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Localization of quantitative trait loci for diapause and other photoperiodically regulated life history traits important in adaptation to seasonally varying environments

VENERA I. TYUKMAEVA,*† PARIS VELTSOS,† JON SLATE,‡ EMMA GREGSON,‡§ HANNELE KAURANEN,* MAARIA KANKARE,* MICHAEL G. RITCHIE,† ROGER K. BUTLIN‡¶ and ANNELI HOIKKALA*

*Department of Biological and Environmental Science, University of Jyväskylä, Survontie 9, PO Box 35, Jyväskylä 40014, Finland, †School of Biology, Dyers Brae, University of St Andrews, Greenside Place, St Andrews Fife KY16 9TH, UK, ‡Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK, §School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK, ¶Sven Lovén Centre for Marine Sciences—Tjärnö, University of Gothenburg, Strömstad SE 452 96, Sweden

Abstract

Seasonally changing environments at high latitudes present great challenges for the reproduction and survival of insects, and photoperiodic cues play an important role in helping them to synchronize their life cycle with prevalent and forthcoming conditions. We have mapped quantitative trait loci (QTL) responsible for the photoperiodic regulation of four life history traits, female reproductive diapause, cold tolerance, egg-to-eclosion development time and juvenile body weight in *Drosophila montana* strains from different latitudes in Canada and Finland. The F2 progeny of the cross was reared under a single photoperiod (LD cycle 16:8), which the flies from the Canadian population interpret as early summer and the flies from the Finnish population as late summer. The analysis revealed a unique QTL for diapause induction on the X chromosome and several QTL for this and the other measured traits on the 4th chromosome. Flies' cold tolerance, egg-to-eclosion development time and juvenile body weight had several QTL also on the 2nd, 3rd and 5th chromosome, some of the peaks overlapping with each other. These results suggest that while the downstream output of females' photoperiodic diapause response is partly under a different genetic control from that of the other traits in the given day length, all traits also share some QTL, possibly involving genes with pleiotropic effects and/or multiple tightly linked genes. Nonoverlapping QTL detected for some of the traits also suggest that the traits are potentially capable of independent evolution, even though this may be restricted by epistatic interactions and/or correlations and trade-offs between the traits.

Keywords: cold tolerance, development time, diapause, *Drosophila montana*, juvenile body weight, photoperiodism

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Introduction

Insects have successfully inhabited various unfavourable environments in temperate and polar zones and evolved several strategies, including migration and different types of dormancy, to survive over the harsh winter period (Bradshaw & Holzapfel 2010). A crucial aspect in any of these strategies is an ability of insects to predict the forthcoming seasonal changes based on environmental cues and to synchronize their life cycles accordingly (Tauber *et al.* 1986). This ability is of special

Correspondence: Venera I. Tyukmaeva, Fax: +44 1334 463366; E-mail: vtyukmaeva@gmail.com

importance for insect species with only one or two generations per year, as well-timed reproduction will lead to a higher survival of both the adults and their offspring (Visser *et al.* 2010). In northern insect species, several life history traits are photoperiodically regulated, but also other cues, such as temperature, light, food, humidity or different combinations of these factors, may play a role in trait regulation (e.g. Tauber *et al.* 1986).

One of the most important adaptations to the seasonally varying environment in northern insect species is photoperiodic diapause, which can take place at either the egg, larval, pupal or adult stage. Diapause syndrome involves drastic changes at physiological, hormonal and behavioural levels, including the storage of energetic reserves, acquisition of a relatively high resistance to environmental stressors, cessation of development and/or reproduction and suppression of metabolism (e.g. Denlinger *et al.* 1988; MacRae 2010), and it often results in lifespan extension and delayed senescence. Photoperiodic adult reproductive diapause has been studied in insect populations by measuring the incidence of diapause (the proportion of females of a given population that enter diapause under short-day conditions), the intensity of diapause (the length of diapause and its dependence on environmental cues) and the critical day length (CDL; the day length where half of the females of a population enter diapause). These studies have shown that the diapause incidence reaches 100% in short day length only in the most northern species and that it is often sensitive to environmental temperature (e.g. Denlinger 2002). Several insect species have been found to show latitudinal clines in their diapause incidence and/or CDL (e.g. Danilevsky 1965; Schmidt & Paaby 2008; Tyukmaeva *et al.* 2011).

Also, several other life history traits, such as cold tolerance (Espinoza *et al.* 2008; Vesala *et al.* 2012a), egg-to-adult development time (Salminen *et al.* 2012; Yadav *et al.* 2014) and juvenile body weight (Hahn & Denlinger 2007; Salminen *et al.* 2012), have been found to be at least partly regulated by photoperiod and temperature, and the effects of diapause may not always be easy to distinguish from the direct effects of these cues. The fact that above-mentioned traits also show latitudinal variation in several species indicates their importance in local adaptation. For example, Gibert *et al.* (2001) found cold tolerance to show latitudinal variation in several *Drosophila* species, and Blanckenhorn & Demont (2004) detected reciprocal clines in development speed and body weight in the dung fly, *Scathophaga stercoraria*. While different life history traits often show correlated changes in latitudinal clines, they can also show trade-offs within populations. For example, a trade-off between development time and juvenile body weight is a typical component of life history models (reviewed in Nylin & Gotthard 1998).

Identifying the loci affecting the photoperiodic regulation of life history traits helps to understand processes involved in local adaptation, as the downstream outputs of population-specific responses to day length are essential to the survival and reproduction of the organisms in seasonally varying environments. Quantitative trait loci (QTL) analyses on diapause and other life history traits have revealed significant QTL for the developmental stage and photoperiod evoking diapause, for example in *Wyeomyia smithii* (Mathias *et al.* 2007; Bradshaw *et al.* 2012), and for the diapause state, development time and wing length in the *Culex pipiens* species complex (Mori *et al.* 2007). In these studies, some QTL for different traits mapped to the same chromosomal regions, suggesting that the traits may be controlled in part by genes with pleiotropic effects and/or by multiple tightly linked loci. Genetic studies on insect cold tolerance, measured as a recovery time from chill coma (CCRT), have revealed major QTL for this trait, for example in *Drosophila melanogaster*. Morgan & Mackay (2006) found CCRT to be associated in this species with a QTL region on chromosome 2, Norry *et al.* (2008) on chromosomes 2 and 3 and Svetec *et al.* (2011) on the X chromosome.

Drosophila montana, a species of the *Drosophila virilis* group, is distributed around the northern hemisphere, and adult females of this species spend the winter in photoperiodically controlled reproductive diapause (Lumme 1978; Tyukmaeva *et al.* 2011). In contrast to *D. melanogaster,* which has only recently been introduced to temperate regions, *D. montana* has adapted to live in seasonally changing environment prevailing at high latitudes and altitudes. Our previous studies on the life history traits of this species have shown that the egg-to-eclosion development time and juvenile body weight of the flies are largely affected by photoperiodic cues during the egg and larval stages, while the developmental pathway of the females (direct development vs. diapause) is regulated by these cues only after eclosion (Salminen *et al.* 2012). Also, the cold tolerance of the flies of this species seems to be affected directly by the photoperiod rather than by the reproductive stage of the females per se (Vesala *et al.* 2012a). In this study, we have performed a QTL analysis on four photoperiodically regulated life history traits in a single day length, which the flies of the parent populations interpret as different times of summer, and where population differences in these traits are notable. In this study, we used *D. montana* flies from Vancouver (Canada; 49°N) and Oulanka (Finland; 66°N) and constructed a linkage map with 53 SNP markers. F2 progenies of the crosses were reared in a 16:8 light:dark (LD) cycle,

which the females with Vancouver alleles are expected to interpret as early summer (June–July) and the females with Oulanka alleles as late summer (late August). The main aim of the study was to compare the genomic positions of QTL for the flies' photoperiodic responses in diapause, cold tolerance, egg-to-eclosion development time and juvenile body weight in a single day length and find out whether they are unique for specific traits and/or shared by several traits. This information helps to understand how the traits are regulated and the extent to which they share a common genetic architecture and therefore indicate the potential for independent evolutionary trajectories.

Material and methods

Parental strains for the QTL crosses and the flies' rearing conditions

The isofemale strains used as parents in QTL crosses were established in 2003, and each of them consisted of the progeny of a single fertilized female collected in Vancouver (strain code Can3F20, Canada, 49′N) or Oulanka (strain code 03F77, Finland, 66′N). The strains have been maintained in half-pint bottles with malt media (Lakovaara 1969) in constant light at 19°C and 60% humidity since their establishment (approximately 65 generations before the start of the experiment). All the experiments were performed on females only, as we can easily determine the diapause state of the females by the developmental state of their ovaries (Fig. 1). It should be noted that maintaining the flies in diapause preventing conditions in the laboratory for years has not been observed to have any effect on the CDL where they enter diapause (Lankinen *et al.* 2013).

Previous studies on *Drosophila montana* have revealed numerous inversion polymorphisms in this species (e.g. Morales-Hojas *et al.* 2007). For the QTL crosses, we chose parental strains with no detectable inversion differences, after tracing possible inversion loops in the salivary glands of hybrid larvae from crosses among several strains from Vancouver and Oulanka (for more information on methods, see, e.g., Schäfer *et al.* 2010).

One generation before performing the first QTL crosses, the flies of the parental strains were allowed to lay eggs for 3 days in half-pint bottles with malt media (several successive bottles), which were then transferred into a constant temperature room in 16°C, 60% humidity and constant light. The flies that developed from these eggs were used as parents for the F1 crosses. Temperature and humidity were kept the same throughout the study, but the LD cycle was changed to 16:8 for phenotypic studies. In *D. montana*, a females' developmental pathway is determined after eclosion with no detectable maternal effects (Salminen *et al.* 2012), and thus, parental flies could be maintained in continuous light (in 16:8 LD cycle, Oulanka females would not have produced progeny).

QTL crosses

Crosses were performed according to an F2 design (Fig. S1, Supporting information). After emergence, parental flies (P) were sexed and kept in constant light (LL) at 16°C until they were sexually mature (about 21 days). To obtain sufficient F2 progeny, we individually mated 3 and 5 pairs of parental flies in the reciprocal crosses between Oulanka females and Vancouver males and Vancouver females and Oulanka males, respectively. The females were allowed to lay eggs for 2 weeks, but every 24 h, they were transferred to fresh vials in order to maintain low egg/larva density. F1 generation flies were maintained in a constant light regime, so that the females would develop ovaries. Sexually mature F1 generation flies were again crossed individually (one female and one male of known parents) to trace the genotypes precisely. F1 females were provided fresh vials for laying eggs every 24 h, and the freshly laid eggs were transferred into 16:8 LD cycle, which the emerging F2 generation females were expected to interpret as early or late summer depending on the sets of alleles that they had inherited from the parent strains. After scoring the females' phenotypes for different life

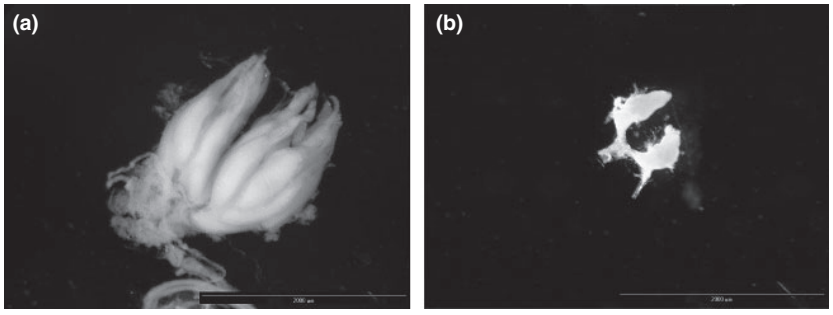

Fig. 1 Developmental stages of females' ovaries: (a) the ovaries of nondiapausing (vitellogenic) and (b) the ovaries of diapausing females.

history traits, they were saved individually in 70% EtOH until DNA was extracted with the Qiagen DNeasy tissue kit (Qiagen, Germany) according to the manufacturer's protocol.

Life history traits

The following methods were used for scoring phenotypes (reproductive diapause, cold tolerance, development time from egg to eclosion and juvenile body weight) in parental strains and their F2 progeny. Differences between the parental strains (at least 100 females measured for each trait) and between the diapausing and nondiapausing F2 females in cold tolerance, development time and juvenile body weight were tested with Mann–Whitney *U*-tests, and correlations between these traits were tested with Spearman's test (R Core Team 2012).

Development time and juvenile body weight. To measure the development time of the flies from egg to eclosion, freshly mated females were allowed to lay eggs in vials with malt media (Lakovaara 1969) for 4 h per day (8:00–12:00) for 14 days (this enabled us to keep larval density low). The vials with parental females were maintained in constant light, while the vials with eggs were transferred into 16:8 LD cycle within 24 h of the egg laying period. When the developing progeny reached the pupal stage, the vials were checked once a day and the newly eclosed flies were sexed under light CO_2 anaesthesia. At the same time, the development time and the juvenile body weight of the females were recorded (scale Mettler Toledo, XS105 DualRange, 0.01 mg).

Cold tolerance. The cold tolerance of the flies was determined with a chill coma recovery time (CCRT) test. Within 1 day of emergence, females were transferred into vials containing 7 mL of yeast–sucrose–agar medium (Rosato & Kyriacou 2006) with some dry yeast on top. When the females reached 21 days of age, the vials were incubated at −7 °C for 16 h, after which the flies were transferred to dishes with lids and separate compartments for individual flies. CCRT was recorded as the time (in seconds) that a fly needed to stand on its legs after returning to room temperature (see Vesala & Hoikkala 2011).

Diapause phenotype. Diapause phenotype of the females was determined on the basis of the developmental stage of their ovaries (Fig. 1), after the females had gone through the CCRT test. On day 22 after emergence, they were transferred to −80°C and stored there until their reproductive status was determined by dissecting the ovaries under a light microscope (details in Tyukmaeva *et al.* 2011).

SNP genotyping

SNP markers occur at a high frequency in the genome and are relatively cheap and less prone to genotyping errors compared to microsatellites (Ball *et al.* 2010). We identified these markers from two 454 transcriptome assemblies based on populations from Vancouver and Oulanka, collected in 2008 and 2009 (P. Veltsos, E. Gregson, B. Morrissey, J. Slate, A. Hoikkala, R. K. Butlin & M. G. Ritchie, in review). SNPs were selected for genotyping based on a minimum coverage of 8 reads in total and a minimum of 3 reads and 20% frequency for the rare variants. A subset of markers was selected after genotyping individuals from the populations where the markers had been identified to ensure good amplification in both populations and even coverage among the chromosomes. There was limited success in finding SNPs in candidate genes even with a direct sequencing approach (P. Veltsos, personal communication), and only eight candidate genes, previously used in a microarray study (Vesala *et al.* 2012b), were included in the SNP assay (see Table S1, Supporting information). Three hundred and eighty-four SNPs were scored with an Illumina VeraCode GoldenGate assay and BeadXpress platform on 96 samples to identify the 96 most informative SNPs for the parental strains used in the experiment. Seven hundred and sixty-eight individuals from the QTL cross were genotyped for these 96 SNP markers and analysed using GENOMESTUDIO 2010.1 (Genotyping module 1.7.4; Illumina, San Diego, CA, USA). Ten markers were discarded due to high levels of genotyping error and 10 more markers due to complete homozygosity. Samples with call rate lower than 0.85 were discarded. In total, 16 flies of the parental and 728 females of the F2 generations were used in further analysis.

Linkage map and QTL analysis

The SNPs were assigned to the five chromosomes of *D. montana* according to their predicted location (Schäfer *et al.* 2010) by cross-referencing with the *Drosophila virilis* scaffolds they aligned with in FLYBASE. The positions of the SNPs within these groups were estimated using CRI-MAP v2.503a (Green *et al.* 1990) with the use of CRIGEN, which split the data into subfamilies to make the linkage mapping faster. Markers showing conflicting linkage were removed from the analysis. Inheritance consistency of the pedigrees was checked with GENOTYPECHECKER, version 2.4 (Paterson & Law 2011). Segregation distortion was detected using a

P-value of 0.05 with Bonferroni correction applied for the number of markers. Seven markers showed significant segregation distortion (12794_164_c2, 12610_303_c2, 12638_371_c1, 14661_51_c1, 12509_821_i, 13040_163_c2 and 12724_270_c2), but were left in the analysis, and the results associated with them were noted. Excluding six of these markers would not have changed the results of QTL analysis significantly, while removing the seventh marker, 12610_303_c2, moved the QTL peak closer to marker 12610_303_c1 (this did not affect the overall results as both of these markers had been developed for the same gene).

We performed separate QTL mapping analysis on each of the four phenotypic traits using the R/QTL package version 1.24–9 (Broman *et al.* 2003) in R software version 2.15.1 (R Core Team 2012). The QTL analyses for three traits (cold tolerance, development time and juvenile body weight) were carried out using multiple imputation mapping as this method deals the best both with missing and with non-normally distributed phenotypic data (Broman & Sen 2009). Diapause was considered as a binary trait (yes/no responses) and was treated as such during interval mapping (the binary method option was selected). For each of the traits, we performed single-QTL and two-QTL scans using 3000 and 1000 permutations, respectively, to establish genomewide $P < 0.05$ logarithm of odds (LOD) significance thresholds for QTL detection. The LOD significance threshold for the X chromosome was estimated separately in each case. Then we proceeded to multiple-QTL mapping where the best-fitting model was selected. 95% approximate Bayesian credible intervals were calculated for the detected QTL.

If two traits have a shared genetic architecture (i.e. if the same genomic locations contribute to genetic variation in both traits), QTL analyses of each trait are expected to show some concordance in test statistic profiles across the genome. To test for an overlap in genomic regions contributing to trait variation, we implemented the permutation method of Keightley & Knott (1999), which compares the correlation in test statistics between two genome scans, while accounting for the autocorrelation between adjacent test locations within each scan. Test locations at 1, 5 and 10 cM intervals were used to measure the correlations between genome scans of two traits.

Markers under significant QTL peaks for each of the studied traits were aligned using the FLYBASE BLAST tool (http://flybase.org/blast/). Gene annotations with molecular function and involvement in biological processes were collected from *Drosophila melanogaster* using BLAST2GO, version .2.5.1 (Conesa *et al.* 2005) to determine whether SNPs present under a QTL fall on genes that could be candidates for photoperiodic regulation of the traits.

Results

Phenotypic differences

Differences in the life history traits between the parental strains were determined in 16:8 LD cycle, where 98% of the females of the Oulanka strain, but only 7% of those of the Vancouver strain entered diapause (Fig. S2, Supporting information). Oulanka females showed high cold tolerance, recovering from chill coma faster (mean ± SE = 490 ± 23 s) than Vancouver females (mean ± SE = 767 ± 42 s). The development time of Oulanka females from egg to eclosion was about 4 days shorter (mean ± SE = 30 ± 0.13 days) than that of the Vancouver females (mean ± SE = 34 ± 0.27 days), while juveniles of the Vancouver strain were heavier (mean ± SE = 2.49 ± 0.02 mg) than those of the Oulanka strain (mean ± SE = 2.40 ± 0.03 mg). Differences between the parental strains in these three traits were significant (diapause incidence: Pearson's chi-squared test = 166.0351, df = 1, *P*-value < 2.2e-16; cold tolerance: $t = -5.4813$, df = 74.598, *P*-value < 0.001; development time: Mann–Whitney *U*-test $W = 3268.5$, *P*-value < 0.001; juvenile body weight: Mann–Whitney *U*-test $W = 2367.5$, *P*-value = 0.008; see also Fig. S2, Supporting information).

Diapausing and nondiapausing F2 progeny females showed significant differences in all other traits (Mann–Whitney *U*-tests: CCRT: $W = 48623.5$, *P*-value < 0.001; development time: $W = 45446.5$, *P*-value < 0.001; juvenile body weight: $W = 38025$, *P*-value < 0.001). Pairwise Spearman's correlations between the traits showed little difference for diapausing and nondiapausing F2 progeny (development time and juvenile body weight: for diapausing rho = 0.10, *P*-value = 0.04, for nondiapausing rho = 0.24, *P*-value < 0.001; development time and CCRT: for diapausing rho = −0.13, *P*-value = 0.01, for nondiapausing rho = −0.17, *P*-value = 0.004; juvenile body weight and CCRT: for diapausing rho = 0.04, *P*-value = 0.42; for nondiapausing rho = 0.04, *P*-value = 0.56).

Linkage map and QTL mapping of the life history traits

Linkage mapping resulted in a total set of 53 markers, on five linkage groups, involving 10, 13, 13 and 13 markers on autosomes 2, 3, 4 and 5, respectively, and four markers on the X chromosome. Even though the number of markers was relatively small, they covered most the chromosomes apart from the X chromosome, where we had difficulties with marker development (P. Veltsos, personal communication). The total length of the map, estimated using the Kosambi function, was

357.2 cM (Fig. 2; the names of the markers, their estimated positions and possible annotation are given in the same order in Table S1, Supporting information). The QTL areas detected in this study are rather large, partly due to the relatively low number of markers, which makes it difficult/impossible to identify genes underlying the studied traits.

The results of the scans using the single-QTL or two-QTL models are shown in Table S2 (Supporting information), while significant QTL peaks in the best-fit models, as well as the model parameters, are presented in Table 1. It should be noted that the QTL for development time at position 1 is associated with the significantly distorted marker 12610_303_c2, and so the power of this QTL might be altered (Zhang *et al.* 2010). For each of the traits, QTL analysis revealed several significant QTL with additive effects. Diapause had a single QTL on the X chromosome at position 8.0, while diapause, development time and body weight had partly overlapping peaks on the 4th chromosome at position 80–93 (Fig. 3, Table 1), where also the fourth trait, cold tolerance, had a suggestive QTL peak. Another apparent 'hot spot' for QTL for the traits was on the 5th chromosome, where development time and juvenile body weight had highly overlapping QTL with very narrow (0.2–4 cM) confidence intervals (CI). Also, cold tolerance had a QTL peak in this region, but it had a very wide CI. Overall, the mapping results for this trait should be treated with caution, as the CIs of both of its QTL covered almost the whole chromosomes. While the QTL for the first-mentioned traits explained 19.5–23.2% of the total variation in traits, they explained only 4.6% of variation in cold tolerance (Table 1).

Genomewide QTL test statistics showed no significant correlation between most pairs of traits (Table S3, Supporting information). However, development time and juvenile body weight did have positively correlated QTL test statistics ($r = 0.81$; P-value = 0.004), indicating that the same genomic locations explained variation in both traits more often than expected by chance.

Discussion

Insect species with a wide geographic distribution are good systems for tracing the genetic basis of photoperiodic regulation of life history traits important in local adaptation. In the present study, *Drosophila montana* females from Vancouver and Oulanka, as well as their F2 progenies carrying different combinations of alleles from these populations, showed high divergence in all studied life history traits when exposed to a single photoperiod, which the females with Vancouver alleles interpret as early summer (time to reproduce) and the ones with Oulanka alleles as late summer (time to prepare for winter).

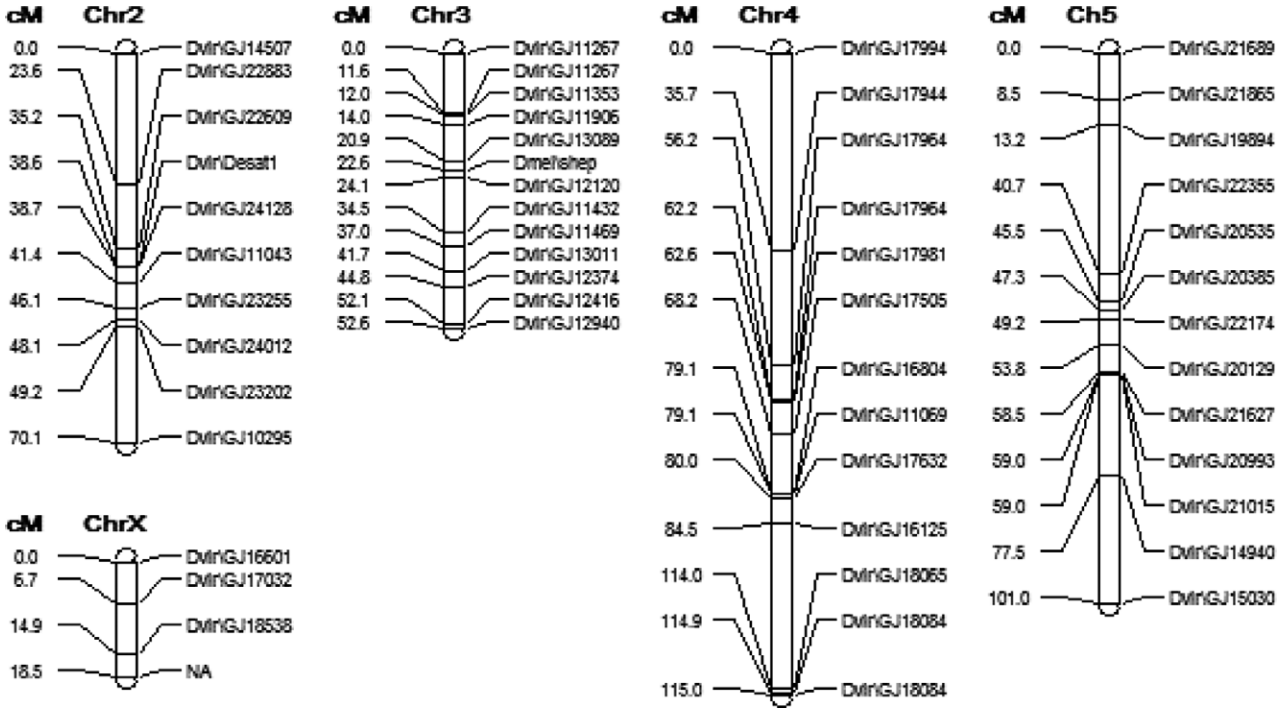

Fig. 2 Linkage map of *D. montana* for the QTL cross. The codes of the genes and markers in different linkage groups are given in Table S1 (Supporting information) in the same order.

Table 1 Summary table for the best-fit model for all traits QTL peaks, their positions, logarithm of odds (LOD) scores and percentage of explained variation

Trait	Chromosome (length)																							
	2 (70.1)				3 (52.6)				4 (115.0)				5 (101.0)				X (18.5)				Model parameters			
	Position	95% CI	LOD	%var	Position	95% CI	LOD	%var	Position	95% CI	LOD	%var	Position	95% CI	LOD	%var	Position	95% CI	LOD	%var	LOD	%var	*P*-value
Diapause									89.0	81;91	17.5	10.2					8.0	6;11	23.4	14.0	36.3	22.7	<0.0001
Cold tolerance	68.0	10;70	3.7	2.5									53.8	25;101	2.9	2.0					6.7	4.6	<0.0001
Development time									1.0	0;3	9.9	5.7	47.3	46;49	7.7	4.4					31.2	19.5	<0.0001
									30.0	26;39	5.3	3.0	54.0	53.8;54	9.2	5.3							
									93.0	76;100	6.5	3.7											
Juvenile body weight	23.6	21;25	7.9	4.3	1.0	0;22	4.4	2.4	80.0	79;82	24.2	14.0	48.0	46;49	5.6	3.0					38.3	23.2	<0.0001
	45.0	34;59	1.5	6.6									53.0	52;54	7.4	4.0							

All life history traits measured in the present study are known to be partly under photoperiodic regulation (e.g. Hahn & Denlinger 2007; Vesala *et al.* 2012a; Yadav *et al.* 2014) and to show plasticity also in response to different temperatures (e.g. Vesala & Hoikkala 2011). Also, the large differences between Vancouver and Oulanka females detected in these traits in the present study are clearly a consequence of the flies' photoperiodic responses, and they would have been much smaller if measured in photoperiods that correspond to early summer in both populations (16:8 LD cycle in Vancouver and 22:2–24:0 LD cycle in Oulanka). Lankinen *et al.* (2013) have found the percentage of diapausing females to vary between 0 and 10% among isofemale strains from Oulanka in 22:2 LD cycle, which corresponds well with the percentage (7%) detected for Vancouver females in the present study. Also, the mean CCRT of 750 s of Oulanka flies in 22:2 LD cycle (Vesala & Hoikkala 2011) fits well with CCRT measured for Vancouver females (767 s) in the present study. Furthermore, Salminen *et al.* (2012) have shown that in Finnish *D. montana* populations, the flies develop more slowly and are heavier as juveniles, that is more similar to Vancouver flies, in photoperiods corresponding to early than to late summer conditions. Thus, the population differences in the studied life history traits are for the most part due to differential responses of the females to the used photoperiod.

Many studies have suggested that the diapause itself, rather than the cues inducing it (e.g. photoperiod), plays a key role in evoking changes in the 'diapause syndrome' traits, which arise along with diapause and enhance survival of the organism in harsh environmental conditions. Cold tolerance is sometimes argued to be one of these traits (Denlinger 1991). However, our earlier studies have shown that seasonal changes in the cold tolerance of *D. montana* females (measured as CCRT) show high plasticity and that these changes are regulated mainly by the day length and temperature and to a lesser degree directly by the females' diapause state (Vesala *et al.* 2012a). Also, females' diapause state cannot affect egg-to-eclosion development time and juvenile body weight, as the diapause induction occurs after eclosion (Salminen *et al.* 2012). Hence, the significant differences that we found between diapausing and nondiapausing F2 females in other life history traits cannot be regarded as being due to a single underlying diapause syndrome. This is also supported by the small differences in the phenotypic correlations of diapausing and nondiapausing F2 females.

The present study revealed significant QTL for all measured life history traits. In total, the QTL effects detected explained about 22.4% of variation in females' propensity to enter diapause, 4.6% of variation in cold

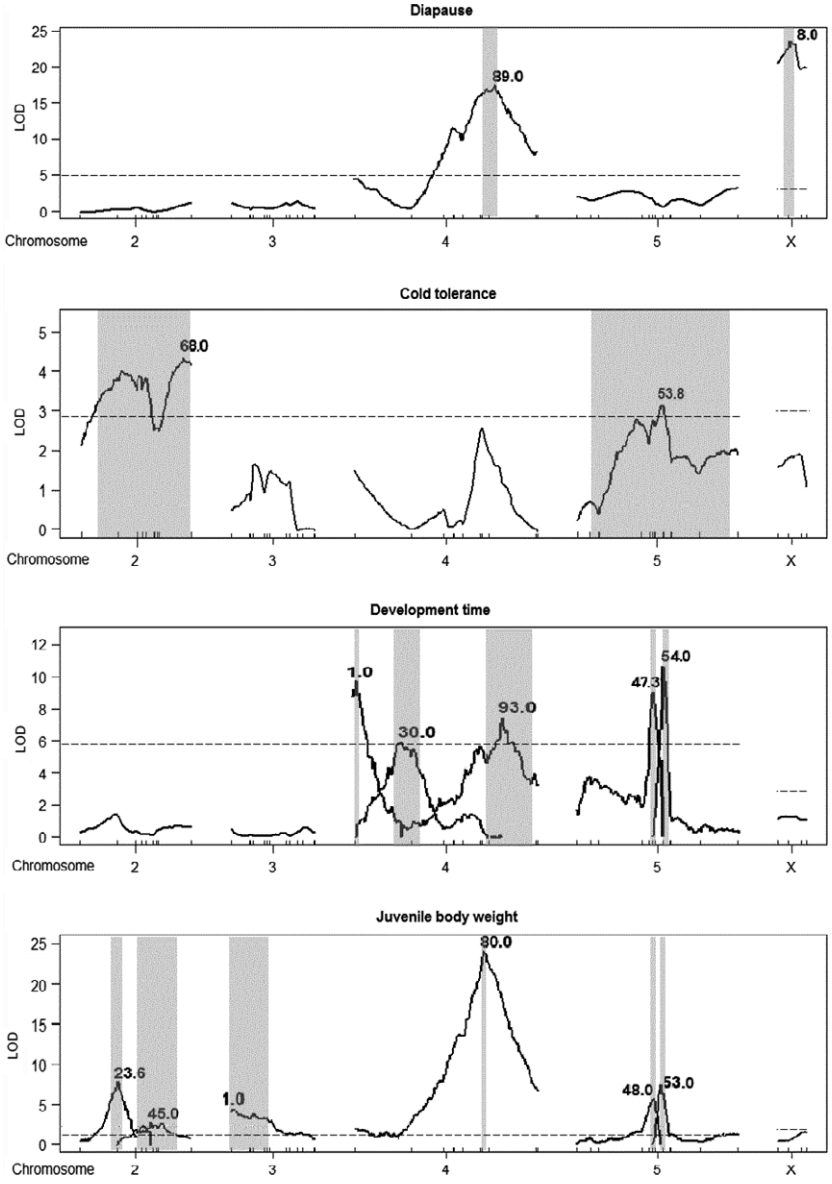

Fig. 3 Significant QTL peaks for diapause, cold tolerance, egg-to-eclosion development time and juvenile body weight. Significance threshold is marked with a dashed line and confidence intervals of QTL with shaded area. Numbers refer to the map position showing highest LOD for the QTL peaks.

tolerance, 19.5% in development time and 23.2% in juvenile body weight. The most interesting finding was a unique QTL peak on the X chromosome, which explained 14% of strain difference in females' diapause induction and which did not overlap with QTL for any other trait. The rest of the traits had several significant QTL on the 2nd, 3rd and 5th chromosome, some of the peaks overlapping with each other, but none of them with diapause induction. The 4th chromosome, however, contained closely localized significant QTL for diapause, development time, juvenile body weight and a suggestive QTL for cold tolerance, which suggests that the traits may not be totally independent. The most tightly overlapping QTL were found on the 5th chromosome for development time and juvenile body weight, which are likely to be interrelated traits (the flies that develop slowly are usually heavier). These were also the only traits where the correlation test of Keightley & Knott (1999) revealed significant QTL associations on a genomewide level. This test is quite conservative as it is based on overall correlations instead of individual cases of coincidence (one major independent effect may make the overall correlation nonsignificant even if other loci have common effects on both traits), and thus, coincident QTL correlations between other traits cannot be totally ruled out.

Diapause QTL on the X and 4th chromosomes support earlier gene localization studies on other *D. virilis* group species. By performing interspecific crosses between *D. virilis* and *Drosophila lummei*, Lumme & Keränen (1978) found the gene(s) affecting diapause induction to be located on the X chromosome. Furthermore,

Lumme (1981) found that the CDL for diapause induction in *Drosophila littoralis* was largely regulated by factor(s) located on the right arm of the fused 3rd and 4th chromosome (corresponding to the 4th chromosome of *D. montana*). QTL detected for specific traits in more distantly related species are difficult to compare with each other due to different karyotypes of the species, but these comparisons may reveal candidate genes that play a role in these traits in a wide range of organisms. For example, studies on *Drosophila melanogaster* have shown the QTL affecting diapause to be located on the 3rd chromosome (Williams *et al.* 2006) and the ones affecting cold tolerance on the 2nd and 3rd chromosome (Morgan & Mackay 2006; Norry *et al.* 2007, 2008). As the chromosomes X, 2, 3, 4 and 5 of *D. virilis* (and also *D. montana*) correspond to the chromosome arms X, 3R, 3L, 2L and 2R of *D. melanogaster* (Lozovskaya *et al.* 1993), the QTL detected for diapause in *D. melanogaster* and *D. montana* are clearly in different genomic regions. Interestingly, Mathias *et al.* (2007) have found sex-linked QTL involved in photoperiodic control of diapause in *Wyeomyia smithii*, which corresponds to our finding in *D. montana*.

The major challenge in linkage map construction and QTL detection is a high incidence of inversion polymorphisms. The regions near inversion break points have been suggested to harbour genes that are targets of spatially varying selection maintaining the inversion polymorphism (Kirkpatrick & Kern 2012). *D. montana* populations, where the parental strains of the QTL study came from, show high inversion polymorphism and also several unique inversions in their chromosomes (Moorhead 1954; Morales-Hojas *et al.* 2007). Contrary to *D. melanogaster* where the majority of inversions have originated in the ancestral African population, *D. montana* populations seem to have gained unique inversions while invading new habitats (Moorhead 1954), which suggests that these inversions might harbour genes beneficial in the new environment (Kirkpatrick & Barton 2006). While the parental strains of our QTL cross did not show detectable differences in their gene arrangement, the region of overlapping QTL for all studied traits on the 4th chromosome coincides with inversion *4 l*, which has been found to be polymorphic in both populations. Furthermore, the QTL for cold tolerance, development time and juvenile body weight on the 5th chromosome fall in the regions of two inversions, *5 l* and *5t*, that are unique to Finnish populations (Morales-Hojas *et al.* 2007).

Our study on diapause and other photoperiodically regulated life history traits is based on the facts that the photoperiodic responses of insects vary at different latitudes and that the same photoperiod may represent different times of summer for the individuals from different populations. The most interesting finding in this study was that the diapause response, which is an on/off trait, has one unique large-effect QTL peak on the X chromosome and another peak that overlaps with the ones for egg-to-eclosion development time and juvenile body weight (and with a nearly significant QTL peak for cold tolerance) on the 4th chromosome. This suggests that the switch to diapause, controlled by a photoperiodic timer (Bradshaw & Holzapfel 2007), has a unique regulatory component and also some common regulatory genes and/or pathways shared with the traits that have been suggested to be controlled by another clock mechanism, a circadian clock (e.g. Espinoza *et al.* 2008; Yadav *et al.* 2014). Overall, information on the photoperiodic control of insects' life cycles, as well as on trade-offs and correlations between the traits, is of utmost importance for understanding adaptation processes occurring in insect populations living in environments with high seasonal variation.

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V.T., A.H., M.R. and R.B. designed the experiment. V.T., E.G. and H.K. collected the data. V.T., J.S., P.V., H.K. and M.K. performed the analyses. The following authors contributed to manuscript writing: V.T., A.H., M.R., R.B., J.S. and M.K.

Data accessibility

Cross design, trait differences, linkage group with the order of markers and results of single-QTL and two-QTL scans for the studied traits uploaded as online Supporting Information.

Input file with genotype and phenotype data for R/QTL is available on Dryad doi:10.5061/dryad.9b65b.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 QTL cross in F2 design.

Fig. S2 Percentages of diapausing females and the means and standard errors of cold tolerance (measured as CCRT), egg to eclosion development time and juvenile body weight of the females of Oulanka and Vancouver strains at 16:8 LD.

Table S1 Linkage group and the order of markers.

Table S2 Results of single-QTL and two-QTL scans for the studied traits.

Table S3 Results of correlation test by the permutation method (Keightley and Knott, 1999).