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QTL analysis of the photoperiodic response and clinal distribution of *period* alleles in *Nasonia vitripennis*

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Abstract

In seasonal environments, organisms synchronize their life cycle with the annual cycle of environmental factors. In many insect species, this includes a diapause response: a timed dormant stage that allows to survive harsh winter conditions. Previously, we have shown that larval diapause in the parasitic wasp *Nasonia vitripennis* is induced by the mother upon exposure to a threshold number of short photoperiods (named *switch point*) and diapause response follows a latitudinal cline in natural populations. Here, we present a QTL analysis using two lines derived from the extremes of this clinal distribution: a northern line from Oulu, Finland and a southern line from Corsica, France. A genomic region on chromosome 1 and one on chromosome 5 were found to be associated with photoperiodic diapause induction. Interestingly, these regions contain the putative clock genes *period*, *cycle* (chromosome 1) and *cryptochrome* (chromosome 5). An analysis of *period* polymorphisms in seven European populations showed a clinal distribution of two main haplotypes that correlate with the latitudinal cline for diapause induction.

Keywords: clock genes, diapause, latitudinal cline, *Nasonia vitripennis*, photoperiodism

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Introduction

Organisms that live in seasonal environments are subject to annual cycles of light, temperature and food availability. Evolutionary theory predicts that natural selection will favour organisms with the ability to cope with these cyclical conditions (Tauber *et al.* 1986; Danks 1987). As a result of regionally different selection pressures, species with a broad distribution are expected to harbour genetic differences that reflect local adaptation (Kawecki & Ebert 2004). Given the tight association between local climatic condition and seasonal photoperiodic changes, photoperiod is an important environmental cue for seasonal responses (Bale & Hayward 2010). Many species respond to short photoperiods by entering, or inducing in their offspring, a physiological state of dormancy called *diapause*, which involves the arrest of development and growth. The factors inducing diapause are typically not unfavourable for reproduction and growth but they anticipate the onset of winter, allowing insects to be already in diapause at the beginning of the adverse season and thus to be prepared for the harsh upcoming conditions. Insect diapause is an adaptive life history trait that is strongly moulded by seasonality, as shown by several studies reporting latitudinal variation in diapause response [reviewed in Hut *et al.* (2013)]. Generally, the incidence of diapause increases with latitude, as winter conditions become progressively harsher. The day length that elicits diapause response in 50% of a given population (*critical photoperiod*, Tauber *et al.* 1986) and their threshold number (*switch point*, Paolucci *et al.* 2013) is positively correlated with latitude. Populations at high latitudes respond to long days, and consequently, they enter in

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diapause earlier in the year compared to populations at lower latitudes that respond to shorter day lengths characteristics of later times of the year. The variable sensitivity to photoperiod allows the maximal exploitation of resources during the short summer in the high latitudes and the long one in the low latitudes. Very well-known studies showing latitudinal variation in diapause related aspects include the study on North American populations of *Drosophila melanogaster* that demonstrates clinal variation in diapause incidence (Schmidt *et al.* 2005) and the study on latitudinal variation in critical photoperiod in the pitcher plant mosquito *Wyeomyia smithii* (Bradshaw & Holzapfel 2001). Although clinal variation in diapause has been observed in different species, information on the underlying genetic basis of such variation remains scarce.

We have shown earlier that photoperiodic induction of diapause in the haplodiploid parasitoid wasp *Nasonia vitripennis* varies geographically over a latitudinal cline (Paolucci *et al.* 2013). In this species, diapause takes place in the larval stage, but its occurrence is determined by the adult mother as a reaction to environmental photoperiodic cues (Saunders 1965). Adult females produce normally developing offspring early in life and switch to the production of diapausing offspring after a critical number of photoperiodic cycles. The number of cycles varies depending on the photoperiodic conditions and determines the *switch point*, which is one of the key features of diapause in *N. vitripennis* (Paolucci *et al.* 2013). The concept of *switch point* is equivalent to the *required day number* (RDN), proposed by Saunders (1973) and defined as 'the number of calendar days in a particular photoperiod treatment which is needed to raise the proportion of diapausing individuals among a day's batch of puparia to 50%' for *Sarcophaga argyrostoma* and similarly for *Nasonia vitripennis* (Saunders 1973, 2002). This required day number varies according to the length of the light phase (Saunders 1966) and can be temperature compensated (Saunders 1971). The switch point/required day number is an expression of the photoperiodic counter, the component of the photoperiodic clock that is used to count the photoperiodic cycles and to transfer this information to the diapause machinery that elicits the response once a threshold number is reached (Paolucci *et al.* 2013). The clinal variation of diapause induction is expressed as variation in the switch point, with early switch points in northern European populations and late switch points in southern populations.

Here, we present a genetic analysis of photoperiodic diapause variation in *Nasonia vitripennis*. Reciprocal crosses between two isofemale lines, originating from a northern (Oulu, Finland) and a southern (Corsica, France) location, were used to investigate the patterns of diapause inheritance and to identify loci involved in switch point variation by QTL mapping analysis. This analysis also revealed a genetic polymorphism in the clock gene *period* that correlates with the clinal maternal photoperiodic diapause induction in seven European populations.

Materials and methods

Experimental lines

Two *Nasonia vitripennis* isofemale lines, each established from a single female originating from Oulu, Finland and Corsica, France (Paolucci *et al.* 2013), were used for the genetic crosses and QTL mapping analysis. Hereafter these lines are named 'northern line' and 'southern line', respectively. *Nasonia* wasps are infected by *Wolbachia* endosymbionts that can cause cytoplasmic incompatibilities between different *Nasonia* species (Breeuwer & Werren 1990). Previous studies have not found differences in infection status in field strains of *Nasonia vitripennis* (Perrot-Minnot *et al.* 1996); therefore, the *Wolbachia* status of the two isofemale lines was not checked. The experimental lines used in this study belong to the species *Nasonia vitripennis* and the crosses did not show any sign of compatibility problems. The lines were maintained on *Calliphora* spp. pupae as hosts in mass culture vials under nondiapause conditions (light:dark cycle 18:6, temperature 20 or 25 °C).

Genetic cross-design

Crosses were set up to investigate the inheritance of diapause induction. Virgin females from the northern line (diploid genotype: NN) and the southern line (diploid genotype: SS) were collected as pupae from the hosts and placed in cotton-plugged 60 mm × 10 mm polystyrene vials together with male pupae from the same line or the other line (haploid male genotype: N or S), resulting in four types of crosses: two intraline crosses N × NN and S × SS, and two reciprocal interline crosses, N × SS and S × NN. Ten vials were set up, each with four females and one male for the intraline crosses, and thirty vials each with four females and one male for the interline crosses. After eclosion and mating, three hosts were provided to each individual female for oviposition. Hosts were kept at 20 °C and continuous light until adult wasp progeny emerged after 3 weeks. Due to the haplodiploid sex determination system, both types of interline crosses produced F_1 diploid female individuals with a hybrid genotype and haploid males with either pure N genotype (from the S × NN cross) or pure S genotype (from N × SS cross).

F_1 individuals were allowed to mate among themselves for 1 day and individual females were subsequently provided with two hosts under diapause inducing photoperiod (light:dark cycle 14:10, 20 °C). This photoperiod was selected as it was previously shown that the variation in diapause response between northern and southern lines is more pronounced at this condition (Paolucci *et al.* 2013). Diapause response was scored for thirty F_1 females from each intraline cross and one hundred females from the interline crosses (NS from N × SS cross and SN from S × NN cross). F_1 females from reciprocal crosses have 50% northern nuclear genome and 50% southern nuclear genome, but differ for the maternally inherited cytoplasm and mitochondrial chromosome, that is either S or N (denoted as 'NS[S]' and 'SN[N]'; Fig. 1A).

To establish the mapping population for the QTL analysis, two males and two females from both the northern and the southern lines were isolated as pupae from the mass culture and placed in pairs in four combinations (N × NN, S × SS, N × SS, S × NN). After eclosion and mating, hosts were provided to each of the four types of females at standard, nondiapausing conditions. Parental individuals were stored in 70% ethanol at −20 °C for genotyping. F_1 females from interline crosses were isolated as virgins to produce F_2 recombinant haploid males (Fig. 1B). Four hundred recombinant males were individually backcrossed to a virgin female from both the southern and northern lines and stored for genotyping. Offspring from these backcrosses developed at 20 °C and continuous light. Four hundred females originated from the north backcross (hereafter termed B_N) and four hundred females originated from the south backcross (hereafter termed B_S) were scored for photoperiodic diapause response upon exposure to 14:10 light:dark cycle and 20 °C.

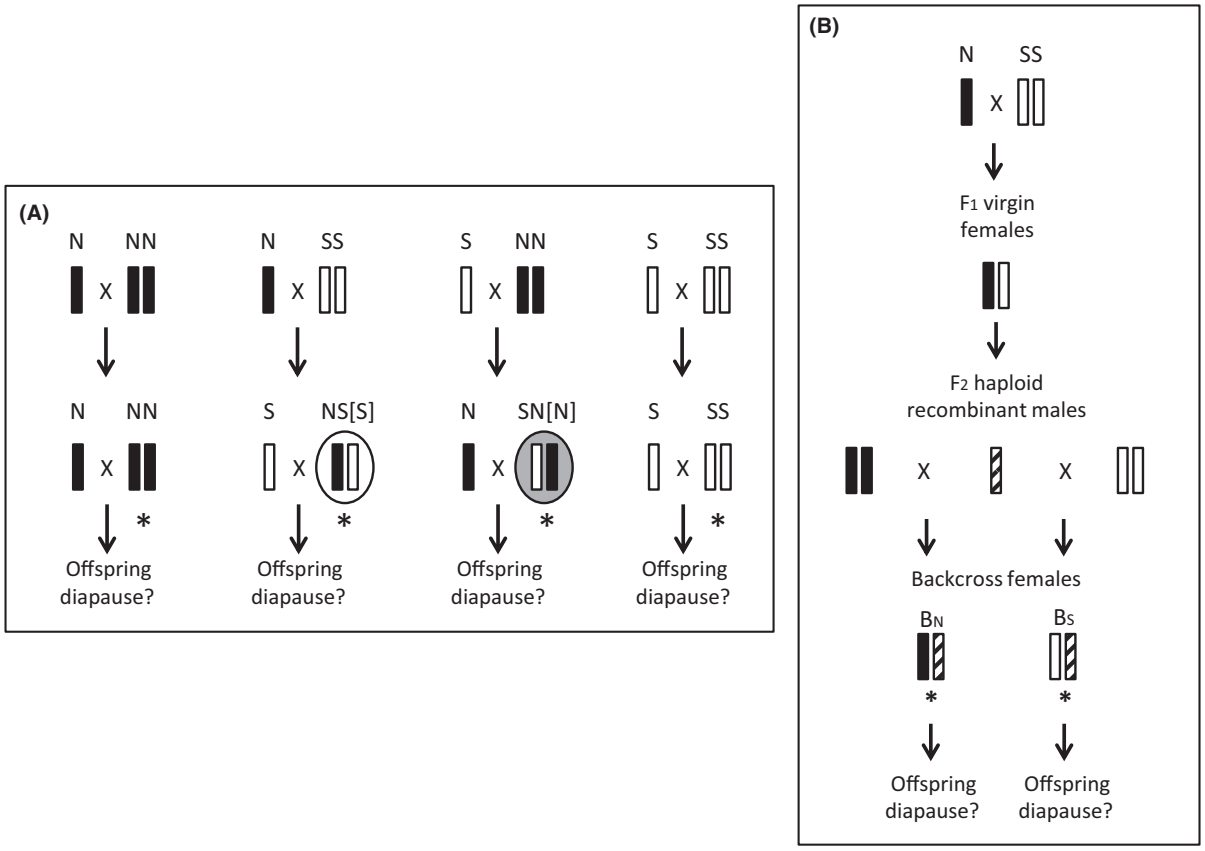

Fig. 1 Scheme of the cross-design. The switch point for diapause induction is measured in females marked with stars. The switch point is determined by scoring diapausing larvae in their offspring. (A) Intraline crosses generate F_1 homozygous NN and SS females (outer crosses in the figure). Reciprocal interline crosses generate F_1 heterozygous NS[S] and SN[N] females with different maternally inherited cytoplasm (inner crosses in the figure). Circle colours indicate cytoplasm, grey is from northern and white from southern line. (B) Simplified cross-design used to generate the mapping population of recombinant individuals. For clarity, only one parental cross is shown. Interline crosses generated F_1 heterozygous females which produced haploid recombinant males used for genotyping. Each F_2 male was backcrossed with females from the northern and southern line to generate B_N and B_S backcross females.

Diapause phenotyping

Single mated females were placed in a vial with two hosts under light:dark cycle 14:10 and 20 °C. Females were exposed to the treatment for the first 20 days of their adult life and provided with two fresh hosts every other day. The parasitized hosts were kept at 20 °C and constant light. Diapause incidence was determined by opening the hosts at 21 days after egg laying and scoring for presence of diapausing larvae (the larvae typically develop into adults in the meantime if not diapausing). As mixed broods, containing diapausing and nondiapausing offspring, are very rare, diapause can be considered a binary trait, so each set of two hosts was scored as either 'diapause' or 'no diapause'. For each female, the diapause response was expressed as *switch point* corresponding to the maternal age in days at which she switches from producing nondiapausing to diapausing offspring (for details on measuring photoperiodic diapause in *Nasonia*, see Paolucci *et al.* 2013). Survival analysis was used to analyse switch points for diapause induction with the R statistical software (R Core Development Team 2012). All phenotypic data were analysed using Cox proportional hazard models implemented in the packages *survival* and *coxme* in R, followed by *post hoc* multiple comparison analysis (generalized linear hypothesis test: *glht* command in the package *multcomp* in R).

Microsatellite and candidate gene genotyping

Genomic DNA was extracted using a standard high salt-chloroform protocol (Maniatis *et al.* 1982). Thirty-five microsatellite markers (Beukeboom *et al.* 2010; Pannebakker *et al.* 2010) were amplified using the Qiagen multiplex PCR kit according to manufacturer's recommendations (PCR profile: 15 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 1.5 min at 57 °C and 1 min at 72 °C, followed by 45 min at 72 °C). The length of the amplified fragments was determined using the Applied Biosystems 3730 DNA Analyzer and analysed using GENE MAPPER version 4.0 (Applied Biosystems, Carlsbad, CA, USA). From this set, twenty-three diagnostic markers were selected to genotype the F_2 recombinant males (Table S1, Supporting information). Selected markers did not show any shared alleles between northern and southern parental individuals.

Diagnostic SNP markers were identified in three candidate genes located in intron 8 of *period* (NasoniaBase reference NV16428-RA), intron 3 of *cycle* (NasoniaBase reference NV13263-RA) and intron 7 of *cryptochrome* (NasoniaBase reference NV13040-RA; Table S2 and Appendix S1, Supporting information). These markers were used to genotype a subset of F_2 recombinant males (98 individuals for *period*, 62 for *cycle*, 71 for *cryptochrome*). The markers did not deviate from a 1:1 segregation (following Bonferroni correction, $P > 0.05$). A linkage map was constructed based on the genotypes of 400 recombinant males with the package R/qtl in the R language (Broman *et al.* 2003). Five linkage groups were found, corresponding to the five chromosomes of *Nasonia*, and the order of markers in the linkage groups was conform to the previously described linkage maps for *Nasonia* (Beukeboom *et al.* 2010; Pannebakker *et al.* 2010; Koevoets *et al.* 2012).

QTL mapping

For each F_2 recombinant male, the probability of the allelic state at every cM map position, conditional to the observed genotype for the segregating microsatellite markers, was estimated using a hidden Markov model, allowing for genotyping errors and missing genotype data, as implemented in the R package R/qtl (Broman & Sen 2009). The QTLs were mapped to the genome using Haley–Knott regressions, involving individual phenotypes of 800 backcross females regressed on the conditional genotype probability of their F_2 sire to carry the northern allele at every cM. Individuals from both backcrosses were analysed jointly in our design, allowing estimation of dominance effects (North Carolina Design III: Kearsey 1980). In the regression model, the genotype probabilities multiplied by the additive and dominant coefficients were included as explanatory variables. A position with minimal residual variance was considered to be a putative QTL. The residual variance of the full model RSS_1, including additive and dominance effects, was used to infer LOD scores, using the formula $LOD = n/2 \log_{10} (RSS_0/RSS_1)$ where n is the sample size and RSS_0 is the variance of the null model (Broman & Sen 2009). The genomewide significance threshold was established by performing 1000 permutations and taking the 5% cut-off as significant threshold value (Churchill & Doerge 1994).

The percentage of phenotypic variance explained by the detected QTLs was determined by building a regression model in which additive and dominant terms of both significant QTLs and all possible interactions were tested as explanatory variables. A chi-squared test for likelihood was used to select the best QTL model containing only significant explanatory factors. The proportion of phenotypic variance explained by the identified QTLs was assessed with the formula $1-RSS_{QTL}/RSS_0$, where RSS_{QTL} is the residual variance of the best QTL model and RSS_0 is the residual variance of the null model without genetic terms (Pannebakker *et al.* 2011). The QTL analysis was performed in two steps. In the first analysis, 23 microsatellite markers

were used. Subsequently, the three SNP markers in the candidate genes were added and a new QTL analysis was performed.

Period haplotypes

The full sequence of *period* with predicted intron/exon structure was downloaded from NasoniaBase (Reference name NV16428-RA, gene ID in GenBank: LOC100121302). Ten primer pairs were designed to amplify gene regions covering parts of exon 3, 7, 8, 10, 11, 12, 14, 15 and 16, representing a subset of the 20 exons present in *period*, and intron 8 (Table S3, Supporting information). These regions were sequenced for male individuals from the northern and southern lines and sequences were aligned using the software for molecular evolutionary genetic analysis MEGA5 (Tamura *et al.* 2011). Synonymous and nonsynonymous SNPs were distinguished based on the PERIOD protein sequence as reference (NCBI ID: XP_001604906.2) and haplotypes were constructed based on nonsynonymous SNPs. In a similar way, the *cycle* locus (NasoniaBase reference NV13263-RA, gene ID in GenBank: LOC100118796) was investigated with seven primer pairs covering regions of exon 2, 4, 8, 9, 10, 11, 12, 13 and 14, representing a subset of the 14 exons of *cycle* (Table S3, Supporting information).

To test the association of the *period* haplotypes with diapause induction, crosses between and within lines were performed to generate F_1 females as described above. After genotyping the parental individuals, the *period* genotype of the F_1 female offspring was determined and individuals of each genotype were tested for their photoperiodic diapause induction following the diapause phenotyping described above (repeated hosting for 10 days). Thirty-five to 42 females from the intraline crosses and 69–100 females from the interline crosses were screened. Survival analysis was used to analyse the switch point for diapause induction. All phenotypic data were analysed using Cox proportional hazard models (package *survival* in the R language) followed by *post hoc* multiple comparison analysis (generalized linear hypothesis test: *glht* command in the package *multcomp* in the R language).

Natural polymorphism in period

Variation in *period* haplotype frequency was investigated by screening 119 female individuals from seven European populations. Photoperiodic diapause induction in these populations followed a latitudinal cline (Paolucci *et al.* 2013). The individuals were collected along a North–South gradient at various locations: Oulu, Finland (OUL: 65°3′40.16″N, 25°31′40.80″E); Turku, Finland (TUR: 61°15′40.53″N, 22°13′23.96″E); Latvia (LAT: 56°51′22.56″N, 25°12′1.38″E); Hamburg, Germany (HAM: 53°36′23.62″N, 10°10′17.74″E); Schlüchtern, Germany (SCH: 50°19′56.10″N, 9°30′47.00″E); Switzerland (SWI: 46°44′9.14″N, 7°6′57.34″E); and Corsica (COR: 42°22′40.80″N, 8°44′52.80″E). Sample sizes for each population ranged between 10 and 25 individuals (Fig. 5). The DNA was isolated from individual females (stored in 70% ethanol and −20 °C) using the standard high salt-chloroform protocol (Maniatis *et al.* 1982). Two primer pairs were used to amplify two representative regions of the *period* gene covering fractions of exon 3, 16 and 17, and a short intron sequence (primer pair Nv_*per*5 and Nv_*per*11 in Table S3, Supporting information). The two sequences from each individual were concatenated resulting in a sequenced fragment of 394 nucleotides. Individual haplotypes were inferred from the sequences using the algorithm PHASE (Clark 1990) implemented in the software for analysis of DNA polymorphism data DNASP5 (Librado & Rozas 2009). Haplotype frequency was estimated for each population. Linear regression (package *lm* in the R language) was used to test for a latitudinal cline in allele frequency for two SNPs in exon 3 and exon 16 and in haplotype frequency for the combination of these SNPs. Given that phenotypic data on switch point for diapause induction were available for the sequenced individuals, the correlation between *period* allele frequencies and haplotype frequencies and mean switch point was tested for each population. Finally, survival analysis was used to test the difference in switch point between different haplotype combinations (genotype) at the *period* locus in sequenced individuals from natural populations.

Results

Inheritance of photoperiodic diapause induction

Adult *N. vitripennis* female offspring from inter- and intraline crosses between the northern and the southern lines showed different diapause switch points upon exposure to a 14:10 circadian light:dark cycle (Cox model, effect of cross: $X^2 = 582.61$, d.f. = 5, $P < 2.2e{-}16$). The mean switch point was 4.23 ± 0.37 (mean ± SE) days for the pure northern line and 12.54 ± 0.54 days for the southern line (multiple comparison, $P < 0.001$, Fig. 2). Crosses between the northern and the southern lines yielded F_1 females with average diapause switch points of 9.04 ± 0.18 and 8.72 ± 0.19 days for NS[S] and SN[N] females, respectively (Fig. 2). These switch points were significantly different from either parental line ($P < 0.001$), but not from each other ($P = 0.98$).

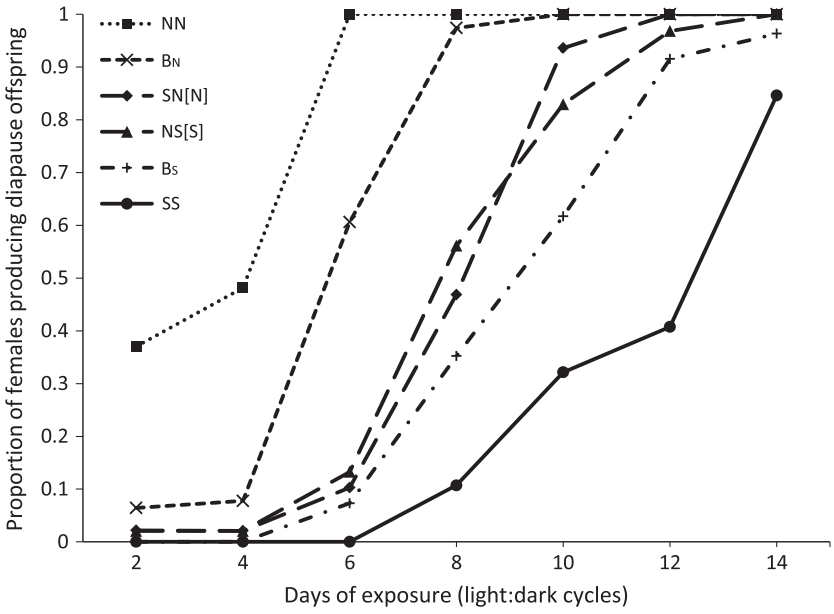

Fig. 2 Diapause response of *Nasonia vitripennis* females under light:dark cycle 14:10. Lines represent the responses of F_1 females from pure crosses (NN[N] and SS[S]), interline crosses with different cytoplasm (NS[N] and SN[S]) and B_N and B_S backcross females. Pure lines have 100% of N or S genome, respectively. F_1 females originated from interline crosses have 50% of S and N genome. Backcross females have 75% of N or S genome and 25% of S or N genome, respectively.

F_2 haploid males were backcrossed with females from both the northern and southern line to yield diploid backcross females (B_N and B_S, respectively). The mean switch points were 6.57 ± 0.08 days for B_N females, and 10.19 ± 0.13 days for B_S females. These values are significantly different from both each other and from the parental and F_1 genotypes (Fig. 2, $P < 0.001$). There is a significant correlation between the relative proportion of the northern and southern genome and the switch point ($Z = 22.16$, Kendall's rank correlation tau = 0.60, $P < 2.2e{-}16$). Together, these results show that maternal photoperiodic diapause induction has a genetic component with additive effects of N and S genotypes, and the absence of cytoplasmic effects.

QTL mapping

The QTL analysis for switch point revealed two significant QTLs: one located on chromosome 1 at 61 cM ($F_{2,699} = 35.13$; $P = 2.9e{-}15$; 1.5LOD interval 49–77 cM) and one on chromosome 5 at 69 cM ($F_{2,699} = 14.21$; $P = 8.9e{-}07$; 1.5LOD interval 33–95 cM; Table 1, Fig. 3). The northern alleles were associated with an earlier switch point in both backgrounds (fast diapause response), and partially dominant over the southern allele (degree of dominance for the QTL on chromosome 1 was d/a = 0.52 and for the QTL on chromosome 5 was d/a = 0.56). As a consequence of dominance, the relative change in switch point associated with the QTLs was lower in the northern background than in the southern background (Table 1, normalized QTL effects). Overall, the percentage of phenotypic variance explained by both QTLs was 13.7% (QTL linear model, $F_{4,697} = 27.714$, $P < 2.2e{-}16$). There was no significant interaction between the two QTLs.

Interestingly, the positions of the clock genes *period* and *cycle* map to the QTL on chromosome 1. Although these genes are separated by approximately 5.8 Mb (Niehuis *et al.* 2010), no recombination between *period* and *cycle* was detected. Another clock gene *cryptochrome* (*cry*) is located on chromosome 5, but fell outside the 1.5 LOD support interval for the identified QTL on this chromosome (Dupuis & Siegmund 1999; Broman & Sen 2009).

Table 1 Genomewide significant QTLs for photoperiodic induction of diapause (switch point) in *Nasonia vitripennis*. The normalized QTL effect is the change in phenotype between homozygous and heterozygous individuals relative to the mean in the northern and southern background. The QTL effect is expressed as additive (a) and dominant (d) effects

Chromosome	Location (cM)	Variance explained (%)	Background	Normalized QTL effect	a	d
1	61 (*per/cycle*)	9.1	North	8.1	−1.51	−0.79
			South	14.3		
5	69	3.9	North	3.4	−1.43	−0.81
			South	8.5		

Period haplotypes

To examine the QTL on chromosome 1 more closely, we chose the *period* gene to generate additional markers to be linked to phenotypic variation in diapause induction and to monitor their segregation in other European populations. Using 10 primer pairs, approximately 2.1 kb of DNA were amplified and sequenced for male individuals from the northern and the southern isofemale line. Fifteen nonsynonymous and 17 synonymous SNPs were identified in 7 exons, and 14 SNPs and two indels in intron 8. From these data three *period* haplotypes could be derived (Table 2 and Table S4, Supporting information for full sequences and all polymorphic sites identified). One of these haplotypes, termed per_S, was found in the southern line and the two other haplotypes (per_{N1} and per_{N2}) in the northern line. The southern haplotype per_S and the northern haplotype per_{N1} are identical for all tested nonsynonymous SNPs except for two SNPs in exon 3 and 16. In contrast, the northern haplotype per_{N2} is different from per_S and per_{N1} for all tested SNPs except for the SNP in exon 3, for which it shares the G with the southern haplotype per_S and the SNP in exon 16, for which it shares the G with the northern haplotype per_{N1} (Table 2). The combination of the two SNPs at the extremes of the sequenced fragment in exon 3 and exon 16 were selected to serve as the identifiers of the three haplotypes. The SNP (G/T) in exon 3 leads to the amino acid polymorphism glycine/valine (Gly/Val): the southern haplotype has the Gly variant and both variants are present in the northern isofemale line. The SNP in exon 16 (A/G) leads to a histidine/arginine polymorphism (His/Arg): the histidine variant is present only in the southern line and the arginine variant only in the northern line (Table 2).

The two selected SNPs do not lie within conserved functional domains and there is no described functional role of these two amino acid substitutions. In the sequence of *cycle*, only one synonymous SNP was detected in exon 2 that did not yield extra information as it was in complete linkage disequilibrium with either per_S or per_{N1} and per_{N2}.

Intraline crosses generated per_{N1N1} and per per_{N2N2} females which are homozygous at both SNPs and per_{N1N2} females that are heterozygous at the SNP in exon 3 and homozygous at the SNP in exon 16. Interline crosses generated per_{N1S} females, which are heterozygous at both SNPs and per_{N2S} females which are homozygous at the SNP in exon 3 and heterozygous at the SNP in exon 16. Females with different northern genotypes showed different switch point for diapause induction (Cox model, effect of genotype: $X^2 = 9.11$, d.f. = 2, $P = 0.01$). Homozygous per_{N1N1} females showed a significantly earlier switch point (6.77 ± 0.20 days) than homozygous per_{N2N2} females (7.71 ± 0.22 days; multiple comparison $P = 0.02$; Fig. 4). Females with per_{N1N2} genotype had a switch point (6.51 ± 0.27 days) significantly different from that of homozygous per_{N2N2} ($P = 0.03$), but not from per_{N1N1}

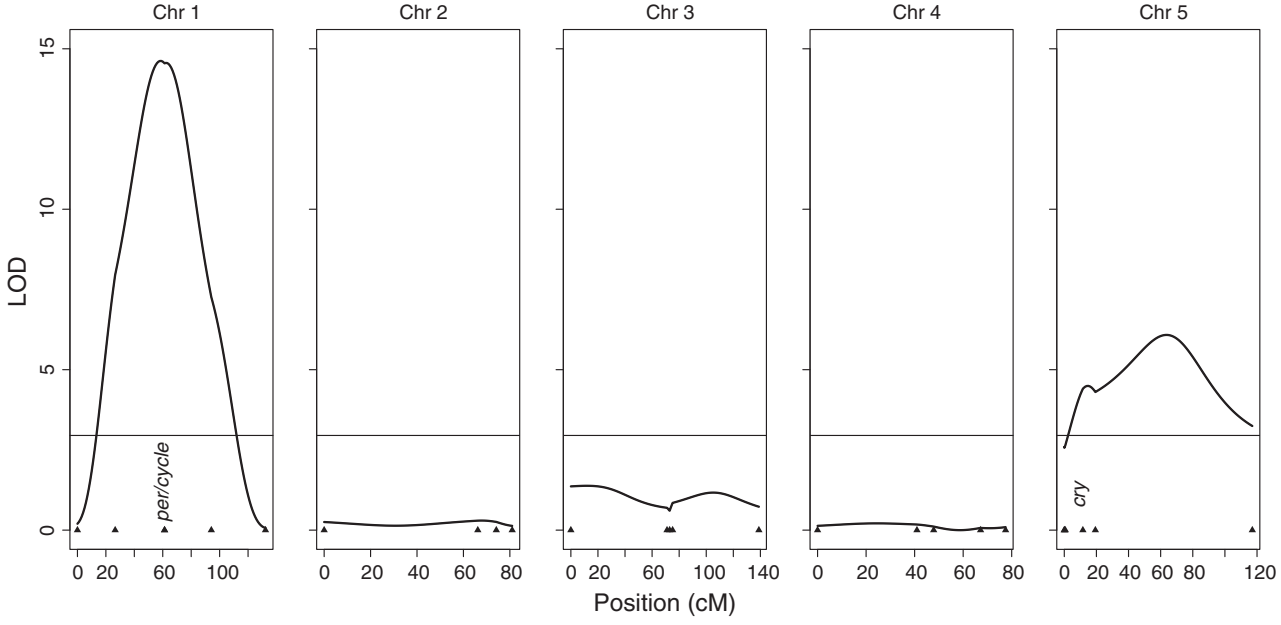

Fig. 3 Locations of QTLs for photoperiodic diapause induction (switch point) in *Nasonia vitripennis*. The horizontal line corresponds to the 5% genomewide significance threshold from permutation tests. The SNP markers in the candidate genes *period*, *cycle* and *cryptochrome* are indicated.

Table 2 Haplotypes of *period* in southern and northern lines based on nonsynonymous SNPs in 7 exons. Dots mean that the nucleotide is the same as the one in the first line at the same position. The SNPs in bold in exon 3 and exon 16 are used to define the three haplotypes. Table S4 in Supporting information shows all polymorphic sites for the three haplotypes and the sequences

Exon	3	10			11		12				14	15			16
Position (NasoniaBase NV16428-RA)	**129**	52	232	254	18	101	12	84	103	175	21	18	37	47	**20**
Haplotype															
per_S	**G (Gly)**	G	G	C	T	T	A	C	C	T	T	G	C	C	**A (His)**
per_{N1}	**T (Val)**	.	.	.	.	.	.	.	.	.	.	.	.	.	**G (Arg)**
per_{N2}	**. (Gly)**	A	T	T	C	G	C	A	A	C	C	T	G	G	**G (Arg)**

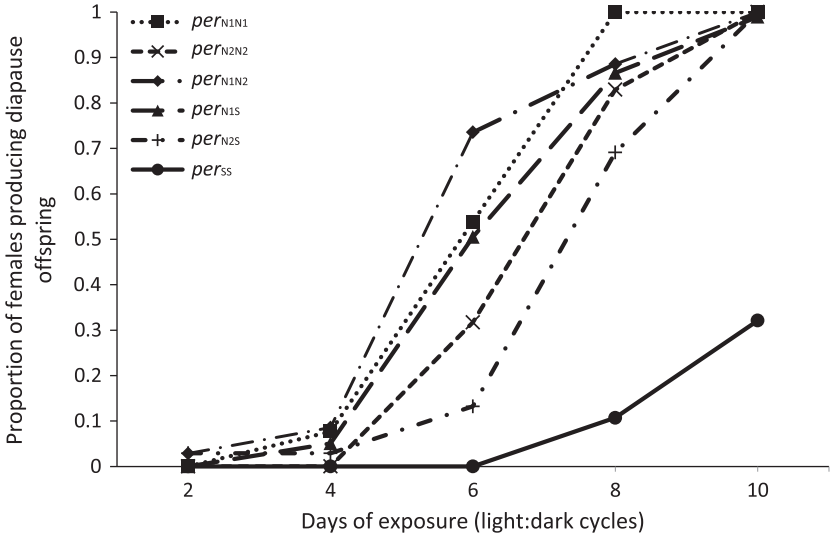

Fig. 4 Diapause response of *Nasonia vitripennis* females with different *period* genotypes under light:dark cycle 14:10. Lines represent the response of F_1 females from intraline crosses (northern) which generated three different *period* genotypes: per_{N1N1}, per_{N1N2}, per_{N2N2} and from interline crosses (north × south) which generated the genotypes per_{N1S} and per_{N2S}. Data for the per_{SS} individuals are from the crossing experiment shown in Fig. 2.

females ($P = 0.99$) indicating dominance of per_{N1} over per_{N2}. This result shows that *period* haplotypes are correlated with different diapause responses (haplotype per_{N1} and per_{N2} associated with early and late switch points, respectively). This was confirmed in the interline crosses where per_{N1S} individuals showed an earlier switch (7.13 ± 0.15 days) compared to per_{N2S} ones (8.23 ± 0.20 days; Cox model $\chi^2 = 10.22$, d.f. = 1, $P = 0.001$; Fig. 4). The switch points of per_{N1S} and per_{N2S} females were both earlier than those of per_{SS} females (12.54 ± 0.54 days; $P < 1e{-}05$) and were similar to the homozygous per_{N1N1} and per_{N2N2}, respectively (per_{N1N1} and per_{N1S} $P = 0.13$; per_{N2N2} and per_{N2S} $P = 0.17$). A partial dominance of both N haplotypes over S haplotype was thus observed.

Natural polymorphism in period and correlation with photoperiodic diapause induction

The conspicuous correlation between *period* haplotypes and photoperiodic diapause induction prompted the investigation of natural allelic variation of *period* in individuals from *N. vitripennis* populations collected along a North–South geographic gradient in Europe. A first analysis of the frequency of the two SNPs (in exon 3 and exon 16) in the full data set yielded an overall frequency of the G (Gly) variant in exon 3 of 0.61 and of the T (Val) variant of 0.39. Similar frequencies were found for the two variants in exon 16: the A (His) variant occurred at a frequency of 0.60 and the A (Arg) variant at 0.40. The frequencies of the two SNPs were subsequently compared between populations (Figure S1, Supporting information) and there was a significant latitudinal cline in frequency for alleles in exon 3 (linear regression: $F_{1,5} = 62.85$, $P < 0.001$, adjusted $R^2 = 0.91$) and exon 16 (linear regression: $F_{1,5} = 29.99$, $P = 0.002$, adjusted $R^2 = 0.83$). The linkage disequilibrium between the two polymorphic sites was high (standardized linkage disequilibrium D′ = 0.905 (Lewontin 1964), Fisher's exact test $P < 0.001$) and the *period* haplotypes were defined based on the combination of the variants at the two focal SNPs alone. Additional polymorphic sites were found in the 394-bp fragment sequenced in 119 individuals, leading to nine haplotypes. However, two haplotypes were highly predominant (overall frequency of 0.78) and all haplotypes were grouped into four main

haplotypes depending on the combination of the SNP variants in exon 3 and 16 (Table S5 and Figure S2, Supporting information for the description of all polymorphic sites and the haplotype frequencies).

The haplotypes per_S and per_{N1} accounted for most of the variation, as their frequencies in the entire data set were 0.58 and 0.34, respectively. Haplotype per_S was present in all populations with a decreasing frequency towards northern latitudes (linear regression: $F_{1,5} = 29.18$, $P = 0.002$, adjusted $R^2 = 0.82$). Haplotype per_{N1} showed an opposite cline with increasing frequency in northern populations (linear regression: $F_{1,5} = 21.28$, $P = 0.006$, adjusted $R^2 = 0.77$) and was absent in the two most southern populations COR and SWI (Fig. 5). Haplotype per_{N2} was rare and observed at low frequencies in northern populations only (total frequency 0.05). Only 5 individuals from three populations had the very rare haplotype defined by the T (Val) variant in exon 3 and G (His) variant in exon 16 (overall haplotype frequency of 0.02). The observed per_S haplotype frequency in populations across the North–South gradient correlates with maternal photoperiodic diapause induction, measured as population mean switch point (Paolucci *et al.* 2013; linear regression, $F_{1,5} = 6.84$, $P = 0.047$, adjusted $R^2 = 0.49$): southern populations have a higher per_S haplotype frequency associated with late switch point for diapause induction.

Haplotypes per_{N1} and per_S are the most common, 87% (104 of 119) of the investigated females had one of the three genotypes: per_{N1N1}, per_{SS} or per_{N1S} (proportions in full data set: 0.25 for per_{N1N1}, 0.13 for per_{N1S}, 0.5 for per_{SS}). The mean switch points for this subset of individuals were 9.23 ± 0.65, 7.38 ± 0.86 and 16.04 ± 1.09 days for per_{N1N1}, per_{N1S} and per_{SS}, respectively (Cox model, effect of genotype: $\chi^2 = 38.49$, d.f. = 2, P = 4.3e−09). These values resemble those obtained for homozygous and heterozygous individuals in the controlled crosses between northern and southern lines and confirm the dominance of haplotype per_{N1} over per_S and the different effect of *period* alleles on diapause per_{N1N1} and per_{N1S} $P = 0.31$; per_{N1N1} and per_{SS} $P <$ 1e−04; per_{N1S} and per_{SS} $P <$ 1e−04; Fig. 6).

Discussion

Our study shows that photoperiodic diapause induction in *N. vitripennis* has a strong genetic component as QTL analysis revealed two genomic loci involved in the variation of this trait: one on chromosome 1 and one on chromosome 5. A more detailed analysis of the *period* gene, located in the QTL on chromosome 1, showed a striking correlation with variation in photoperiodic induction of diapause.

With the first crosses, the relative role of maternal nuclear and cytoplasmic effects on diapause variation

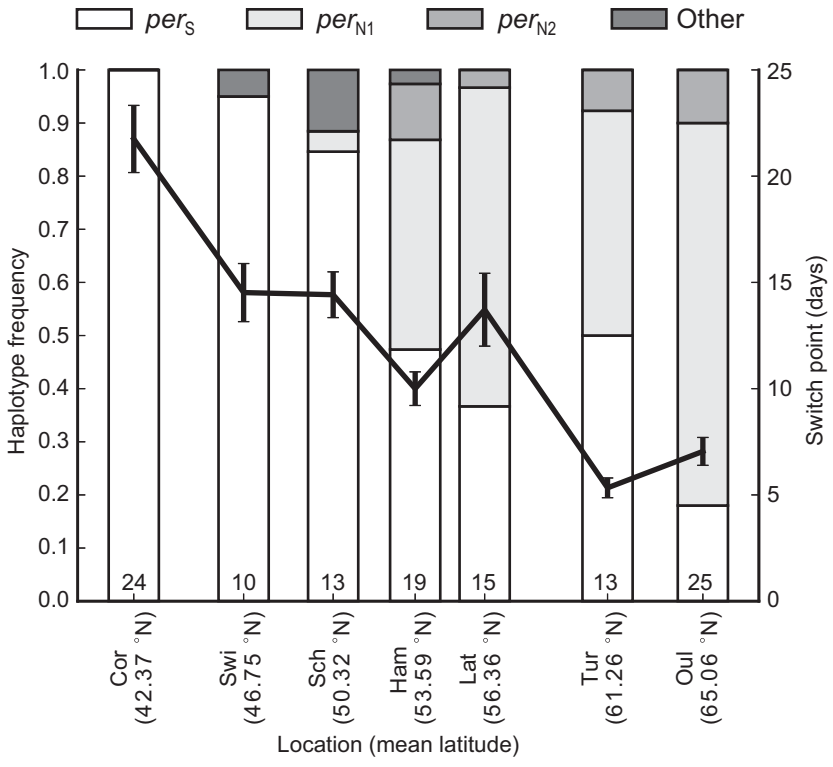

Fig. 5 Latitudinal cline of *period* haplotype frequency in *Nasonia vitripennis* natural populations from seven locations in Europe (sample size shown at the bottom of each bar) and correlation with mean population switch point for diapause induction measured under light:dark 14:10 (Paolucci *et al.* 2013). Only females that reached the switch point during the experiment were considered for the calculation of the mean population switch point. Error bars represent standard error.

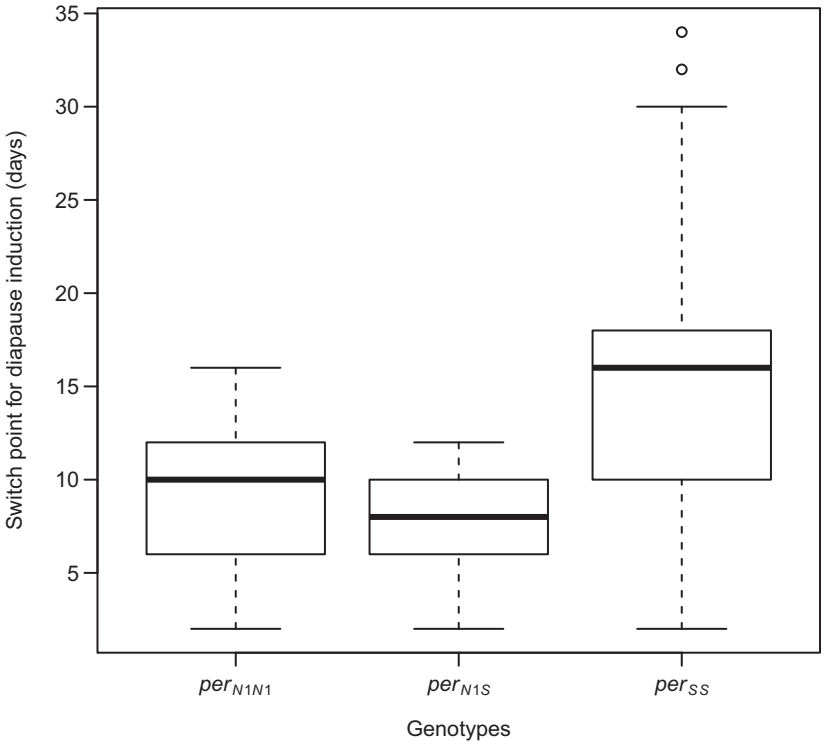

Fig. 6 Diapause switch point in *Nasonia vitripennis* females with different *period* genotypes. Individuals from seven European locations are grouped based on their genotype at the *period* locus. Switch point for each female was measured under light:dark 14:10 (Paolucci *et al.* 2013). Females that did not reach the switch point during the experiment were excluded. Rectangular boxes represent the interquartile range (IQR) of the data, the horizontal lines inside the boxes are the median value, and the whiskers extend to the most extreme data point which is no more than 1.5 times IQR. Dots are outliers.

was investigated. No significant differences in switch point were found between F_1 females of reciprocal interline crosses, indicating the absence of cytoplasmic effects and identifying the maternal genome as the primary cause of between-line variation in diapause response. This result was confirmed by the significant correlation between the relative proportion of northern and southern genome in the backcross females and their switch point. Overall, our findings confirm that difference in diapause is based on genetic variation for diapause timing, expressed as switch point. Figure 2 shows that the proportion of females producing diapause offspring did not differ between pure lines, F_1 and backcross females during the first days of adult life (days 2–6) and later in life (day 14). As the largest phenotypic variation was observed between days 6 and 12, it can be concluded that natural selection acts on maternal sensitivity to environmental cues resulting in different timing of diapause in the offspring. Thus, studying the molecular and genetic basis of adaptive diapause variation should focus on the mechanisms underlying maternal switch point timing, which represents the phenotypic trait directly linked to detection and interpretation of photoperiod. In *Nasonia*, other factors can play a role in diapause induction. Practically all individual females have the capacity to induce diapause in their offspring at some point in their life, even under long photoperiods. For example, old females tend to produce diapausing offspring in the last days of their life, probably as a consequence of senescence. However, our previous analysis showed that lifespan does not depend on photoperiod and its variation does not follow a latitudinal cline, confirming that the variation in switch point does not correlate with variation in adult lifespan (Paolucci *et al.* 2013). This confirms that *Nasonia* is able to modulate the innate capacity to induce diapause, depending on specific environmental cues.

The QTL with the highest LOD score is located on chromosome 1 and accounts for 9.1% of the phenotypic variation, while the QTL on chromosome 5 explains 3.1% of variation. The QTLs cover large segments of both chromosomes and contain numerous potential genes. However, a reiteration of the QTL analysis including markers in the clock genes *period* and *cycle* revealed that these genes coincided with the central position of the QTL peak, suggesting that these genes or closely linked loci account for most of the explained phenotypic variance. Unfortunately, the diagnostic SNP markers in *period* and *cycle* segregated as a single marker in the F_2 backcrosses and we could not disentangle the relative importance of the two genes.

To more specifically assess the possible involvement of *period* or closely linked loci in diapause induction, the segregation of three haplotypes of the *period* gene in *N. vitripennis* field lines were compared to latitudinal variation in photoperiodic diapause response. This analysis revealed a latitudinal cline in *per* haplotype frequencies that significantly correlates with the latitudinal

cline in photoperiodic diapause induction (Paolucci *et al.* 2013). If natural selection acts along a latitudinal geographic gradient to result in the proper timing for diapause response, selection of the optimal photoperiodic counter (threshold level of photoperiodic cycles inducing diapause response) would be expected. Genes involved in the counter machinery are thus under selection and are expected to show correlated polymorphisms, as is the case for *period* in this study. Although clinal variation in allelic frequency can also be the result of neutral processes (e.g. genetic drift), previous analysis of populations along the North–South gradient, showed that genetic differentiation at neutral marker loci did not correlate with latitude (Paolucci *et al.* 2013). Therefore, the latitudinal cline in *period* polymorphism may well result from selection pressure at this locus in response to environmental conditions. In addition, the association between *period* genotypes and switch point in the individuals from the field lines is consistent with the results of the laboratory crosses and the dominance of the northern allele over the southern allele. However, we cannot exclude that the role of *period* in influencing photoperiodic response is affected by the interaction with other genes and with the genomic background. An introgression experiment in which the different *per* alleles are crossed into a common genetic background is required to better determine the specific role of *period* in diapause. In addition, more analyses including higher resolution polymorphisms (e.g. SNPs) are needed to clarify the relative involvement of different genes in diapause induction as well as potential hitch-hiking effects which will provide information on how selection may act at specific loci.

Clinal variation in clock genes has previously been found in *Drosophila melanogaster* (Costa *et al.* 1992; Sawyer *et al.* 1997; Tauber *et al.* 2007; Fabian *et al.* 2012) and in the European corn borer moth *Ostrinia nubilalis* (Levy *et al.* 2015). Here, we found a correlation between *period* variation and diapause induction. Together with the fact that *period* is under the QTL peak on chromosome 1, this is suggestive for a link between the circadian clock and the photoperiodic clock (Emerson *et al.* 2009). Such link has been initially hypothesized by Bünning (1936) and has been addressed by numerous studies (reviewed by Koštál 2011; Saunders & Bertossa 2011; Goto 2013; Meuti & Denlinger 2013), using different methodologies including expression studies, use of mutants (Koštál & Shimada 2001) and gene knock down through RNAi techniques (Ikeno *et al.* 2010), but the topic is still subject of vivid debate. Although these studies show directly or indirectly that clock genes play a role in photoperiodic diapause, it is still unknown whether the circadian clock forms the basis for the seasonal photoperiodic diapause response, or whether the clock genes have pleiotropic effects on both traits (Hut *et al.* 2013). A study on *Drosophila triauraria* showed that allelic variation in *timeless* and *cryptochrome* is associated with the incidence of adult reproductive diapause but *period*, *cycle* and *clock* did not show any association (Yamada & Yamamoto 2011). In another study using northern and southern strains of the pitcher plant mosquito *W. smithii*, the gene *timeless* was found to epistatically interact with a significant QTL for critical photoperiod inducing larval diapause (Mathias *et al.* 2007). This suggests that the role of *timeless* in photoperiodic timer (expressed as critical photoperiod) is independent from its function in the circadian clock. Recently, Pegoraro *et al.* (2014) demonstrated the involvement of *period*, *timeless* and *Clock* in seasonal phenotypes using arrhythmic clock mutant strains of *Drosophila melanogaster* that showed altered chill coma recovery time (CCRt) after exposure to specific photoperiods, further confirming a link between seasonal phenotype and circadian clock (Pegoraro *et al.* 2014).

Photoperiodism in *Nasonia* has received considerable attention in the past (Saunders 1965, 1966, 1970), and recently, Bertossa *et al.* (2014) showed the oscillating expression of *period* in *Nasonia* under different photoperiods. Additionally, while this study was being prepared, Mukai & Goto (2016) reported a functional involvement of *period* in photoperiodic diapause response in *Nasonia vitripennis*, using an RNAi approach. They demonstrated that *period* knockdown in adult females leads to a significantly lower proportion of diapausing offspring when exposed to short photoperiods with a later switch point compared to the control group. However, the *per* RNAi wasps were still able to induce diapause in their offspring when they were exposed to low temperature (which is known to trigger diapause). These results show that *period* affects the perception of the photoperiod, but is not involved in the maternal physiological mechanisms that lead to the production of diapausing offspring (Mukai & Goto 2016). These results, however, do not elucidate the role of *period* allelic variation. As we show here that variation in *period* correlates with variation in switch point for diapause induction, our results, combined with the study of Mukai & Goto (2016), strongly justify to further investigating the functional genetics behind the role of *period* allelic variation in adaptive photoperiodic diapause induction.

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Conflict of interest

The authors declare that there are no conflicts of interests.

References

Bale JS, Hayward SAL (2010) Insect overwintering in a changing climate. *Journal of Experimental Biology*, **213**, 980–994.

Bertossa RC, van de Zande L, Beukeboom LW, Beersma DGM (2014) Phylogeny and oscillating expression of *period* and *cryptochrome* in short and long photoperiods suggest a conserved function in *Nasonia vitripennis*. *Chronobiology International*, **31**, 749–760.

Beukeboom LW, Niehuis O, Pannebakker BA *et al.* (2010) A comparison of recombination frequencies in intraspecific versus interspecific mapping populations of *Nasonia*. *Heredity*, **104**, 302–309.

Bradshaw WE, Holzapfel CM (2001) Genetic shift in photoperiodic response correlated with global warming. *Proceedings of the National Academy of Sciences*, **98**, 14509–14511.

Breeuwer JAJ, Werren JH (1990) Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature*, **346**, 558–560.

Broman KW, Sen S (2009) *A Guide to QTL Mapping with R/qtl*. Springer, New York.

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, **19**, 889–890.

Bünning E (1936) Die endonome Tagershythmik als Grundlage der photoperiodischen Reaktion. *Berichte Deutsche Botanische Gesellschaft*, **54**, 590–607.

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics*, **138**, 963–971.

Clark AG (1990) Inference of haplotypes from PCR-amplified samples of diploid populations. *Molecular Biology and Evolution*, **7**, 111–122.

Costa R, Peixoto AA, Barbujani F, Kyriacou CP (1992) A latitudinal cline in a *Drosophila* clock gene. *Proceedings of the Royal Society B - Biological Sciences*, **250**, 43–49.

Danks H (1987) *Insect Dormancy: An Ecological Perspective*. Biological Survey of Canada, Ottawa, Canada.

Dupuis J, Siegmund D (1999) Statistical methods for mapping quantitative trait loci from a dense set of markers. *Genetics*, **151**, 373–386.

Emerson KJ, Bradshaw WE, Holzapfel CM (2009) Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. *Trends in Genetics*, **25**, 217–225.

Fabian DK, Kapun M, Nolte V *et al.* (2012) Genome-wide patterns of latitudinal differentiation among populations of *Drosophila* melanogaster from North America. *Molecular Ecology*, **21**, 4748–4769.

Goto SG (2013) Roles of circadian clock genes in insect photoperiodism. *Entomological Science*, **16**, 1–16.

Hut R, Paolucci S, Dor R, Kyriacou CP, Daan S (2013) Latitudinal clines: an evolutionary view on biological rhythms. *Proceedings of the Royal Society B - Biological Sciences*, **280**, 20130433, 1–9.

Ikeno T, Tanaka SI, Numata H, Goto SG (2010) Photoperiodic diapause under the control of circadian clock genes in an insect. *BMC Biology*, **8**, 116, 1–9.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.

Kearsey MJ (1980) The efficiency of the North Carolina Expt. III and the selfing, backcrossing series for estimating additive and dominance variation. *Heredity*, **45**, 73–82.

Koevoets T, Niehuis O, van de Zande L, Beukeboom LW (2012) Hybrid incompatibilities in the parasitic wasp genus *Nasonia*: negative effects of hemizygosity and the identification of transmission ratio distortion loci. *Heredity*, **108**, 302–311.

Koštál V (2011) Insect photoperiodic calendar and circadian clock: independence, cooperation, or unity? *Journal of Insect Physiology*, **57**, 538–556.

Koštál V, Shimada K (2001) Malfunction of circadian clock in the non-photoperiodic diapause mutants of the drosophilid fly, *Chymomyza costata*. *Journal of Insect Physiology*, **47**, 1269–1274.

Levy RC, Kozak GM, Wadsworth CB, Coates BS, Dopman EB (2015) Explaining the sawtooth: latitudinal periodicity in a circadian gene correlates with shifts in generation number. *Journal of Evolutionary Biology*, **28**, 40–53.

Lewontin RC (1964) The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics*, **49**, 49–67.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Maniatis T, Fritsch EF, Sambrook J (1982) *Molecular Cloning: A Laboratory Manual*, 11th edn. Cold Spring Harbor Laboratory Press, New York.

Mathias D, Jacky L, Bradshaw WE, Holzapfel CM (2007) Quantitative trait loci associated with photoperiodic response and stage of diapause in the pitcher-plant mosquito, *Wyeomyia smithii*. *Genetics*, **176**, 391–402.

Meuti ME, Denlinger DL (2013) Evolutionary links between circadian clocks and photoperiodic diapause in insects. *Integrative & Comparative Biology*, **53**, 131–143.

Mukai A, Goto SG (2016) The clock gene *period* is essential for the photoperiodic response in the jewel wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Applied Entomology and Zoology*, **51**, 185–194.

Niehuis O, Gibson JD, Rosenberg MS *et al.* (2010) Recombination and its impact on the genome of the haplodiploid parasitoid wasp *Nasonia*. *PLoS ONE*, **5**, e8597.

Pannebakker BA, Niehuis O, Hedley A, Gadau J, Shuker DM (2010) The distribution of microsatellites in the *Nasonia* parasitoid wasp genome. *Insect Molecular Biology*, **19**, 91–98.

Pannebakker BA, Watt R, Knott SA, West SA, Shuker DM (2011) The quantitative genetic basis of sex ratio variation in *Nasonia vitripennis*: a QTL study. *Journal of Evolutionary Biology*, **24**, 12–22.

Paolucci S, van de Zande L, Beukeboom LW (2013) Adaptive latitudinal cline of photoperiodic diapause induction in the parasitoid *Nasonia vitripennis* in Europe. *Journal of Evolutionary Biology*, **26**, 705–718.

Pegoraro M, Gesto JS, Kyriacou CP, Tauber E (2014) Role for circadian clock genes in seasonal timing: testing the Bünning hypothesis. *Plos Genetics*, **10**, e1004603.

Perrot-Minnot MJ, Guo LR, Werren JH (1996) Single and double infections with *Wolbachia* in the parasitic wasp *Nasonia vitripennis*: effects on compatibility. *Genetics*, **143**, 961–972.

R Core Development Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Saunders DS (1965) Larval diapause of maternal origin: induction of diapause in *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae). *Journal of Experimental Biology*, **42**, 495–508.

Saunders DS (1966) Larval diapause of maternal origin II: the effect of photoperiod and temperature on *Nasonia vitripennis*. *Journal of Insect Physiology*, **12**, 569–581.

Saunders DS (1970) Circadian clock in insect photoperiodism. *Science*, **168**, 601–603.

Saunders DS (1971) The temperature compensated photoperiodic clock "programming" development and pupal diapause in the flesh-fly *Sarcophaga argyrostom*. *Journal of Insect Physiology*, **17**, 801–812.

Saunders DS (1973) The photoperiodic clock in the flesh-fly, *Sarcophaga argyrostoma*. *Journal of Insect Physiology*, **19**, 1941–1954.

Saunders DS (2002) *Insect Clocks*, 3rd edn. Elsevier, Amsterdam.

Saunders DS, Bertossa RC (2011) Deciphering time measurement: the role of circadian "clock" genes and formal experimentation in insect photoperiodism. *Journal of Insect Physiology*, **57**, 557–566.

Sawyer L, Hennessy J, Peixoto A, Rosato E (1997) Natural variation in a *Drosophila* clock gene and temperature compensation. *Science*, **278**, 2117–2120.

Schmidt PS, Matzkin L, Ippolito M, Eanes WF (2005) Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. *Evolution*, **59**, 1721–1732.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Tauber MJ, Tauber CA, Masaki S (1986) *Seasonal Adaptations of Insects*. Oxford University Press, USA.

Tauber E, Zordan M, Sandrelli F *et al.* (2007) Natural selection favors a newly derived timeless allele in *Drosophila melanogaster*. *Science*, **316**, 1895–1898.

Yamada H, Yamamoto MT (2011) Association between circadian clock genes and diapause incidence in *Drosophila triauraria*. *PLoS ONE*, **6**, e27493.

S.P., L.Z. and L.W.B. conceived and designed the overall study, interpreted the data and wrote the manuscript; L.S. performed genetic crosses and phenotypic measurements; S.P. performed the genotypic characterization; C.J.V. designed the QTL cross-design; S.P., L.S. and C.J.V. analysed the data.

Data accessibility

All phenotypic data on photoperiodic diapause induction, genotypic data for QTL analysis and DNA sequences are archived in Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.220j6.

Supporting information

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

Fig. S1 Latitudinal cline in SNP frequency in two non-synonymous SNPs in exon 3 and exon 16 in the *period* gene of *Nasonia vitripennis*.

Fig. S2 Frequency of *period* haplotypes in *Nasonia vitripennis* natural populations from seven locations in Europe.

Table S1 Multiplex sets of primers for amplifying microsatellite markers selected for QTL analysis.

Table S2 Primers for diagnostic SNP markers in candidate genes used in QTL analysis.

Table S3 Primers for amplifying exon regions of *period* and *cycle*.

Table S4 Haplotypes of *period* in southern and northern lines.

Table S5 Haplotypes of *period* in 119 female individuals from seven European locations.

Appendix S1 Sequencing protocol.