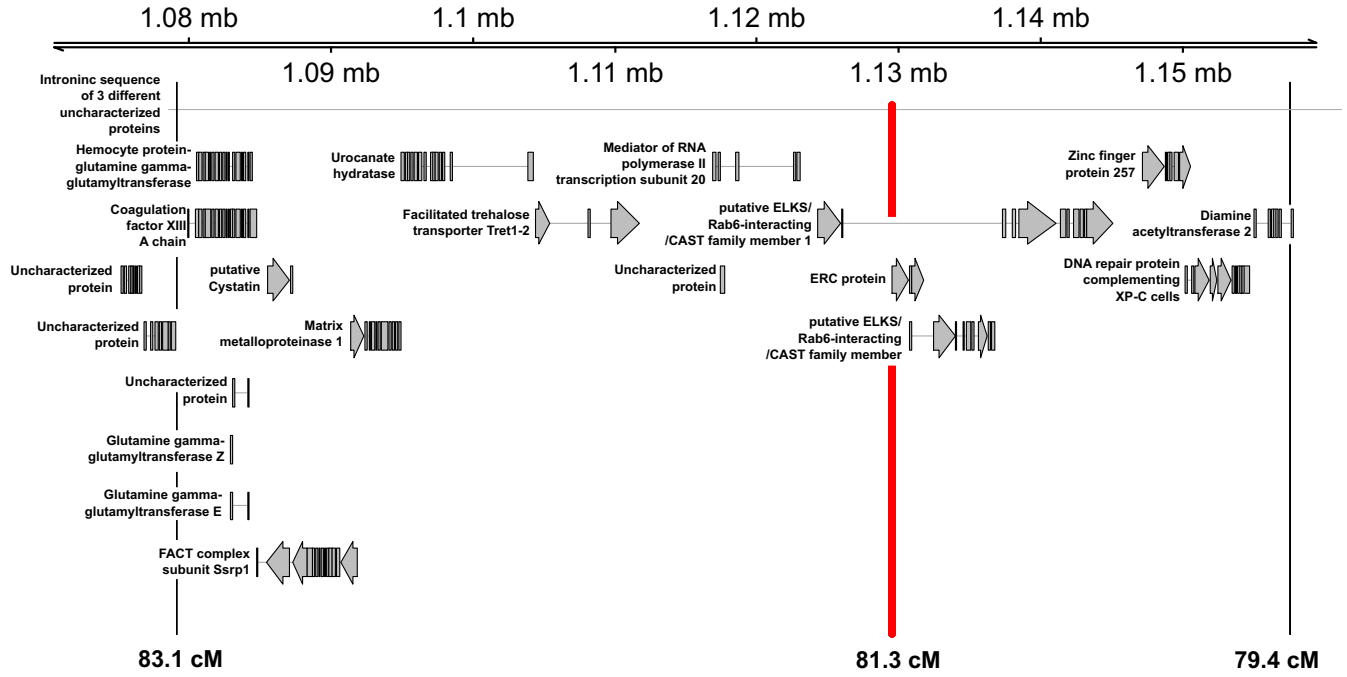

FIGURE 4 Genomic region of interest for the QTL at LG 3. Top: Genomic position (in megabase-pairs) on genomic scaffold. The red vertical line indicates the position of the central QTL marker at 81.3 cM in the genome. The two black vertical lines indicate the position of the two adjacent markers (83.1 and 79.4 cM) that confine the genomic region of interest. Genes are shown by a schematic representation of their intron-exon structure

However, the QTLs on LG 5 and 3, which had been already identified during the single QTL scan, stood out in having stronger effects on diapause termination than the other QTLs.

The QTL on LG 5 was associated with a strong differentiation in average diapause termination fraction between genotypes. Clones homozygous for the German allele of this QTL showed low diapause termination fractions (Figure 1b), largely independent of photoperiod. One possible interpretation of this observation is that the QTL on LG 5 points to a gene causing a low diapause termination fraction without adaptive value, similar to the two recessive infertility alleles already identified in the panel (Routtu, Jansen, Colson, De Meester, & Ebert, 2010). Alternatively, reduced diapause termination fractions have been described as an adaptive bet-hedging strategy in *Daphnia* populations from unpredictable habitats (Cáceres & Tessier, 2003; Waterkeyn et al., 2013). If a fraction of the diapausing population does not hatch from the protective ephippia at the start of a growing season, it will survive in case of an unpredictable catastrophic event (e.g., drying or freezing of a pond) and can exploit beneficial conditions in the next growing season, thereby increasing its long-term fitness (Starrfelt & Kokko, 2012). Based on the currently available data, it is not possible to distinguish between these two scenarios. In this context, it is noteworthy that the marker order of the genetic map did not agree with the genome sequence around the QTL on LG 5 (Supporting Information Figure S11). As the reference genome was based on the sequence of the Finnish clone from the mapping panel from which the genetic map was developed, this could reflect a structural genomic polymorphism, e.g., an inversion, between the Finnish and the German clone (Routtu et al., 2014). While our data do not allow us to investigate this further and exclude the possibility of e.g., errors in the genetic map or new genome assembly, inversions have been repeatedly shown to be involved in the adaptive divergence in the timing of life history transition (Wellenreuther & Bernatchez, 2018), including diapause termination in insects (Ragland et al., 2017; Wadsworth et al., 2015). Thus, this finding might indicate a worthwhile possibility for future research regarding a role of the QTL on LG 5 in local adaptation of diapause termination in one of the source habitats.

The other major QTL on LG 3 explained the highest amount of variation in diapause termination fraction in the multiple QTL and was involved in three of the four pairwise interactions that were detected in this study. Together with the epistatic interaction between the QTLs on LGs 1 and 8, this supported our second hypothesis about the relevance of epistatic interactions for the genetic control of diapause termination in *D. magna*. Interestingly, interactions between the combination of homozygous alleles from different backgrounds caused an increase (Figure 2a) or decrease (Figure 2b) in diapause termination fraction depending on the loci involved.

The nature of the detected epistatic interactions hence indicates a possible avenue for admixture facilitated evolution of diapause termination (Krehenwinkel, Rödder, & Tautz, 2015): For example, introgression of a German homozygous allele of the QTL at LG 2 in Finnish background could lead to an increase DTF diapause termination fraction (see Figure 2a). Although admixture between two source populations used to construct the mapping panel (Routtu et al., 2014) is rather unlikely due the vast geographic distance between the Germany and Finland, previous findings indicate that diapause termination patterns also vary between populations of *Daphnia* at a micro-geographic scale (De Meester & De Jager, 1993). Thus, we speculate that adaptation of diapause termination timing in response to anticipated climate change (Dupuis & Hann, 2009) might be facilitated by an increase in adaptive potential via adaptive introgression of relevant alleles from adjacent populations. Adaptive introgression would leave overall genomic structure of the local population intact, thereby preserving habitat related adaptive variation (Nolte, Gompert, & Buerkle, 2009). Our findings on the genomic architecture of diapause termination in *Daphnia* might also hint a possible explanation for an emerging pattern in diapause induction studies in insects, where diapause induction was found to be inherited as a single locus trait at one photoperiod, but as a multilocus trait at other photoperiods (Fu, Chen, Xiao, He, & Xue, 2015; Pruisscher et al., 2017). Our findings indicate that different alleles at one QTL can modulate and largely negate the effect of another QTL on DTF (Figure 2). Hence, observed patterns of single vs multilocus inheritance at different photoperiods (Fu et al., 2015; Pruisscher et al., 2017), could result from QTLs only expressed depending on photoperiod, especially if such QTLs interact with many other loci (like the QTL on LG 3). In turn, we speculate that evolutionary changes of the critical photoperiod for the expression of such QTL might release cryptic variation on which selection might act (Ghalambor, McKay, Carroll, & Reznick, 2007) and hence raise the adaptive potential for diapause termination timing.

In this context, the relevance of the locus on LG 3 was also underlined by the identification allele specific effects of photoperiod on diapause termination fraction (Figure 1b). The photoperiod independent, strong separation of our data set into clones with either high or low diapause termination fraction complicated the detection of the comparably weaker effect of photoperiod on diapause termination (see Supporting Information). Yet, our analysis indicated a locus specific interaction with photoperiod for the QTL on LG 3 in agreement with our third hypothesis. Photoperiod has repeatedly been shown to serve as a seasonal cue for the adaptive seasonal timing of life-history in many species (Bradshaw & Holzapfel, 2007, 2008), including cladocerans (Jones & Gilbert, 2016). The genomic region of interest for this QTL spanned <100 kb (Figure 4). Directly at the marker position there are annotations for three isoforms of the same gene, the *ELKS/Rab6 interacting/Cast family member* (also known as *ERC protein*). While the region of interest includes additional genes whose potential functional relevance for diapause cannot be disproven (e.g., the trehalose transporter *Tret 1–2* [Hengherr, Heyer, Brümmer, & Schill, 2011]), most of these genes are closer to the genomic position of the adjacent markers for the genetic map positions of 79.4 or 83.1 cM (Figure 4), or do not have any function assigned (*uncharacterized protein* in Figure 4). In turn, the role of the *ERC protein* as a top candidate for the photoperiod dependent termination of diapause is also reinforced by functional considerations. The insect homolog of *ERC protein*, the gene *bruchpilot*, has

been intensively studied in *Drosophila*, where it was identified as a structural protein of the presynaptic active zone (Wagh et al., 2006), which is also involved in the modulation of signal transmission including the regulation of photosensitivity in photoreceptor synapses (Sugie et al., 2015). Moreover, *bruchpilot* was recently found to be in a light dependent way degraded by the photopigment *cryptochrome* (Damulewicz et al., 2017), analogous to the well known light dependent degradation of the circadian gene *timeless*, which is involved in photoperiod dependent diapause induction in *Drosophila* (Sandrelli et al., 2007). There is a long-standing debate, if the circadian clock and the photoperiodic seasonal clock in control of diapause are the same, or distinct (Bradshaw & Holzapfel, 2010). The identification of *bruchpilot* as a gene not pertaining to the core circadian clock, but interacting with it (Górska-Andrzejak et al., 2013), might contribute to a better understanding as how these important biological clocks are coupled (Denlinger et al., 2017). Overall, our findings suggest that we identified a candidate gene for the observed differentiation in diapause termination, as well as for the perception of photoperiod as a cue for season.

5 | CONCLUSION

This study contributes to the elucidation of the genetic architecture for diapause termination in the water flea *Daphnia*. The integration of QTL mapping results with genomic data allowed the delineation of the candidate gene *bruchpilot* for diapause termination in response to photoperiod, and the genomic locations of the remaining QTLs lay the foundation for revealing further candidate genes in the future. Our results, together with corresponding findings of diapause induction (Roulin et al., 2016, 2013), now establish *Daphnia* as an ideal study system for the ecological genomics of diapause timing. Given the long tradition of *Daphnia* as a model organism in ecology and evolution (Lampert, 2011), this can be extended to the analysis of well characterized natural populations. This will allow to study the effect of genetic architecture in constraining or facilitating the evolution of diapause timing in response to environmental changes in conjunction with its implications for ecosystem functioning.

ACKNOWLEDGEMENTS

TC was supported by grants CZ 230 1/1 and CZ 230 2/1 by the German Research Foundation. DE and PDF were supported by the Swiss National Science Foundation and the University of Basel. LDM was supported by KU Leuven Research Fund projects PF/2010/07 and C16/2017/002, and by FWO project G0C3818. We acknowledge support in ephippia production by Carla Denis & Melissa Schepens and discussion with Tom Pinceel.

AUTHOR CONTRIBUTIONS

T.C. conceived the study together with L.D.M. and D.E. and performed the laboratory work for diapause termination assessment with substantial support from E.vdB. J.R. designed the mapping panel and genetic map used in this study. P.D.F. contributed unpublished genome assembly data. T.C. performed data analysis wrote the manuscript with help from L.D.M. and D.E. All authors gave valuable suggestions and final approval for publication.

DATA ACCESSIBILITY

The full data set on diapause termination fractions and number of analysed embryos for the assessed clones of the mapping panel are provided in the electronic supplementary to this publication. These data are provided together with the genotypic marker information for the respective clones and can directly be loaded in the accompanying QTL analysis script to reproduce the results of this study.

ORCID

Till Czypionka https://orcid.org/0000-0001-7655-2897
Peter D. Fields https://orcid.org/0000-0003-2959-2524

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Bradshaw, W. E., Emerson, K. J., Catchen, J. M., Cresko, W. A., & Holzapfel, C. M. (2012). Footprints in time: comparative quantitative trait loci mapping of the pitcher-plant mosquito, *Wyeomyia smithii*. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1747), 4551–4558. https://doi.org/10.1098/rspb.2012.1917

Bradshaw, W. E., & Holzapfel, C. M. (2001). Genetic shift in photoperiodic response correlated with global warming. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(25), 14509–14511. https://doi.org/10.1073/pnas.241391498

Bradshaw, W. E., & Holzapfel, C. M. (2007). Evolution of animal photoperiodism. *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 1–25. https://doi.org/10.1146/annurev.ecolsys.37.091305.110115

Bradshaw, W. E., & Holzapfel, C. M. (2008). Genetic response to rapid climate change: It's seasonal timing that matters. *Molecular Ecology*, *17*(1), 157–166. https://doi.org/10.1111/j.1365-294X.2007.03509.x

Bradshaw, W. E., & Holzapfel, C. M. (2010). What season is it anyway? Circadian tracking *vs.* photoperiodic anticipation in insects. *Journal of Biological Rhythms*, *25*(3), 155–165. https://doi.org/10.1177/0748730410365656

Broman, K. W., Wu, H., Sen, S., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics (Oxford, England)*, *19*(7), 889–890. https://doi.org/10.1093/bioinformatics/btg112

Cáceres, C. E., & Tessier, A. J. (2003). How long to rest: The ecology of optimal dormancy and environmental constraint. *Ecology*, *84*(5), 1189–1198. https://doi.org/10.1890/0012-9658(2003)084[1189:HLTRTE]2.0.CO;2

Czypionka, T., Reeves, G., Vanhamel, M., & De Meester, L. (2016). Assessing hatching rates and the timing of hatching from plankton resting stages—an accurate and cost effective high throughput approach. *Limnology and Oceanography: Methods*, *14*(11), 718–724. https://doi.org/10.1002/lom3.10125

Damulewicz, M., Mazzotta, M. G., Sartori, E., Rosato, E., Costa, R., & Pyza, E. M. (2017). Cryptochrome is a regulator of synaptic plasticity

in the visual system of *Drosophila melanogaster*. *Frontiers in Molecular Neuroscience*, *10*, 165. https://doi.org/10.3389/fnmol.2017.00165

De Meester, L., & De Jager, H. (1993). Hatching of *Daphnia* sexual eggs. I. Intraspecific differences in the hatching responses of *D. magna* eggs. *Freshwater Biology*, *30*(2), 219–226. https://doi.org/10.1111/j.1365-2427.1993.tb00803.x

Denlinger, D. L., Hahn, D. A., Merlin, C., Holzapfel, C. M., & Bradshaw, W. E. (2017). Keeping time without a spine: What can the insect clock teach us about seasonal adaptation? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1734), 20160257. https://doi.org/10.1098/rstb.2016.0257

Donnelly, A., Caffarra, A., Kelleher, C. T., O'Neill, B. F., Diskin, E., Pletsers, A., … Sparks, T. H. (2012). Surviving in a warmer world: Environmental and genetic responses. *Climate Research*, *53*(3), 245–262. https://doi.org/10.3354/cr01102

Dopman, E. B., Pérez, L., Bogdanowicz, S. M., & Harrison, R. G. (2005). Consequences of reproductive barriers for genealogical discordance in the European corn borer. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(41), 14706–14711. https://doi.org/10.1073/pnas.0502054102

Dupuis, A. P., & Hann, B. J. (2009). Climate change, diapause termination and zooplankton population dynamics: An experimental and modelling approach. *Freshwater Biology*, *54*(2), 221–235. https://doi.org/10.1111/j.1365-2427.2008.02103.x

Edwards, M., & Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*(7002), 881–884. https://doi.org/10.1038/nature02808

Emerson, K. J., Dake, S. J., Bradshaw, W. E., & Holzapfel, C. M. (2009). Evolution of photoperiodic time measurement is independent of the circadian clock in the pitcher-plant mosquito, *Wyeomyia smithii*. *Journal of Comparative Physiology A*, *195*(4), 385–391. https://doi.org/10.1007/s00359-009-0416-9

Flatt, T., & Heyland, A. (2011). *Mechanisms of life history evolution: The genetics and physiology of life history traits and trade-offs*, 1st ed. Oxford, UK: Oxford University Press.

Forrest, J., & Miller-Rushing, A. J. (2010). Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3101–3112. https://doi.org/10.1098/rstb.2010.0145

Fu, S., Chen, C., Xiao, L., He, H., & Xue, F. (2015). Inheritance of diapause in crosses between the northernmost and the southernmost strains of the Asian corn borer *Ostrinia furnacalis*. *PLoS ONE*, *10*(2), e0118186. https://doi.org/10.1371/journal.pone.0118186

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, *21*(3), 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x

Gienapp, P., Teplitsky, C., Alho, J. S., Mills, J. A., & Merilä, J. (2008). Climate change and evolution: Disentangling environmental and genetic responses. *Molecular Ecology*, *17*(1), 167–178. https://doi.org/10.1111/j.1365-294X.2007.03413.x

Górska-Andrzejak, J., Makuch, R., Stefan, J., Görlich, A., Semik, D., & Pyza, E. (2013). Circadian expression of the presynaptic active zone protein bruchpilot in the lamina of *Drosophila melanogaster*. *Developmental Neurobiology*, *73*(1), 14–26. https://doi.org/10.1002/dneu.22032

Hahne, F., & Ivanek, R. (2016). Visualizing genomic data using Gviz and Bioconductor. In E. Mathé & S. Davis (Eds.), *Statistical genomics* (pp. 335–351). New York, NY: Humana Press. doi:10.1007/978-1-4939-3578-9_16.

Hairston, N. G., & Walton, W. E. (1986). Rapid evolution of a life history trait. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(13), 4831–4833. https://doi.org/10.1073/pnas.83.13.4831

Hand, S. C., Denlinger, D. L., Podrabsky, J. E., & Roy, R. (2016). Mechanisms of animal diapause: Recent developments from nematodes, crustaceans, insects, and fish. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *310*(11), R1193–R1211. https://doi.org/10.1152/ajpregu.00250.2015

Hansen, T. F. (2013). Why epistasis Is important for selection and adaptation. *Evolution*, *67*(12), 3501–3511. https://doi.org/10.1111/evo.12214

Hengherr, S., Heyer, A. G., Brümmer, F., & Schill, R. O. (2011). Trehalose and vitreous states: Desiccation tolerance of dormant stages of the Crustaceans *Triops* and *Daphnia*. *Physiological and Biochemical Zoology*, *84*(2), 147–153. https://doi.org/10.1086/658499

Jones, N. T., & Gilbert, B. (2016). Changing climate cues differentially alter zooplankton dormancy dynamics across latitudes. *Journal of Animal Ecology*, *85*(2), 559–569. https://doi.org/10.1111/1365-2656.12474

Jones, T. R., Kang, I., Wheeler, D. B., Lindquist, R. A., Papallo, A., Sabatini, D. M., … Carpenter, A. E. (2008). Cell Profiler Analyst: Data exploration and analysis software for complex image-based screens. *BMC Bioinformatics*, *9*, 482. https://doi.org/10.1186/1471-2105-9-482

Krehenwinkel, H., Rödder, D., & Tautz, D. (2015). Eco-genomic analysis of the poleward range expansion of the wasp spider *Argiope bruennichi* shows rapid adaptation and genomic admixture. *Global Change Biology*, *21*(12), 4320–4332. https://doi.org/10.1111/gcb.13042

Kunte, K., Shea, C., Aardema, M. L., Scriber, J. M., Juenger, T. E., Gilbert, L. E., & Kronforst, M. R. (2011). Sex chromosome mosaicism and hybrid speciation among Tiger Swallowtail butterflies. *PLOS Genetics*, *7*(9), e1002274. https://doi.org/10.1371/journal.pgen.1002274

Lampert, W. (2011). *Daphnia: Development of a model organism in ecology and evolution*. Oldendorf, Germany: International Ecology Institute.

Mathias, D., Jacky, L., Bradshaw, W. E., & Holzapfel, C. M. (2007). Quantitative trait loci associated with photoperiodic response and stage of diapause in the pitcher-plant mosquito, *Wyeomyia smithii*. *Genetics*, *176*(1), 391–402. https://doi.org/10.1534/genetics.106.068726

Meuti, M. E., & Denlinger, D. L. (2013). Evolutionary links between circadian clocks and photoperiodic diapause in insects. *Integrative and Comparative Biology*, *53*(1), 131–143. https://doi.org/10.1093/icb/ict023

Miner, B. E., De Meester, L., Pfrender, M. E., Lampert, W., & Hairston, N. G. (2012). Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1735), 1873–1882. https://doi.org/10.1098/rspb.2011.2404

Nolte, A. W., Gompert, Z., & Buerkle, C. A. (2009). Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. *Molecular Ecology*, *18*(12), 2615–2627. https://doi.org/10.1111/j.1365-294X.2009.04208.x

Paolucci, S., Salis, L., Vermeulen, C. J., Beukeboom, L. W., & van de Zande, L. (2016). QTL analysis of the photoperiodic response and clinal distribution of period alleles in *Nasonia vitripennis*. *Molecular Ecology*, *25*(19), 4805–4817. https://doi.org/10.1111/mec.13802

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Pruisscher, P., Larsdotter-Mellström, H., Stefanescu, C., Nylin, S., Wheat, C. W., & Gotthard, K. (2017). Sex-linked inheritance of diapause induction in the butterfly *Pieris napi*. *Physiological Entomology*, *42*(3), 257–265. https://doi.org/10.1111/phen.12194

Pruisscher, P., Nylin, S., Gotthard, K., & Wheat, C. W. (2018). Genetic variation underlying local adaptation of diapause induction along a cline in a butterfly. *Molecular Ecology*, *27*(18), 3613–3626. https://doi.org/10.1111/mec.14829

R Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Ragland, G. J., Doellman, M. M., Meyers, P. J., Hood, G. R., Egan, S. P., Powell, T. H. Q., … Feder, J. L. (2017). A test of genomic modularity among life-history adaptations promoting speciation with gene flow. *Molecular Ecology*, *26*(15), 3926–3942. https://doi.org/10.1111/mec.14178

Roulin, A. C., Bourgeois, Y., Stiefel, U., Walser, J.-C., & Ebert, D. (2016). A photoreceptor contributes to the natural variation of diapause induction in *Daphnia magna*. *Molecular Biology and Evolution*, *33*(12), 3194–3204. https://doi.org/10.1093/molbev/msw200

Roulin, A. C., Mariadassou, M., Hall, M. D., Walser, J.-C., Haag, C., & Ebert, D. (2015). High genetic variation in resting-stage production in a metapopulation: Is there evidence for local adaptation? *Evolution*, *69*(10), 2747–2756. https://doi.org/10.1111/evo.12770

Roulin, A. C., Routtu, J., Hall, M. D., Janicke, T., Colson, I., Haag, C. R., & Ebert, D. (2013). Local adaptation of sex induction in a facultative sexual crustacean: Insights from QTL mapping and natural populations of *Daphnia magna*. *Molecular Ecology*, *22*(13), 3567–3579. https://doi.org/10.1111/mec.12308

Routtu, J., Hall, M. D., Albere, B., Beisel, C., Bergeron, R. D., Chaturvedi, A., … Ebert, D. (2014). An SNP-based second-generation genetic map of *Daphnia magna* and its application to QTL analysis of phenotypic traits. *BMC Genomics*, *15*(1), 1033. https://doi.org/10.1186/1471-2164-15-1033

Routtu, J., Jansen, B., Colson, I., De Meester, L., & Ebert, D. (2010). The first-generation *Daphnia magna* linkage map. *BMC Genomics*, *11*, 508. https://doi.org/10.1186/1471-2164-11-508

Sandrelli, F., Tauber, E., Pegoraro, M., Mazzotta, G., Cisotto, P., Landskron, J., Kyriacou, C. P. (2007). A molecular basis for natural selection at the timeless Locus in *Drosophila melanogaster*. *Science*, *316*(5833), 1898–1900. https://doi.org/10.1126/science.1138426.

Schmidt, P. S., Matzkin, L., Ippolito, M., & Eanes, W. F. (2005). Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. *Evolution*, *59*(8), 1721–1732. https://doi.org/10.1111/j.0014-3820.2005.tb01821.x

Sorgeloos, P., Bossuyt, E., Laviña, E., Baeza-Mesa, M., & Persoone, G. (1977). Decapsulation of *Artemia* cysts: A simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture*, *12*(4), 311–315. https://doi.org/10.1016/0044-8486(77)90209-5

Starrfelt, J., & Kokko, H. (2012). Bet-hedging - a triple trade-off between means, variances and correlations. *Biological Reviews*, *87*(3), 742–755. https://doi.org/10.1111/j.1469-185X.2012.00225.x

Stenseth, N. C., & Mysterud, A. (2002). Climate, changing phenology, and other life history traits: Nonlinearity and match–mismatch to the environment. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(21), 13379–13381. https://doi.org/10.1073/pnas.212519399

Stoks, R., Geerts, A. N., & De Meester, L. (2014). Evolutionary and plastic responses of freshwater invertebrates to climate change: Realized patterns and future potential. *Evolutionary Applications*, *7*(1), 42–55. https://doi.org/10.1111/eva.12108

Sugie, A., Hakeda-Suzuki, S., Suzuki, E., Silies, M., Shimozono, M., Möhl, C., … Tavosanis, G. (2015). Molecular remodeling of the presynaptic active zone of *Drosophila* photoreceptors via activity-dependent feedback. *Neuron*, *86*(3), 711–725. https://doi.org/10.1016/j.neuron.2015.03.046

Tauber, E., Zordan, M., Sandrelli, F., Pegoraro, M., Osterwalder, N., Breda, C., … Costa, R. (2007). Natural selection favors a newly derived *timeless* allele in *Drosophila melanogaster*. *Science*, *316*(5833), 1895–1898. https://doi.org/10.1126/science.1138412

Urbanski, J., Mogi, M., O'Donnell, D., DeCotiis, M., Toma, T., & Armbruster, P. (2012). Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient. *The American Naturalist*, *179*(4), 490–500. https://doi.org/10.1086/664709

Vandekerkhove, J., Declerck, S., Brendonck, L., Conde-Porcuna, J. M., Jeppesen, E., & Meester, L. D. (2005). Hatching of cladoceran resting eggs: Temperature and photoperiod. *Freshwater Biology*, *50*(1), 96–104. https://doi.org/10.1111/j.1365-2427.2004.01312.x

Vanoverbeke, J., & De Meester, L. (2009). Within season short-term hatching delays suggest risk-spreading behaviour in populations of the freshwater cladoceran *Daphnia*. *Ecoscience*, *16*(4), 441–451. https://doi.org/10.2980/16-4-3254

Varpe, Ø. (2017). Life history adaptations to seasonality. *Integrative and Comparative Biology*, *57*(5), 943–960. https://doi.org/10.1093/icb/icx123

Wadsworth, C. B., & Dopman, E. B. (2015). Transcriptome profiling reveals mechanisms for the evolution of insect seasonality. *Journal of Experimental Biology*, *218*(22), 3611–3622. https://doi.org/10.1242/jeb.126136

Wadsworth, C. B., Li, X., & Dopman, E. B. (2015). A recombination suppressor contributes to ecological speciation in *Ostrinia* moths. *Heredity*, *114*(6), 593–600. https://doi.org/10.1038/hdy.2014.128

Wagh, D. A., Rasse, T. M., Asan, E., Hofbauer, A., Schwenkert, I., Dürrbeck, H., … Buchner, E. (2006). Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in *Drosophila*. *Neuron*, *49*(6), 833–844. https://doi.org/10.1016/j.neuron.2006.02.008

Walsh, M. R. (2013). The link between environmental variation and evolutionary shifts in dormancy in zooplankton. *Integrative and Comparative Biology*, *53*(4), 713–722. https://doi.org/10.1093/icb/ict035.

Waterkeyn, A., Pottelbergh, N. V., Vanoverbeke, J., Vanschoenwinkel, B., Meester, L. D., & Brendonck, L. (2013). Constitutive but no *Triops*-induced differences in bet-hedging strategies for hatching in *Daphnia*. *Hydrobiologia*, *715*(1), 29–35. https://doi.org/10.1007/s10750-012-1299-2

Wellenreuther, M., & Bernatchez, L. (2018). Eco-evolutionary genomics of chromosomal inversions. *Trends in Ecology & Evolution*, *33*(6), 427–440. https://doi.org/10.1016/j.tree.2018.04.002

Williams, C. M., Ragland, G. J., Betini, G., Buckley, L. B., Cheviron, Z. A., Donohue, K., … Visser, M. E. (2017). Understanding evolutionary impacts of seasonality: An introduction to the symposium. *Integrative and Comparative Biology*, *57*(5), 921–933. https://doi.org/10.1093/icb/icx122

Winder, M., & Schindler, D. E. (2004a). Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology*, *85*(8), 2100–2106. https://doi.org/10.1890/04-0151

Winder, M., & Schindler, D. E. (2004b). Climatic effects on the phenology of lake processes. *Global Change Biology*, *10*(11), 1844–1856. https://doi.org/10.1111/j.1365-2486.2004.00849.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Czypionka T, Fields PD, Routtu J, van den Berg E, Ebert D, De Meester L. The genetic architecture underlying diapause termination in a planktonic crustacean. *Mol Ecol*. 2019;28:998–1008. https://doi.org/10.1111/mec.15001