Adaptive latitudinal cline of photoperiodic diapause induction in the parasitoid Nasonia vitripennis in Europe

Paolucci, S.; van de Zande, L.; Beukeboom, L. W.

Published in:
Journal of Evolutionary Biology

DOI:
10.1111/jeb.12113

JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12113

Adaptive latitudinal cline of photoperiodic diapause induction in the parasitoid *Nasonia vitripennis* in Europe

S. PAOLUCCI, L. VAN DE ZANDE & L. W. BEUKEBOOM

Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen, Groningen, The Netherlands

Keywords:

diapause;
latitudinal cline;
life history;
Nasonia vitripennis;
photoperiod;
seasonal adaptation;
switch point.

Abstract

Living in seasonally changing environments requires adaptation to seasonal cycles. Many insects use the change in day length as a reliable cue for upcoming winter and respond to shortened photoperiod through diapause. In this study, we report the clinal variation in photoperiodic diapause induction in populations of the parasitoid wasp *Nasonia vitripennis* collected along a latitudinal gradient in Europe. In this species, diapause occurs in the larval stage and is maternally induced. Adult *Nasonia* females were exposed to different photoperiodic cycles and lifetime production of diapausing offspring was scored. Females switched to the production of diapausing offspring after exposure to a threshold number of photoperiodic cycles. A latitudinal cline was found in the proportion of diapausing offspring, the switch point for diapause induction measured as the maternal age at which the female starts to produce diapausing larvae, and the critical photoperiod for diapause induction. Populations at northern latitudes show an earlier switch point, higher proportions of diapausing individuals and longer critical photoperiods. Since the photoperiodic response was measured under the same laboratory conditions, the observed differences between populations most likely reflect genetic differences in sensitivity to photoperiodic cues, resulting from local adaptation to environmental cycles. The observed variability in diapause response combined with the availability of genomic tools for *N. vitripennis* represent a good opportunity to further investigate the genetic basis of this adaptive trait.

Introduction

In temperate and polar zones, light–dark periods and temperature show seasonal fluctuations. Species living in such areas synchronize their life cycle with annual cycles of environmental factors to optimally use the resources available during the favourable season for their growth and reproduction and to survive during harsh conditions. Organisms cope with seasonal change in a variety of ways, including different types of physiological, behavioural and developmental adaptations. Such long-term adaptations usually involve genetic changes, combined with plastic responses and physiological adjustments that are characteristic of short-term responses (Gienapp *et al.*, 2008; Hoffmann & Sgrò, 2011).

Many insect species spend the unfavourable season in a physiological state of dormancy called diapause mediated by neuro-hormonal signals, in which development is arrested, metabolic activity reduced and resistance to environmental challenges increased (Tauber *et al.*, 1986). The developmental stage at which diapause occurs ranges from embryo to adult, but each species has a genetically determined and specific expression of the diapause syndrome (Tauber *et al.*, 1986; Danks, 1987), Commonly, insects show facultative diapause in which specific developmental stages are sensitive to environmental stimuli. Only when cues for approaching seasonal change (for example upcoming winter) are perceived, insects respond adaptively by entering diapause (Tauber *et al.*, 1986). The evolutionary significance of the diversity in diapausing stages among insects has hardly been addressed. This requires

Correspondence: Silvia Paolucci, Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 11103, 9700 CC Groningen, The Netherlands.
Tel.: +31 (0)50 363 2336; fax: +31 50 363 2412;
e-mail: s.paolucci@rug.nl

comparative studies in connection with related life-history traits in a variety of insect species.

Various environmental factors can induce diapause response (Tauber *et al.*, 1986), but the majority of species living in temperate zones use the change in daily light:dark cycles (photoperiod). This is the most reliable cue for seasonal change as it remains constant over geological time (Bale & Hayward, 2010) and is correlated with other factors such as temperature, moisture and food availability (Tauber *et al.*, 1986). Photoperiod changes gradually and consistently along a latitudinal gradient, and insects at different latitudes have evolved specific responses to the prevailing photoperiods. The critical photoperiod, corresponding to the day length at which 50% of individuals in a population enters diapause, is longer towards higher latitudes (Kurota & Shimada, 2003; Wang *et al.*, 2011).

Despite the large number of studies on latitudinal variation of diapause response, its genetic regulation and the evolutionary mechanisms behind it are still poorly understood. To get insight into these mechanisms, it is important to investigate each aspect of diapause and particularly the genetic variation underlying variation in the sensitive stage during which the information from the environmental cues is perceived. The fruit fly *Drosophila melanogaster* has been extensively used as a model species for studying the molecular basis of adult reproductive diapause and the silk moth *Bombyx mori* for induction of egg diapause (Schiesari *et al.*, 2011). These studies show that information from the environment is processed by specific brain regions that stimulate hormone production, which in turn activates pathways for signal transduction, development and stress resistance (Nelson *et al.*, 2010). Natural selection can act on different levels of the pathway leading to diapause. For example, the observed geographical variation in diapause induction can be determined by genetic variation for detection of environmental cues. Alternatively (or simultaneously), selection can act on hormone production or on the regulation of signal transduction that induce the diapause response. To understand the genetic basis of adaptation to seasonal environmental cycles, it is crucial to elucidate those aspects of diapause that constitute the target of natural selection and vary in relation to the environment. This requires insect species with robust and clear photoperiodic responses, distinct sensitive and responsive stages and well-developed genomic tools (Emerson *et al.*, 2009).

The haplodiploid Hymenopteran genus *Nasonia* is emerging as new model system in evolutionary biology (Beukeboom & Desplan, 2003; Werren *et al.*, 2010). *Nasonia* are small gregarious parasitoid wasps that parasitize the pupal stage of various blowfly species. There are four closely related species, of which *Nasonia vitripennis* has a cosmopolitan distribution and therefore can cope with a wide range of climatic conditions (Darling & Werren, 1990). *Nasonia* has a facultative diapause which occurs at the fourth larval instar just before pupation and which is well defined and easy to measure (Saunders, 1965). Interestingly, it is induced by the adult female which therefore represents the sensitive stage. In general, when adult females are exposed to short-day conditions (short photoperiod), they initially produce normal developing larvae and switch to production of diapausing larvae after exposure to a critical number of light:dark cycles. Under long photoperiodic conditions, the switch occurs later or not at all. The larval diapause is very strong and remains until specific environmental conditions, such as increasing temperature, induce further development (Saunders, 1962, 1966). Saunders used *N. vitripennis* extensively for studying the mechanisms of photoperiodism and proposed that photoperiodism consists of two separate components: a photoperiodic timer which measures the length of the day (or night), and a photoperiodic counter which counts the number of cycles. The counter uses the information from the timer to elicit the response after a threshold number of photoperiodic cycles has been reached (Saunders, 2002). Studying geographical variation of this threshold level during the sensitive stage will shed light onto the proximate mechanisms underlying diapause variation and the genetics of seasonal adaptation. In this respect, *Nasonia* represents an excellent model system since it provides the opportunity to study variation in the perception of environmental conditions during the sensitive stage (adult female) and how this is translated into different response in the offspring.

Here, we describe the latitudinal variation in photoperiodic induction of diapause using seven *N. vitripennis* populations collected along a latitudinal gradient in Europe, as a first step towards unravelling the genetic architecture of this important life-history trait and its variation. For all populations, we measured the critical photoperiod (expression of the photoperiodic timer), the number of photoperiodic cycles required for the induction of diapause (photoperiodic counter) and the overall proportion of diapausing offspring produced during the entire life of adult females exposed to different photoperiodic regimes. We link these results to molecular data on genetic divergence of populations to evaluate the role of selection in maintaining the observed variation.

Materials and methods

Field collection

In summer 2009, *Nasonia* wasps were collected from seven locations along a latitudinal gradient in Europe from northern Finland to Corsica, covering a range of about 23 degrees (Fig. 1. OUL: 65°3′40.16″N, 25°31′40.80″E; TUR: 61°15′40.53″N, 22°13′23.96″E; LAT: 56°51′22.56″N, 25°12′1.38″E; HAM: 53°36′23.62″N, 10°10′17.74″E; SCH: 50°19′56.10″N, 9°30′47.00″E; SWI: 46°44′9.14″N, 7°6′57.34″E; COR: 42°22′40.80″N, 8°44′

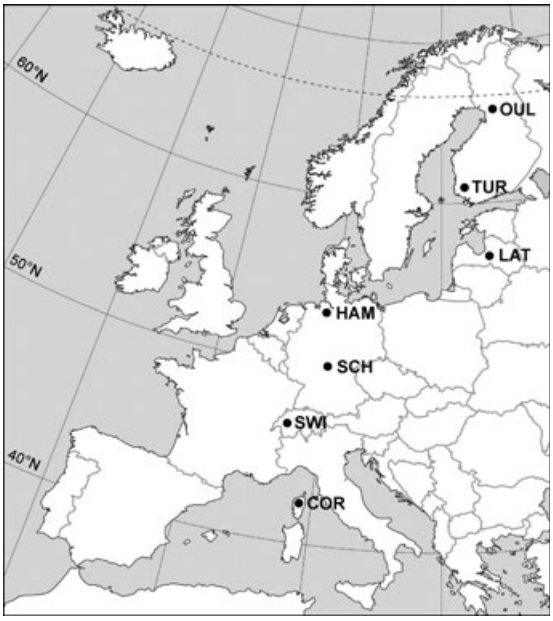

Fig. 1 Map of sampling locations in Europe. From North to South: Finland, Oulu (OUL); Finland, Turku (TUR); Latvia (LAT); Germany, Hamburg (HAM); Germany, Schlüchtern (SCH); Switzerland (SWI); France, Corsica (COR).

52.80″E). Wasps were collected from bird nests in nest boxes. The nest boxes were mainly used by great tits (*Parus major*), blue tits (*Parus caeruleus*) and flycatchers (*Ficedula hypoleuca*). At least two sampling sites, each including several nest boxes, were visited at each location to increase the diversity of samples within the same geographical location. Wasps were collected in three ways. The main sampling technique consisted of removal of nests at least 5 days after the birds had fledged and subsequent dissection for fly pupae that might have been parasitized. The second sampling methodology involved the use of baits consisting of mesh bags with approximately 25 laboratory-raised fly pupae (*Calliphora spp*.) that were placed in nest boxes for a few days to attract *Nasonia*. They were subsequently taken to the laboratory for further development and checked for wasp emergence. Thirdly, adult wasps could also be collected directly in the field from the nest material or on the baits. Table 1 provides an overview of the total number of nest boxes that were sampled at each location. Wasps collected within a nest box may be genetically related because a single female can parasitize several fly pupae within one nest, although most nests are colonized by multiple unrelated founding females (Grillenberger *et al.*, 2008).

Establishment of isofemale lines

Isofemale lines were established from females collected directly from the field, or from nest and bait emergences. The pool of natural pupae obtained from a single nest box or bait was maintained in a vial until emergence of flies or wasps. Flies were discarded, while emerged wasps were left for 1 day in order to allow mating within the patch. Single females were subsequently isolated in a plastic vial and supplied with hosts (*Calliphora* spp. pupae). As *Nasonia* females typically mate once, a maximum of three different alleles per gene will segregate in each line because of haplodiploidy, but this number may be reduced due to mating among relatives (Grillenberger *et al.*, 2008). In some cases, multiple lines had to be established from one nest box when not enough females were available because too few nest boxes or baits yielded wasps. Isofemale lines were maintained in mass culture vials at diapause-preventing conditions (long photoperiod, temperature = 20–25 °C). Throughout this article, the term 'population' will be used for the pool of isofemale lines established from each location.

Some wild-caught females from northern latitudes (OUL and TUR) produced only diapausing offspring after field collection and therefore could not be used

Table 1 Summary of field collection showing the number of nest boxes that yielded wasps in each sampling location and isofemale lines used in the experiment. For each locality, the number of used nest boxes is given and the number of used isofemale lines established from nest material, baits and wild-caught females in those nests. Lines used in the second experiment are shown in the panel on the right. Location abbreviations are explained in Fig. 1.

	Location	COR	SWI	SCH	HAM	LAT	TUR	OUL	TUR	OUL
Fieldwork	Nest boxes inspected	129	87	106	60	70	55	84		
	Nest boxes that yielded wasps*	23	12	6	7	11	13	29		
Experiment	Nest boxes used	8	12	6	7	11	11	19	5	15
	Lines from nest material	0	22	22	18	20	6	10	0	0
	Lines from baits	0	2	0	6	4	5	0	0	0
	Lines from wild-caught females	25	2	0	2	2	10	16	6	18
	Total	25	26	22	26	26	21	26	6	18

*from natural host puparia, baits or as adult female individuals.

immediately in the first experiment. These lines were established as 'diapause lines' and maintained under diapause conditions (temperature = 5 °C, constant darkness). After a few months, they were transferred to 20 °C and light:dark (LD) cycle 18 : 6 to break diapause and subsequently used in a second experiment (see below) to determine their diapause response.

Photoperiodic induction of diapause

Twenty-one to 26 isofemale lines per location were used to measure diapause response under different photoperiods. Given that the main goal of this experiment was to investigate latitudinal variation in photoperiodic response rather than variation within local geographical populations, the number of independent lines was maximized and lines were considered replicates within a location. The details about number of isofemale lines and their source for each location are given in Table 1. Individuals from the first or second generation after field collection were used. Prior to their use, they were kept under standard conditions (25 ± 1 °C, LD 18 : 6) in mass cultures. Newly emerged individuals were allowed to mate among themselves (siblings) for 1 day. Single females were subsequently placed in cotton-plugged 60 mm × 10 mm polystyrene tubes with two hosts. Wasps were distributed over eight incubators corresponding to the eight treatments used in the experiment, resulting in a sample of 21–26 individuals per location in every condition. Each treatment consisted of a constant photoperiod (hours of light in 24 h). The following treatments were applied: LD 8 : 16, LD 10 : 14, LD 12 : 12, LD 13 : 11, LD 14 : 10, LD 15 : 9, LD 16 : 8, LD 18 : 6 (Light intensity: 100–200 lum/sqf). All treatments were at constant temperature (T = 20 ± 1 °C) and humidity (50%–55% RH).

Females were exposed to the treatments for their entire life and the two hosts were replaced every other day. Parasitized hosts were transferred to a new vial and cultured at 20 ± 1 °C and constant light. This ensured standardized developing conditions of offspring from all individuals in all treatments. Females were re-hosted until death providing additional data about adult longevity under different LD regimes.

Diapause scoring

Diapause in *Nasonia* occurs at the fourth instar larval stage. Typically, females produce normal developing larvae at the beginning of their life and switch to production of diapausing larvae later in life after exposure to a certain number of LD cycles. Diapausing larvae arrest their development and resume it only after having been kept under diapause-maintaining conditions for at least 2 months (temperature ~4 °C, constant darkness). Normal developing larvae emerge from the host as adults after 21 days at 20 °C. Thus, diapause can easily be determined by opening the hosts after 21 days and scoring for presence of larvae.

A total of 20 987 broods were scored for diapause. Diapause was measured as a binary trait: each set of two hosts provided in a 2-days interval was scored as 'diapause' when only diapausing larvae were present or 'no diapause' when only adult offspring emerged. In the case of mixed broods, if 50% or more individuals were diapausing larvae, they were counted as 'diapause broods', otherwise as 'nondiapause broods'. Mixed broods were rare and typically only occurred in one or two hostings around the switch point. Very few hosts were empty or yielded flies (nonparasitized) and were excluded from the dataset.

For each female, two parameters were measured: *the production of diapause offspring* which corresponds to the proportion of diapausing broods relative to the total number of broods in her life and the *switch point* measured as the maternal age at which the female switches from producing nondiapausing to diapausing offspring during the sequential hosting (i.e. the required number of days of exposure to a particular LD regime for the occurrence of the switch). For each population, the *critical photoperiod* for diapause induction was estimated as the photoperiod corresponding to 50% of diapause response (see statistical analysis). The correlation between all parameters and latitude was measured.

Photoperiodic induction of diapause in 'diapause lines' from northern locations

In a second experiment, additional isofemale lines (Table 1) were used that had been established as 'diapause lines' right after collection (see above). These lines were from the two most northern locations and needed to be added to the analysis to prevent a biased photoperiodic response measurement. Females were taken out of diapause after 2 months storage at 4 °C and cultured for an extra generation under standard conditions (25 ± 1 °C, LD 18 : 6). The experimental set-up was the same as the first experiment, except that only five LD treatments were applied (LD 13 : 11, LD 14 : 10, LD 15 : 9, LD 16 : 8, LD 18 : 6) and females were re-hosted every other day until day 20 of adult life.

Effect of laboratory culture on photoperiodic diapause induction

Our experiments to determine the natural variation of photoperiodic diapause response were performed using individuals from the first or second generation after field collection in order to keep the genetic make-up of the different isofemale lines as close as possible to the original state. In a separate experiment, a possible effect of adaptation to lab condition was investigated by re-testing a number of isofemale lines from three locations (COR, HAM and OUL) after having been maintained

for 13–14 generations in the lab. In addition, replicates of the same lines that had been kept in diapause from the second or third generation after field collection (for about 10 months) served as controls. After synchronization and standardization of culturing conditions (25 ± 1 °C, LD 18 : 6) for two generations, adult females were exposed to five LD treatments (LD 13 : 11, LD 14 : 10, LD 15 : 9, LD 16 : 8, LD 18 : 6) and re-hosted every other day for the first 20 days of adult life. Diapause was subsequently scored as previously described using 10–16 lines per location.

Microsatellite genotyping for analysis of population differentiation

The wild-caught adult females and those used to establish the isofemale lines were stored in 70% Ethanol at −20 °C prior to DNA extraction and molecular analysis. Genomic DNA was isolated from individual females using a standard high salt-chloroform protocol (Maniatis *et al.*, 1982). Fifteen to 26 individuals per location originating from different nest boxes within each location were selected. Genetic differentiation between populations was established using eleven polymorphic microsatellite markers (Nv26, Nv107, Nv118, Nv200, Nv205, Nv229, Nv301, Nv303, Nv319, Nv320, Nv322) (Beukeboom *et al.*, 2010; Pannebakker *et al.*, 2010; Beukeboom, unpublished) that 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 T_A = 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 v4.0 (Applied Biosystems, Carlsbad, CA, USA).

Statistical analysis

Statistical analysis of all data on photoperiodic diapause induction was performed using the R statistical software (R Development Core Team, 2012). The general technique for building up a statistical model followed the standard model simplification procedure starting from full model with all possible factors and interactions and proceeding by removing nonsignificant explanatory factors. The fit of the models was assessed after comparing the likelihood of different models with Chi-squared test.

Proportion of diapause production

To analyse the data on variation in lifetime proportion of diapause, we used a generalized linear mixed effect model (R package *lme4*). The response variable is a matched pair of counts of nondiapause broods and diapause broods, which is interpreted as the proportion of broods that were in diapause for each female. Location and treatment were included as fixed explanatory variables, the random effects were nested as follow: nest box (from which each isofemale line was established) nested within the location nested within LD treatment using the binomial error distribution. The correlation between latitude and proportion of diapause was analysed with a series of mixed effect logistic regressions for each treatment independently. In these models, latitude was fitted as fixed continuous explanatory variable and nest box nested in location as the random effect. The error distribution is binomial and the link function is logit. The validity of the model was assessed through comparison between the model with latitude and the null model with only random effect (likelihood ratio test). The odds ratios are calculated from the coefficients estimated in the model.

Switch point for diapause induction

In the second analysis, we investigated the switch point for diapause induction in different treatments for different populations. Survival analysis was used to analyse the time of switch as response variable. This is expressed as the maternal age at the switch point which corresponds to the number of LD cycles experienced by the adult female before the switch would occur. Data were analysed using Cox proportional hazard mixed effects model (package *coxme* in R), and data were censored for individuals that did not live long enough to reach the switch point. In the first model, location and treatment were the fixed effects, the random effects were specified as above. The effect of latitude on switch point within each treatment was analysed separately to check for a correlation between latitude and switch point. Hazard ratios were obtained from these models as estimates of the difference in the rate of switching to diapause for every unit of the predictor variable 'latitude' (one degree unit). A positive value of the hazard ratio means that the risk of switching to diapause per unit of time increases for every degree of latitude, which indicates that the chance of inducing diapause is early in life (higher rate) at higher latitudes and later in life at low latitudes. This in turn results in a negative correlation between switch point and latitude.

Survival analysis (Cox proportional hazard mixed effects model) was also applied in a similar way to test the effect of treatment and location on longevity of wasps. In this case, there were no censored data as all wasps eventually died. Correlation between latitude and life span was also tested with the same type of survival analysis.

Critical photoperiod for diapause induction

For the estimation of the critical photoperiod of each population, we focused mainly on 10-day-old females

because this age point coincided with the largest variation in response between populations. A mixed effect logistic regression model was fitted for specific age points, considering diapause as a binary response variable, treatment and location as fixed effect, and nest box nested within location nested within treatment as random effects. The critical photoperiod for each population was established after fitting an incidence function model (generalized linear model with binomial error) based on diapause incidence as function of photoperiod which yielded the photoperiod corresponding to 50% of diapause response. The diapause incidence is defined as the proportion of females of a given population producing diapausing offspring at a certain age and under specific condition.

The correlation between critical photoperiod and latitude was determined with linear regression analysis (*lm* in stats package in R) between estimated critical photoperiod and mean latitude of origin of each population. Data on photoperiodic diapause induction in northern lines subsequently added to the data set and data on diapause response of lines maintained in the laboratory for several generations were analysed using the same types of statistical methods.

Population differentiation analysis

Population structure of *N.vitripennis* European populations was determined with the software Fstat (Goudet, 2001) from 11 microsatellite markers. Gene diversity was estimated per locus and per population using an unbiased estimate of genetic variability (gene diversity, Nei, 1987) as implemented in Fstat. Single and multilocus F_{ST} values were calculated according to Weir & Cockerham (1984) and SE were obtained after jackknifing over populations or loci. The same procedure was used to obtain pairwise F_{ST} values.

Pairwise tests of differentiation were performed following G-statistic and significant values are considered at the nominal level of 0.05 after Bonferroni correction. The pairwise F_{ST} values and the approximate geographical distance (km) between geographical locations were used for the isolation by distance analysis performed with a Mantel test for matrix correlation.

Results

Latitudinal cline for proportion of diapause production

Diapause was induced in *N. vitripennis* larvae after exposure of females from the maternal generation to different LD regimes, confirming that photoperiod represents an important diapause-inducing factor. All tested isofemale lines produced diapausing larvae under at least one of the applied LD regimes. In general, the overall production of diapausing broods was higher for females exposed to short photoperiods and decreased with longer photoperiods (Fig. 2; mixed effect logistic regression model, effect of treatment: $\chi^2 = 42.43$, $P = 4.29\text{e-}07$). There was also a clear effect of geographical origin (mixed effect logistic regression model, effect of location: $\chi^2 = 142.22$, $P < 2.2\text{e-}16$). In addition, for all LD regimes a positive correlation was found between latitude and proportion of diapausing broods (mixed effect logistic regression models, all *P*-values are < 0.05, odds ratios are given in Fig. 2). This indicates a latitudinal cline for photoperiodic diapause induction in *N. vitripennis*. As the diapause production was measured under the same experimental conditions, the observed variation in response reflects genetic differences in sensitivity to specific photoperiods between different populations.

Latitudinal cline for switch point (photoperiodic counter)

Adult *N. vitripennis* females exposed to experimental LD regimes initially produced normal developing offspring and switched to the production of diapausing offspring later in life. For each female, the switch is pronounced and rapid; broods containing a mixture of diapausing larvae and nondiapausing larvae are rare and occur only for one or 2 days around the switch.

Eighty-five per cent of all tested females lived long enough to pass the switch point. For COR, the most southern population, 69 of 200 females did not switch, when exposed to long photoperiods and also showed the shortest life span. The required day number for the switch was dependent on the applied LD regime (Fig. 3) and the geographical location of the population. For all populations, adult females under short photoperiods switched to the production of diapause broods earlier compared with long photoperiods. In addition, for each regime, individuals from southern latitudes switched later than those from northern latitudes, indicating a correlation between latitude and switch point. The effect of latitude was more pronounced at intermediate photoperiods (LD 14 : 10 and LD 15 : 9) corresponding to the largest variation in switch point across locations. This is reflected by a significant effect of the interaction between location and treatment (survival analysis, Cox mixed effects model, effect of treatment × latitude: $\chi^2 = 77.03$, $P = 0.0007$). This correlation between latitude and switch point was supported by a nonparametric survival analysis, using a series of Cox models for each treatment separately. In all treatments, latitude and switch point were negatively correlated (survival analysis, Cox mixed effects models, all *P* values < 0.05, hazard ratios are given in Fig. 3) meaning that southern individuals switch later in life compared with northern individuals.

To investigate whether the observed cline in switch point is caused by latitudinal differences in female life

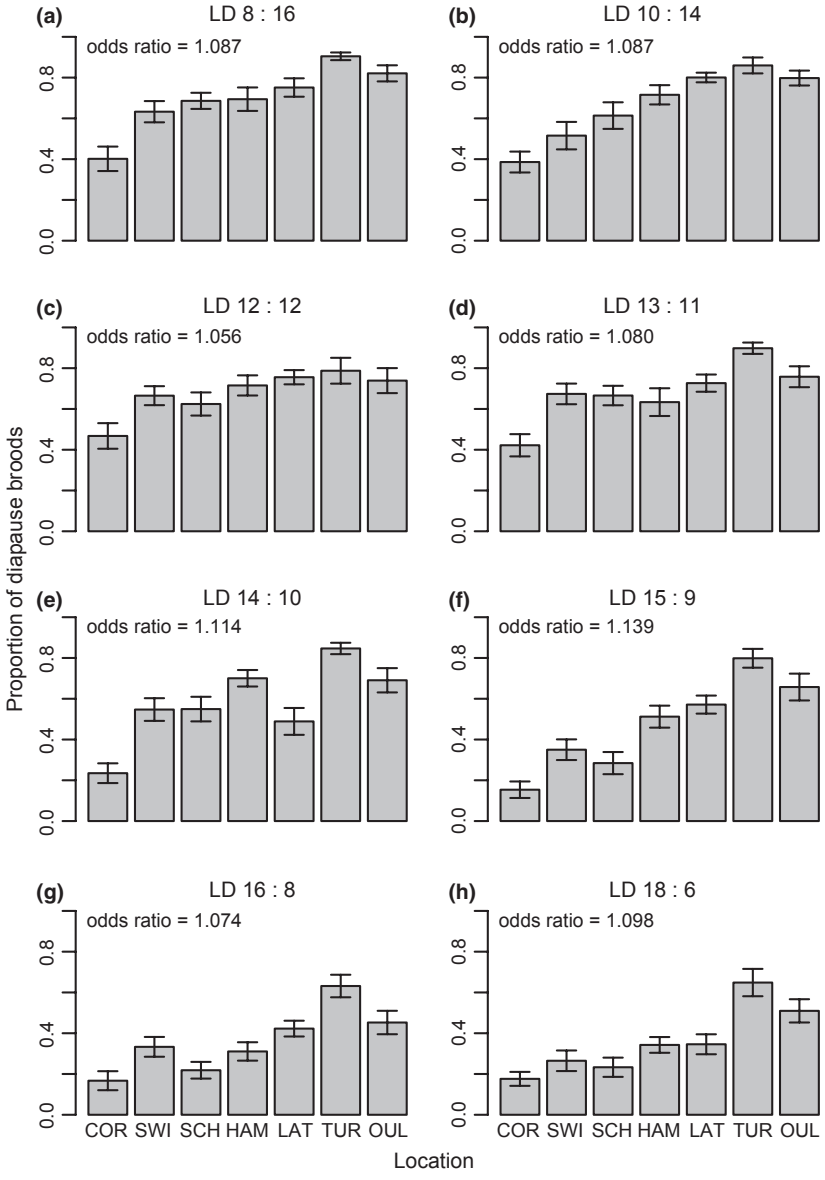

Fig. 2 Proportion of diapausing broods relative to the total number of broods produced by females (mean ± SE) from seven geographical locations under applied LD treatments. The odds ratios obtained from the coefficients estimated by the logistic regression model are given in each panel. The sample size is 21–26 individuals in each location and treatment.

span, we measured adult longevity at 20 °C. There was no effect of LD treatment (survival analysis, Cox mixed effects model, effect of treatment: $\chi^2 = 5.37$, $P = 0.61$) meaning that photoperiod itself does not affect life span in *N. vitripennis*. Considerable variation, however, was found between individuals from different locations (Fig. 4): mean adult longevity ranged from 26.3 (COR) to 36.2 (LAT) days and there was a significant effect of location (survival analysis, Cox mixed effects model, effect of location: $\chi^2 = 36.526$, $P = 2.18e\text{-}06$). Although populations differed in their mean life span, there was no correlation between longevity and latitude (survival analysis, Cox mixed regression model, effect of latitude: $\chi^2 = 0.99$, $P = 0.32$) indicating that the observed differences between locations are not due to the latitudinal cline. These results imply that the observed variation in switch point is not affected by variation in longevity.

Latitudinal cline for critical photoperiod (photoperiodic timer)

The effect of photoperiod on diapause induction for 10-day-old females was chosen for comparison of critical photoperiod as this time point showed the largest variation in response between locations. For the construction of photoperiodic response curves (Fig. 5), the incidence of diapause is defined as the percentage of individuals in a given population that switched to the production of diapausing larvae under a certain condition. In line with the results shown in the first section, geographical origin

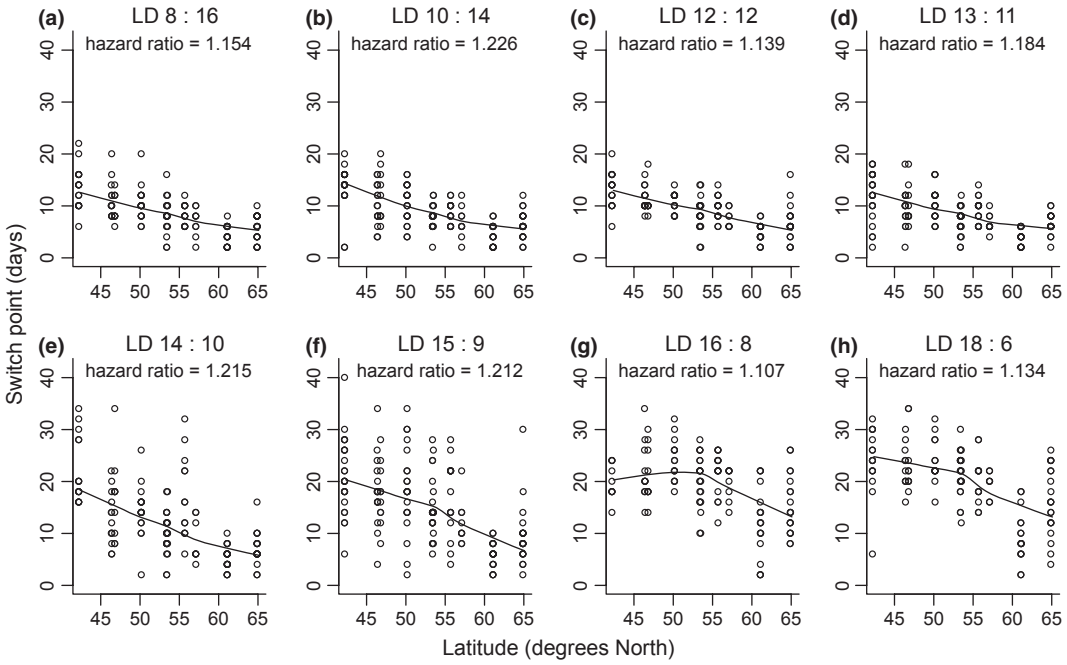

Fig. 3 Switch point for diapause induction of females from different latitudes exposed to different LD treatments. Maternal age at switch point is shown for those females that reached the switch. The fitted curves in each panel are computed by Loess smoothing. Hazard ratios obtained from the statistical model are given in each panel. The sample size is 21–26 individuals in each location and treatment.

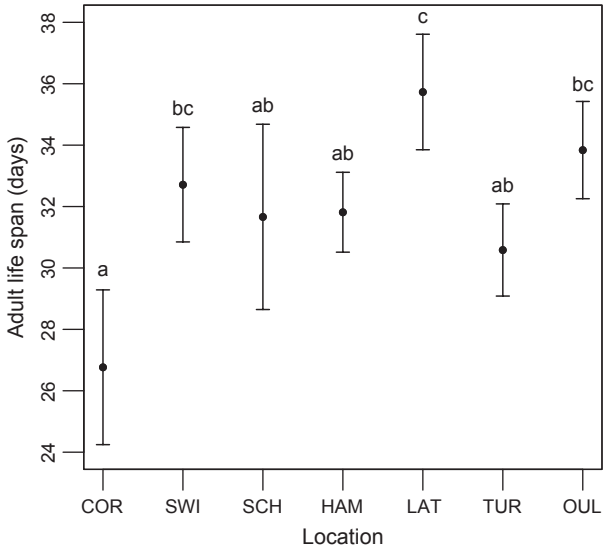

Fig. 4 Life span of females from the seven locations (mean ± SE). Data from different LD treatments are pooled (there is no significant effect of treatment), which increased the sample size in each location to 160–208 individuals per location. Letters indicate significant differences (*post hoc* multiple comparison for survival analysis, $P < 0.05$).

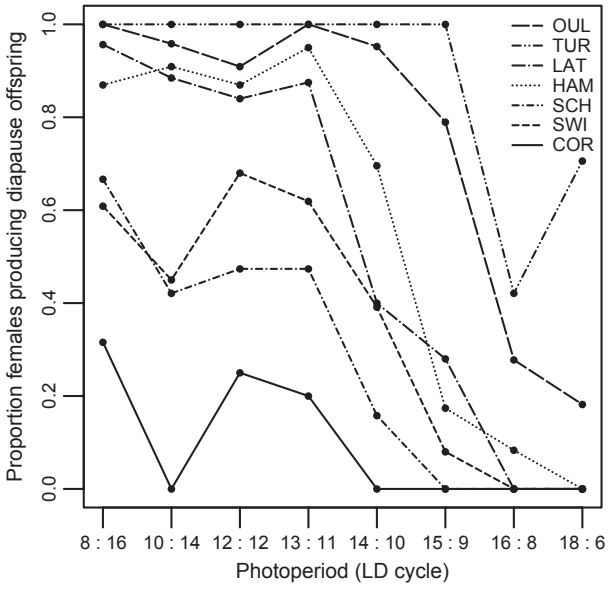

Fig. 5 Photoperiodic response curves for diapause induction in 10-day-old *Nasonia vitripennis* females from seven geographical locations. The sample size is 21–26 individuals in each location and treatment.

and LD regimes had an effect on diapause induction (mixed effect logistic regression model, effect of location: $\chi^2 = 140.06$, $P < 2.2e\text{-}16$; effect of treatment: $\chi^2 = 42.81$, $P = 3.64e\text{-}07$). Overall, diapause induction was high in populations from northern locations and decreased in more southern populations.

The photoperiodic response curves for the different populations have similar shapes reflecting high diapause incidence at short photoperiods, low incidence at long photoperiods and an abrupt change at intermediate photoperiods (in the range between LD 13 : 11 and 16 : 8). The response curve for the three most southern populations for LD 10 : 14 deviates from the expected trend and showed an unexpected low diapause response. We explain this as an experimental incubator effect, in which deviant temperature and/or humidity delayed diapause in the three most southern populations. As these populations generally have weaker response compared with northern populations their switch point may be more sensitive to small changes in environmental conditions.

The critical photoperiod was estimated for each population after fitting an incidence function model based on diapause incidence as function of photoperiod, excluding the LD 10 : 14 in all populations to obtain a better fit of the model. As not all populations reached 100% of diapause incidence under the experimental conditions at the chosen age point, the critical photoperiod was defined as the photoperiod at which diapause occurred in 50% of the females that produced diapausing offspring. The estimated critical photoperiods ranged from 10.5 (COR) to 18.0 h (TUR) and showed positive correlation with the mean latitude of population origin. This reflects a clear latitudinal cline for critical photoperiod inducing diapause in *N. vitripennis* (Fig. 6).

To confirm the conclusions for 10-day-old females, the critical photoperiod in each population was estimated using data from day 16. Figure 6 shows the linear regressions between latitude and estimated critical photoperiod using data from 10-day-old (linear regression: $F = 18.8$, $P = 0.007$, estimated slope: 0.286 ± 0.066, adjusted $R^2 = 0.75$) and 16-day-old females (linear regression: $F = 12.49$, $P = 0.016$, estimated slope: 0.25 ± 0.069, adjusted $R^2 = 0.66$). The diapause incidence for 16-day-old females reached 100% in all populations in short photoperiods and estimated critical photoperiods are longer for all populations. However, the same pattern as for 10-day-old females is evident, the critical photoperiod increases to the north.

The estimation of critical photoperiod for the TUR population was more complicated due to the high incidence of diapause in this population also at very long photoperiods (LD treatment 18 : 6). In this regard, the TUR population deviates substantially from the latitudinal cline showing a critical photoperiod longer than the OUL population.

Additional data on 'high diapause' northern lines

A number of isofemale lines from the most northern locations (OUL, TUR) could not be tested in the first experiment because they immediately produced diapausing larvae after collection. After emergence from diapause, females were exposed to five LD regimes for the first 20 days of their adult life. All surviving females in all treatments reached the switch point for diapause induction before day 20, most of them switched before day 10, indicating a very fast diapause response typical of northern populations. The switch point was not significantly different for the individuals from the two locations (survival analysis, Cox mixed effects model, effect of location: $\chi^2 = 1.00$, $P = 0.314$). The LD regime had a significant effect (survival analysis, Cox mixed effects model, effect of location: $\chi^2 = 13.06$, $P = 0.008$): the switch occurred later under longer photoperiods in accordance with the data for these populations from the previous experiment.

We estimated a new critical photoperiod for the most northern populations, combining data from 10 day-old-females from the first and the second experiment and re-calculated the linear regression between latitude and critical photoperiod to test for a latitudinal cline in critical photoperiod (linear regression: $F = 26.14$, $P = 0.004$, estimated slope: 0.314 ± 0.061, adjusted $R^2 = 0.81$) (Fig. 6). In the new data set, the estimated critical photoperiod of the OUL population was 16.9 h, about 0.9 h (54 min) longer than the critical photoperiod estimated with the first data set. This indicates that the OUL lines used in the first experiment likely constituted

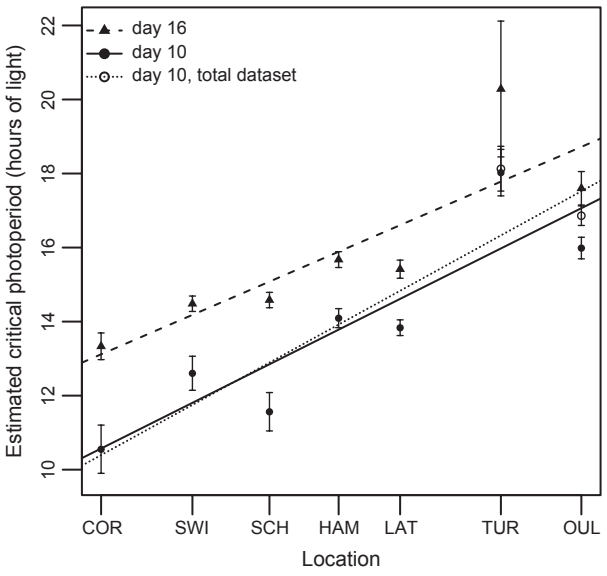

Fig. 6 Linear regressions between estimated critical photoperiod (±SE) and latitudinal origin of populations for 10- and 16-day-old females (sample size is 21–26 individuals in each location). The difference between the regression lines for 10-day-old females is due to the inclusion or exclusion of high diapause lines from the two northern populations (see text for details). When the high diapause lines are included the sample size for TUR is 27 and for OUL is 44 individuals. The seven locations are spaced along the *x*-axis according to the mean latitude (degrees) of origin.

a biased sample because the lines with highest proportion of diapausing offspring and long critical photoperiods were not yet included. The new estimated critical photoperiod for the TUR population did not differ from the one estimated using the first dataset and it was quite long, again consistent with a very northern type of diapause response.

Effect of laboratory culture on photoperiodic diapause induction

The photoperiodic response after maintenance in the lab for 5–6 generations and for 13–14 generations after field collection was compared to the initial response. After 13–14 generations of lab maintenance still a clear difference between locations was observed as well as the effect of treatment (mixed effect logistic regression model for data of 10-day-old females, effect of location: $\chi^2 = 115.66$, $P < 2.2e\text{-}16$; effect of treatment: $\chi^2 = 30.85$, $P < 3.29e\text{-}06$) (Fig. 7). The northern population (OUL) showed high diapause incidence and long critical photoperiod, the southern location (COR) low diapause incidence and very short critical photoperiod. The response of the HAM population was intermediate. The persistent variation in diapause shows that photoperiodic response was not affected by lab culturing (at least for 13–14 generations) (mixed effect logistic regression model for data of 10-day-old females, effect of lab maintenance: $\chi^2 = 3.65$, $P = 0.16$) and confirms that the variation is based on genetic differences.

Population structure and differentiation

The eleven microsatellites used for the population genetic analysis showed substantial polymorphism, allele numbers ranging from 10 (Nv303) to 27 (Nv319) (Table 2). Single-locus F_{ST} values (Weir & Cockerham, 1984) ranged from 0.025 (Nv205) to 0.152 (Nv322) with an average F_{ST} over all loci of 0.069 (95% confidence intervals: 0.049, 0.098 after bootstrapping over loci) (Table 2). Pairwise comparisons of F_{ST} values between locations revealed an appreciable level of genetic differentiation between populations (Table 3). The extreme southern and northern populations (COR, OUL) and the population from Latvia (LAT) are significantly different from all other populations, except for the comparison SWI-OUL.

The isolation by distance analysis (Mantel test for matrix correlation) showed a slightly significant correlation between pairwise F_{ST} and geographical distance ($R^2 = 0.153$, $P = 0.035$), that is mainly caused by the two extreme populations COR and OUL. When excluding either the COR or the OUL population, the correlation is not significant (data without COR: $R^2 = 0.01$, $P = 0.36$, data without OUL: $R^2 = 0.08$, $P = 0.18$).

Discussion

This study shows a latitudinal cline in three aspects of maternal photoperiodic diapause induction for field lines of *N. vitripennis*: the proportion of diapausing offspring, the switch point (photoperiodic counter) and the critical photoperiod (an expression of the photoperiodic timer). The observed variation indicates that photoperiod is an important environmental factor for diapause induction. The fact that the populations from different latitudes responded differently to the applied LD regimes indicates an important genetic component for diapause induction. Therefore, we conclude that maternal diapause induction in *N. vitripennis* is an evolutionary adaptive response to a periodically fluctuating environment for which photoperiod is one of the key cues.

In our study, we found a positive correlation between latitude and proportion of diapausing individuals. The observed pattern is likely due to differential

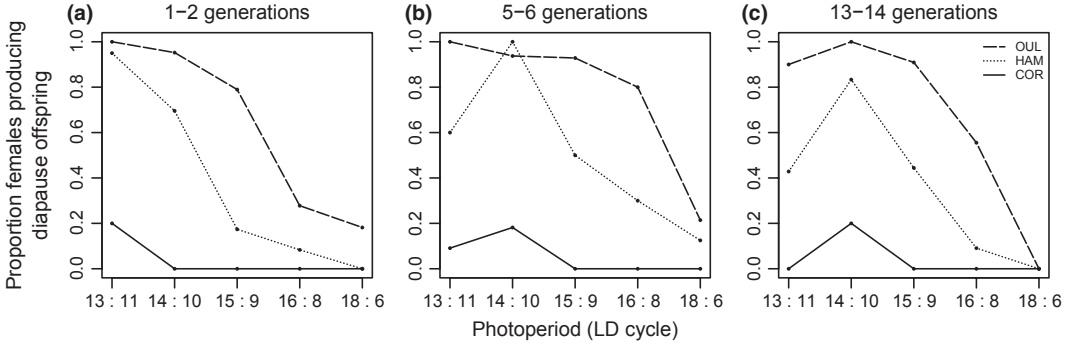

Fig. 7 Effect of laboratory culture on photoperiodic diapause induction in three populations (COR, HAM, OUL). Photoperiodic response curves are constructed with data from 10-day-old females which were selected from lines maintained in the laboratory for 1–2 generations after field collection (a), 5–6 generations (b) and 13–14 generations (c). The sample size is 10–16 individuals in each location and treatment.

Table 2 Population genetic data of the seven sampling locations. Shown are the number of alleles sampled, the expected heterozygosity (H_T; gene diversity) and single-locus F_{ST} values for each of 11 microsatellite markers. The bottom line averages values over all markers. Standard errors (SE) were obtained after jackknifing over populations or loci. *n* = sample size (individuals genotyped).

Marker	No. alleles	H_T COR (*n* = 18)	H_T SWI (*n* = 18)	H_T SCH (*n* = 15)	H_T HAM (*n* = 17)	H_T LAT (*n* = 20)	H_T TUR (*n* = 22)	H_T OUL (*n* = 26)	F_{ST} ± SE
Nv26	18	0.455	0.885	0.783	0.888	0.833	0.918	0.779	0.093 ± 0.052
Nv107	23	0.894	0.91	0.896	0.89	0.936	0.916	0.743	0.041 ± 0.026
Nv118	16	0.898	0.905	0.814	0.852	0.917	0.857	0.797	0.062 ± 0.017
Nv200	18	0.858	0.89	0.86	0.912	0.882	0.923	0.696	0.061 ± 0.035
Nv205	22	0.946	0.902	0.913	0.933	0.872	0.913	0.792	0.025 ± 0.021
Nv229	25	0.857	0.909	0.807	0.91	0.896	0.945	0.825	0.072 ± 0.024
Nv301	19	0.936	0.932	0.859	0.841	0.79	0.94	0.74	0.059 ± 0.028
Nv303	10	0.625	0.773	0.58	0.5	0.561	0.687	0.742	0.113 ± 0.055
Nv319	27	0.833	0.905	0.792	0.917	0.898	0.945	0.869	0.076 ± 0.019
Nv320	19	0.9	0.897	0.868	0.877	0.837	0.832	0.887	0.029 ± 0.009
Nv322	12	0.512	0.801	0.814	0.728	0.721	0.818	0.688	0.152 ± 0.079
Average	19	0.792	0.883	0.817	0.841	0.831	0.881	0.778	0.069 ± 0.011 over loci

Table 3 Pairwise F_{ST} values between European populations for all loci combined.

	COR	SWI	SCH	HAM	LAT	TUR	OUL
COR	–	0.0821*	0.1022*	0.1074*	0.1089*	0.089*	0.1541*
SWI		–	0.0135	0.0141	0.0369*	0.0093	0.0438
SCH			–	0.0339	0.0873*	0.0416	0.0889*
HAM				–	0.0665*	0.0389*	0.0645*
LAT					–	0.0388*	0.0856*
TUR						–	0.0743*
OUL							–

*Significant values at the nominal level of 5% after Bonferroni correction following G-statistics (as implemented in Fstat).

adaptation to local environments as a result of natural selection. In northern regions, winters are characterized by severe climatic conditions that do not allow normal development and reproduction, while in southern regions winters are mild and might allow survival without diapause. Thus, selection for diapause response in the North is expected to be stronger than in the South resulting in a higher proportion of diapausing individuals. A similar latitudinal cline has been previously described in other species (Schmidt *et al.*, 2005; Scharf *et al.*, 2010; Leisnham *et al.*, 2011). For example, in *D. melanogaster*, the incidence of adult diapause was positively correlated with latitude in populations in Eastern North America (Schmidt *et al.*, 2005).

We also found a positive correlation between latitude and critical photoperiod. Together with the results of latitudinal variation in the proportion of diapausing individuals discussed above, this cline in critical photoperiod provides strong indications for adaptive evolution of diapause in *N. vitripennis* and stresses the importance of photoperiod as the main cue for approaching seasonal change. As northern winters arrive earlier in the year compared with southern ones, populations at high latitudes have to be able to respond swifter to longer photoperiod than southern ones for a timely induction of diapause. The same type of cline was observed for natural populations of the pitcher plant mosquito *Wyeomyia smithii*, for which critical photoperiod also varied along a latitudinal gradient in North America (Bradshaw & Lounibos, 1977). Similar results were obtained in species with diapause at different developmental stages like *Drosophila montana* which possesses an adult reproductive diapause (Tyukmaeva *et al.*, 2011) and the butterfly *Sericinus montelus* which has a pupal diapause (Wang *et al.*, 2011). The estimated critical photoperiod could be used as an indication for the time of the year during which populations start producing diapause offspring. In our case, the long critical photoperiod estimated for OUL occurs approximately on August 11th, whereas the short critical photoperiod of COR corresponds to October 24th. This reflects a difference in timing of diapause induction according to local seasonality. However, different environmental factors play a role in diapause response in nature and may modify the date at which it is beneficial to start producing diapause offspring.

A central aspect of photoperiodic diapause response in *N. vitripennis* is the switch point, measured as the maternal age at which the adult female switches to the production of diapausing offspring. The switch point

corresponds to the number of inductive LD cycles accumulated during the sensitive stage necessary for inducing the diapause response and it varies according to latitude and photoperiod. The early switch of northern populations in short photoperiods might represent an evolutionary adaptation to the very rapid seasonal change characteristic of high latitudes. Individuals in these environments respond fast enough to photoperiodic change to be able to produce diapausing offspring that will survive winter conditions. Accordingly, southern females inhabit environments characterized by a gradual seasonal change and have a late switch point which allows the production of normal developing offspring during the major part of adult life. As the environmental conditions are generally favourable for a long period, the chance that the offspring will survive and reproduce within the same season is high. Field experiments on induction of diapause could provide information on the voltinism of *N. vitripennis* in different localities, necessary for understanding the importance of latitudinal variation in switch point.

Photoperiodic diapause response in *N. vitripennis* is a threshold trait whose phenotypic expression is discontinuous and the two phenotypic classes can easily be recognized: in every oviposition event, diapausing and nondiapausing broods can be clearly distinguished and mixed broods are rare, indicating a fast physiological change. The adult female is sensitive to photoperiodic cycles and accumulates the stimuli every day until a specific threshold level is reached and the physiological cascade that leads to the diapause response is activated. The threshold level that activates diapause response depends on the type of stimuli and on the origin of the line. We could rule out an effect of senescence on variation in switch point, as described by Reznik *et al.* (2002) and Yang *et al.* (2007), by showing that life span does not vary under different photoperiodic conditions. In addition, the switch to diapause induction represents the manner in which day length expresses its effect on diapause production. Nonetheless, the strong effect of photoperiod could mask a possible effect of ageing which (if present) could be investigated under constant light in populations from different latitudes.

The described variation in switch point indicates that in *Nasonia*, clinal adaptation applies to a cue perceived during the sensitive stage (the mother) that is being effectuated in the next generation. In evolutionary terms, natural selection operates on the threshold number of photoperiodic cycles required to elicit the diapause response and generates the observed latitudinal clines as a consequence of the environmental cycles and photoperiods that characterize each latitude.

Our results are consistent with those of Saunders who identified photoperiod as the main diapause-inducing factor in laboratory lines of *Nasonia* (Saunders, 1966). In his pioneering studies, Saunders also observed a difference in diapause response in two lab strains originated from two geographical locations. However, his studies on diapause induction in *N. vitripennis* did not consider natural variation in diapause response. Therefore, our work complements the studies of Saunders by demonstrating for the first time the importance of adaptive photoperiodic response in diapause induction in *N. vitripennis* in nature. We further showed that natural selection generates the clinal variation through its action on the threshold level of LD cycles required for diapause induction. Following the proposal of Saunders (2002) to divide photoperiodism into two components, timer and counter, we observed that both components show a latitudinal cline by themselves and are correlated. Interestingly, the relationship between timer and counter was consistent over the different latitudes indicating that the photoperiodic machinery becomes increasingly sensitive to short days (or long nights) towards high latitudes. Short photoperiods, measured by the counter, trigger an earlier switch point in northern populations compared with southern ones. This shows the interrelationship of the timer-counter system and may indicate an overlapping genetic architecture, perhaps involving the circadian clock genes.

Interestingly, we neither found a line completely lacking the ability to undergo diapause nor one that entered into obligate diapause at every generation regardless of environmental conditions. This observation confirms that the difference in diapause response between the lines is based on variation in sensitivity to photoperiod, and not a developmentally fixed pattern to undergo diapause *per se*. However, it cannot be excluded that strains exist which do not possess a photoperiodic diapause, for example, at latitudes lower than our southernmost location where photoperiod may not represent a good cue for seasonal change. Moreover, some strains with obligate diapause could be present at very high latitudes outside the studied range, where extremely short summers and rapid seasonal change do not allow more than one generation per year. During our fieldwork, we were able to collect a single individual in a location in a very northern latitude (Finland, Kilpisjärvi, 69°2′39.44″N, 20°48′11.88″E). This female was used to establish an isofemale line but only produced diapausing larvae and could not be included in our experiments. This shows that *N. vitripennis* has extended its geographical range to extreme latitudes making it a cosmopolitan species which has adapted to many different climatic conditions. *N. vitripennis* represents therefore a powerful model to investigate the genetic basis of variation in diapause and other adaptive traits.

Population differentiation analysis using pairwise F_{ST} comparisons based on microsatellite markers showed an appreciable level of genetic differentiation between some of the tested populations. Previous studies indicated that gene flow between *N. vitripennis* populations in North America is high within a range of about

100 km and limited between populations that are separated by 300 km or larger distances, particularly if large bodies of water or mountain ranges are present between the locations (Grillenberger *et al.*, 2009). In our study, the F_{ST} values measured between distant locations are similar to the ones previously reported for North American populations confirming that gene flow is restricted. Studies in other insect species demonstrated that local adaptation can lead to clinal variation in different phenotypic traits despite a high level of gene flow measured with neutral genetic markers (Demont *et al.*, 2008; Sarup *et al.*, 2009; Tyukmaeva *et al.*, 2011). Comparisons of populations at similar geographical distances show that our measured F_{ST} values are generally larger than those of the dung fly *Scatophaga stercoraria* and the northern malt fly *D. montana*. For example, the F_{ST} value for yellow dung fly populations from Finland and Germany (about 1500 km) was 0.011 (Demont *et al.*, 2008), whereas the value for our OUL and HAM populations, at similar distance, was 0.064. Similarly, F_{ST} values between populations of *D. montana* in Finland were negligible in all pairwise comparisons, including the most southern and northern populations (845 km) that have F_{ST} value of 0.006 (Tyukmaeva *et al.*, 2011), whereas in our case the F_{ST} value between TUR and OUL (450 km) was 0.07. The difference between flies and *Nasonia* is likely caused by the subdivided population structure of *Nasonia* due to a patchy distribution of its host (Grillenberger *et al.*, 2008) and the inability of the flightless males to disperse.

The measured genetic differentiation does not correlate entirely with the latitudinal cline for diapause response and the overall isolation by distance effect was mainly due to the two most distant populations. Hence, the observed geographical variation in diapause appears to be in a selection – migration balance rather than due to neutral processes (e.g. genetic drift) (Leinonen *et al.*, 2008). When more information is available about the genetic basis of diapause in *N. vitripennis*, population genomic studies may reveal signatures of selection in the genes involved. The reported natural variation in photoperiodic diapause combined with the genomic tools available for this species offers an excellent opportunity to investigate the genetic basis of diapause and its adaptive variation.

In conclusion, the present work represents the first study on variation of photoperiodic diapause induction in *N. vitripennis* in relation to natural seasonal cycles in different geographical locations and provides indications for adaptive evolution of this trait. The importance of the switch point for diapause induction in the establishment of the latitudinal cline indicates that future studies aiming to identify the genetic basis of diapause variation should focus on the sensitive maternal stage, on the perception of photoperiodic cues and on processing of the information derived from these cues.

Acknowledgments

This study was funded by the Marie Curie Initial Training Network SPECIATION 'Understanding the evolutionary origin of biological diversity'. We thank Maaria Kankare and all participants in the network for helpful and stimulating discussions. The field work was supported by a grant from LAPBIAT2 (Finland) and from the Schure-Beijerinck-Popping Funds (The Netherlands). We thank the people who were involved during the field work for giving us the opportunity to visit their nest boxes or collecting nest material for us: Antero Järvinen, Markku Orell, Tapio Eeva, Miia Koivula, Indrikis Krams, Mikus Abolins-Abols, Ralph Peters, Karl-Heinz Schmidt, Philippe Christe, Fabrice Helfenstein, Bruno Massa, Saleta Perez-Vila, Wenwen Diao and Sjors Beukeboom. We also thank Joop Prijs, Anna Rensink, Rogier Houwerzijl for taking care of the field material upon arrival in Groningen, the members of the Evolutionary Genetics Group at Groningen University for help in the laboratory and for useful discussions on statistical analysis of the data and two anonymous reviewers for helpful comments on a previous version of the manuscript.

References

Bale, J.S. & Hayward, S.A. 2010. Insect overwintering in a climate change. *J. Exp. Biol.* **213**: 980–994.

Beukeboom, L.W. & Desplan, C. 2003. Quick guide. *Nasonia*. *Curr. Biol.* **13**: R860–R860.

Beukeboom, L.W., Niehuis, O., Pannebakker, B.A., Koevoets, T., Gibson, J.D., Shuker, D.M. *et al.* 2010. A comparison of recombination frequencies in intraspecific versus interspecific mapping populations of *Nasonia*. *Heredity* **104**: 302–309.

Bradshaw, W.E. & Lounibos, L.P. 1977. Evolution of dormancy and its photoperiodic control in pitcher-plant mosquitoes. *Evolution* **31**: 546–567.

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

Darling, D.C. & Werren, J.H. 1990. Biosystematics of *Nasonia* (Hymenoptera, Pteromalidae): two new species reared from birds' nests in North-America. *Ann. Entomol. Soc. A.* **83**: 352–370.

Demont, M., Blanckenhorn, W.U., Hosken, D.J. & Garner, T.W. 2008. Molecular and quantitative genetic differentiation across Europe in yellow dung flies. *J. Evol. Biol.* **21**: 1492–1503.

Emerson, K.J., Bradshaw, W.E. & Holzapfel, C.M. 2009. Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. *Trends Genet.* **25**: 217–225.

Gienapp, P., Teplitsky, C., Alho, J.S., Mills, J.A. & Merilä, J. 2008. Climate change and evolution: disentangling environmental and genetic responses. *Mol. Ecol.* **17**: 167–178.

Goudet, J. 2001. *FSTAT, A Program to Estimate and Test Gene Diversities and Fixation Indices*. [2.9.3], Available at http://www2.unil.ch/popgen/softwares/fstat.htm.

Grillenberger, B.K., Koevoets, T., Burton-Chellew, M.N., Sykes, E.M., Shuker, D.M., Van de Zande, L. *et al.* 2008. Genetic structure of natural *Nasonia vitripennis* populations:

validating assumptions of sex-ratio theory. *Mol. Ecol.* **17**: 2854–2864.

Grillenberger, B.K., Gadau, J., Bijlsma, R., Van de Zande, L. & Beukeboom, L.W. 2009. Female dispersal and isolation-by-distance of Nasonia vitripennis populations in a local mate competition context. *Entomol. Exp. Appl.* **132**: 147–154.

Hoffmann, A.A. & Sgrò, C.M. 2011. Climate change and evolutionary adaptation. *Nature* **470**: 479–485.

Kurota, H. & Shimada, M. 2003. Geographical variation in photoperiodic induction of larval diapause in the bruchid beetle, *Bruchidius dorsalis*: polymorphism in overwintering stages. *Entomol. Exp. Appl.* **107**: 11–18.

Leinonen, T., O'Hara, R.B., Cano, J.M. & Merilä, J. 2008. Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. *J. Evol. Biol.* **21**: 1–17.

Leisnham, P.T., Towler, L. & Juliano, S.A. 2011. Geographic variation of photoperiodic diapause but not adult survival or reproduction of the invasive mosquito *Aedes albopictus* (Diptera: Culicidae) in North America. *Ann. Entomol. Soc. Am.* **104**: 1309–1318.

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

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY, USA.

Nelson, R.J., Denlinger, D.L. & Somers, D.E. 2010. *Photoperiodism. The Biological Calendar*. Oxford University Press, USA, New York.

Pannebakker, B.A., Niehuis, O., Hedley, A., Gadau, J. & Shuker, D.M. 2010. The distribution of microsatellites in the *Nasonia* parasitoid wasp genome. *Insect Mol. Biol.* **19**(Suppl. 1): 91–98.

R Development Core Team 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, [www document]. URL http://www.R-project.org.

Reznik, S.Y., Kats, T.S., Umarova, T.Y. & Voinovich, N.D. 2002. Maternal age and endogenous variation in maternal influence on photoperiodic response in the progeny diapause in *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae). *Eur. J. Entomol.* **99**: 175–179.

Sarup, P., Frydenberg, J. & Loeschcke, V. 2009. Local adaptation of stress related traits in *Drosophila buzzatii* and *Drosophila simulans* in spite of high gene flow. *J. Evol. Biol.* **22**: 1111–1122.

Saunders, D.S. 1962. The effect of the age of female *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae) upon the incidence of larval diapause. *J. Insect Physiol.* **8**: 309–318.

Saunders, D.S. 1965. Larval diapause of maternal origin: induction of diapause in *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae). *J. Exp. Biol.* **42**: 495–508.

Saunders, D.S. 1966. Larval diapause of maternal origin II: the effect of photoperiod and temperaure on *Nasonia vitripennis*. *J. Insect Physiol.* **12**: 569–581.

Saunders, D.S. 2002. *Insect Clocks*, 3rd edn. Elsevier, Amsterdam.

Scharf, I., Bauerfeind, S.S., Blanckenhorn, W.U. & Schäfer, M.A. 2010. Effects of maternal and offspring conditions on growth, development and diapause in latitudinal yellow dung fly populations. *Clim. Res.* **43**: 115–125.

Schiesari, L., Kyriacou, C.P. & Costa, R. 2011. The hormonal and circadian basis for insect photoperiodic timing. *FEBS Lett.* **585**: 1450–1460.

Schmidt, P.S., Matzkin, L., Ippolito, M. & Eanes, W.F. 2005. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. *Evolution* **59**: 1721–1732.

Tauber, M.J., Tauber, C.A. & Masaki, S. 1986. *Seasonal Adaptations of Insects*. Oxford University Press, USA.

Tyukmaeva, V.I., Salminen, T.S., Kankare, M., Knott, K.E. & Hoikkala, A. 2011. Adaptation to a seasonally varying environment: a strong latitudinal cline in reproductive diapause combined with high gene flow in *Drosophila montana*. *Ecol. Evol.* **1**: 160–168.

Wang, X.P., Yang, Q.S., Dalin, P., Zhou, X.M., Luo, Z.W. & Lei, C.L. 2011. Geographic variation in photoperiodic diapause induction and diapause intensity in *Sericinus montelus* (Lepidoptera: Papilionidae). *Insect Sci.* **19**: 295–302.

Weir, B.S. & Cockerham, C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Werren, J.H., Richards, S., Desjardins, C.A., Niehuis, O., Gadau, J., Colbourne, J.K. *et al.* 2010. Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science* **327**: 343–348.

Yang, D., Lai, X.T., Sun, L. & Xue, F.S. 2007. Parental effects: physiological age, mating pattern, and diapause duration on diapause incidence of progeny in the cabbage beetle, *Colaphellus bowringi* Baly (Coleoptera: Chrysomelidae). *J. Insect Physiol.* **53**: 900–908.

Received 9 October 2012; accepted 14 December 2012