TITLE: Sex-linked inheritance of diapause in the Green-veined White, Pieris napi

RUNNING TITLE: Sex-linked inheritance of diapause

Peter Pruisscher¹; Helena Larsdotter-Mellström¹,²; Sören Nylin¹; Christopher W. Wheat¹; and Karl Gotthard¹

₁: Department of Zoology, Stockholm University, Svante Arrhenius väg 18b, S-106 91 Stockholm, Sweden
₂: Centre for Evolutionary Biology, The University of Western Australia (M092), 35 Stirling Highway, Crawley WA 6009, Australia

peter.pruisscher@zoologi.su.se
helena.mellstrom@uwa.edu.au
soren.nylin@zoologi.su.se
chris.wheat@zoologi.su.se
karl.gotthard@zoologi.su.se

Corresponding author: Peter Pruisscher, Department of Zoology, Stockholm University, Svante Arrhenius väg 18b, S-106 91 Stockholm, Sweden, peter.pruisscher@zoologi.su.se, orcid.org/0000-0002-6987-5839
ABSTRACT (max 250 words)

Many temperate insects survive harsh environmental conditions, such as winter, by entering a state of developmental arrest. This diapause state is predominantly induced by photoperiod. The photoperiod varies with latitude and has led to local adaptation in the photoperiodic induction of diapause in many insects. In order to understand the rapid evolution of the photoperiodic threshold it is important to investigate and understand the underlying genetic mechanisms. In this paper the genetic basis of photoperiodic diapause induction is investigated in the Green-veined White butterfly *Pieris napi*, by assaying diapause induction in a range of conditions for a Swedish and Spanish population. Furthermore, the inheritance of diapause induction was assessed in reciprocal F1 and F2 hybrid crosses between the two populations. The southern population showed a highly dynamic response to photoperiod, while the northern populations showed a high incidence of diapause, regardless of photoperiod. The hybrid crosses revealed a very strongly sex-linked decision of diapause, and a diapause incidence that was highly dependent on photoperiod, revealing the importance of assaying a range of conditions in diapause inheritance studies. The results indicate a strongly heritable diapause induction with a major component on the Z-chromosome, as well as a minor effect of the autosomal background.

Keywords (max 6): photoperiodic response, diapause induction, inheritance, Lepidoptera, sex-linked

INTRODUCTION (Full paper max 7500 words)

Life cycle regulation is vital for organisms that occur in seasonal habitats. In temperate areas, organisms that do not migrate must possess adaptations that allow them to survive long periods of harsh conditions such as winter. Most temperate insects persist under adverse conditions by entering a state of developmental arrest called diapause, which is associated with physiological changes related to accumulation of energy and increased cold tolerance (Tauber & Tauber 1976; Tauber et al. 1986; Pullin et al. 1991; Koštál 2006; Hahn & Denlinger 2010). In many species diapause is induced at a given life stage regardless of environmental input (Tauber et al. 1986) and this obligate diapause typically results in a life cycle with one generation per year. However, it is also very common that temperate insects have a facultative diapause that allows them to produce several directly developing generation per year with only the last generation entering diapause before the arrival of harsh conditions (Lees 1955). In these insects, diapause is an alternative developmental pathway that is initiated in response to seasonal cues in advance of the onset of adverse conditions, the most common of which are temperature and photoperiod (Hahn & Denlinger 2010). This plastic induction of alternative developmental pathways represents an important life history adaptation in temperate insects.
Photoperiod, defined as the hours of light in a day, is a reliable environmental cue that is consistent between seasons and years, and consequently is the predominant cue for initiating diapause in temperate environments (Tauber et al. 1986). The photoperiod varies with latitude and this has led to local adaptation in the photoperiodic induction of diapause (Bradshaw 1976). Moreover, as the length of the favourable season typically is decreasing from south to north many species show latitudinal variation in the number of generation produced per year, expressed as variation in voltinism (Levy et al. 2015). These two factors lead to variation in the photoperiodic threshold along latitudes, most notably for species in which diapause is facultative. Common garden laboratory studies have demonstrated that these population differences in voltinism typically are due to heritable variation in the photoperiodic threshold for diapause induction (Bradshaw 1976; Kimura 1988; Hahn & Denlinger 2010; Paolucci et al. 2013; Levy et al. 2015; Aalberg Haugen & Gotthard 2015).

Diapause induction exhibits strong patterns of local adaptation and appears to evolve rapidly in response to climate change (Bradshaw & Holzapfel 2001), and in invasive species that experience novel conditions (Gomi 2007; Urbanski et al. 2012). This rapid response is a consequence of strong selection on life cycle regulation, but may also be influenced by the explicit genetic architecture of the trait. Evolution of a trait can be governed by an oligogenic architecture where selection acts on few genes with large effect sizes (Roff 1996), or alternatively a polygenic architecture with an infinitesimal number of genes of small effect sizes. As the photoperiodic threshold in diapause induction in general seem to follow a continuous gradient over latitude, it is possible that the latter architecture is most likely for diapause induction. Furthermore, the location of the loci on the genome has an effect; selection is able to act more strongly on beneficial mutations occurring on sex-chromosomes, as recessive mutations are exposed to selection in the heterogametic sex in comparison to recessive loci residing on autosomes, where they remain hidden from selection unless two parents carrying a recessive locus pass it on to their offspring (Charlesworth et al. 1987). Thus, selection can act upon sex-linked variation faster than the autosome, and this faster X-effect of sex-linked mutations allows for them to spread through a population faster than mutations present on autosomes, causing potentially rapid evolution of sex-linked traits and population divergence (Meisel & Connallon 2013). As many traits are disproportionally sex-linked in Lepidoptera (Sperling 1994), this can be a potentially powerful force in shaping the genetic architecture of diapause induction. But, in order to understand the rapid evolution of the photoperiodic threshold it is important to investigate and understand the underlying genetic mechanisms.
The inheritance of diapause induction has been studied in many species through reciprocal crossing of divergent populations and species in an attempt to understand its underlying genetic architecture. Evidence from crosses suggests different genetic architectures responsible for adaptive clines in photoperiodic diapause induction, expressed as single locus Mendelian inheritance (Doležel et al. 2005; Suwa & Gotoh 2006; Han & Denlinger 2009; Kawakami et al. 2010), polygenic or oligogenic inheritance with incomplete dominance (McCoy et al. 1968; Kurahashi & Ohtaki 1977; Sims 1983; Söderlind & Nylin 2011; Raina et al. 2011; Xia et al. 2012; Chen et al. 2012; Lehmann et al. 2016), and in several species there is evidence of oligogenic inheritance with a clear sex-linked component (Lumme & Keränen 1978; Kimura & Yoshida 1995; Ikten et al. 2011; Chen et al. 2014). The inheritance of diapause induction can also be modified by other components, such as maternal effects (McWatters & Saunders 1997). Recent papers have also revealed strong gene by environment interaction effects, where inheritance patterns and dominance are dependent on which photoperiod insects are reared in (Fu et al. 2015; Xiao et al. 2015). Fu et al. (2015) argue this would ensure that the right developmental pathway is chosen even if there would be gene flow between populations. The different modes of inheritance of diapause induction show the complexity and the need for further study to understand this phenotype.

In this study we investigate the inheritance of diapause induction in the Green-veined White butterfly Pieris napi (Lepidoptera, Pieridae), which enters diapause in the pupal stage and uses photoperiod as the predominant cue for regulating diapause induction (Lees & Archer 1980; Hahn & Denlinger 2010; Kivelä et al. 2015). Using two distinct populations, one that facultatively induces diapause to produce between two and three generations per year, and one northern population that is univoltine and shows no direct development in the field, we aimed to answer two questions. First, what is the photoperiodic response of these natural populations? To answer this, we characterized the diapause incidence of these two populations under different photoperiods in order to document the natural variation that is present in this trait, and to investigate whether the univoltine population has retained the capacity to develop directly. Second, what is the mode of inheritance of diapause induction? To address this, we generated reciprocal F1 hybrid crosses and assayed them for their diapause incidence under different environmental conditions to study the mode of inheritance. In addition, we generated four F2 hybrid backcrosses where F1 hybrids were crossed back to the northern population, and assayed at a single condition to further investigate the inheritance of diapause induction.

METHODS
In short, wild-caught Abisko, Sweden (NxN) and Barcelona, Spain (SxS) populations were reared under six different photoperiods to assay diapause incidences in 2013. An independent set of Abisko individuals was reared into diapause and crossed with a newly caught population sample of Barcelona in 2014 to generate F1 hybrids under two environmental conditions, as well as produce another generation of within-population samples. In 2015, the F1 hybrids and pure population samples were used to generate F2 backcrosses, as well as pure population controls. Furthermore, an independent set of F1 hybrids was generated in two environmental conditions from newly caught Barcelona and Abisko individuals to further characterize the diapause response. The experimental setup is described in detail below.

Mated *P. napi* adult females were collected in summer 2013 from Spain (Barcelona, 41°N, hereafter designated as southern), and northern Sweden (two adjacent localities Abisko and Kiruna, 68°N, hereafter designated as northern or Abisko). Females were kept individually in one-litre cups where they were allowed to lay eggs on *Alliaria petiolata*. The southern population was caught earlier in the season and was reared into direct development for one generation in order to synchronize with the northern population. Offspring of both populations were fed a mixture of wild *A. petiolata* and *Armoracia rusticana* and randomly assigned to one of six treatments of LD 8:16, LD 11:13, LD 14:10, LD 17:7, LD 20:4, or LD 23:1 at 20°C to estimate the effect of photoperiod on diapause incidence. Total sample and family sizes are recorded in Table 1.

An independent sample of northern females (Abisko, Sweden 68°N) was also collected in 2013, and again from the southern population (Barcelona, Spain, 41°N) in summer 2014. Eggs and offspring were generated as described above. Larvae of the 2013 northern population were reared under continuous light (LD 24:0, 23°C) and individuals that entered diapause were stored in cold conditions until the following year to synchronize with the 2014 southern individuals. This design created one generation of selection for high diapause incidence in order to reduce polymorphism in the diapause response of the northern population used in the subsequent crosses, as the initial survey of the pure populations showed some incidence of direct development under these extreme conditions in the northern population (~25%, data not shown).

Offspring of each line were crossed to generate southern SxS (crosses are designated female first, then male) and northern NxN offspring and reared under LD 8:16 at 20°C for use in subsequent crosses. Adults were also used to generate two types of F1 hybrids: SxN and NxS. Hybrid offspring were reared under a split brood design, where half of the individuals were
reared for assessing diapause induction under LD 23:1 and 20°C, and the other half were reared under LD 8:16 and 17°C to ensure diapause development. Preliminary analysis showed a very asymmetric response on these F1 hybrids and indicated the need for an extra experiment to properly test any Z-linked influence on diapause induction. Adult females were therefore collected again from the same populations in summer 2015, and their offspring were reared into diapause under LD 8:16, 17°C. In the following spring SxN and NxS hybrids were generated and reared under LD 18:6, 23°C, as well as LD 12:12, 17°C. These conditions were selected to further explore in which conditions offspring from the crosses entered diapause.

Individuals from the initial F1 cross in LD 8:16 at 20°C were taken out of diapause conditions following spring 2015. Eclosing adults were mated in order to generate four reciprocal F2 backcrosses to the northern population (NxNS, NSxN, NxSN and SNxN) while also generating SxS and NxN crosses to control for rearing effects. Larvae were reared in groups of ± 100 individuals synchronized by two laying days in L40xW30xH50 cm Pyjama cages, while being fed ad-lib A. petiolata and A. rusticana. Families within and between crosses were randomly distributed in cages placed in a climate controlled room at LD 23:1 and 20°C. In Lepidoptera, females are the heterogametic sex and females exhibit no recombination (Traut & Marec 1997; Sahara et al. 2012). Therefore, it is only in crosses with male hybrids (i.e. NxNS and NxSN) that there will be recombination between the northern and southern chromosomes and any potential Z-linked effects will be most apparent, allowing an assessment of whether diapause incidence is inherited in a sex-linked manner or governed by additive autosomal elements. Consequently, sample sizes were doubled in the NxNS and NxSN crosses compared with cross with female hybrids, i.e. NSxN and SNxN. For this and all other experiments described here developmental pathway (direct or diapause) was recorded for each individual as well as family of origin. All cups and cages were checked daily, with newly pupated individuals collected and placed in individual cups with a unique identifier. The directly developing individuals eclosed within 2-3 weeks in their individual cups, while individuals that did not show any signs of development after three weeks were deemed to be in diapause. Total sample and family sizes for the F1 and F2 crosses are recorded in Table 2.

All statistical tests were performed using JMP v12.01 (SAS). To compare proportions of diapausing versus direct developing individuals in the pure populations, a generalized linear mixed model was used, assuming a binomial distribution (diapause or not), and a logit link function. Photoperiod, population and their interaction were used as explanatory variables. Family was added as random factor.
For the F1 hybrid crosses diapause incidence at each photoperiod was compared to expected diapause incidences under a Z-linked as well as autosomal additive genetic architecture of diapause induction. No dominance was assumed. Estimates were derived from the pure populations and compared by Fisher’s exact test. If the diapause decision is completely sex-linked, the hemizygous females in the F1 hybrids should show a developmental choice in concordance with the paternal population of origin, while the males heterozygous for the Z-chromosome should show an intermediate response. In the case of additive autosomal inheritance, all hybrid individuals are expected to behave similarly and intermediate to the pure populations. The F1 hybrids reared under LD 18:6 did not have a corresponding sampling point in the pure populations and instead was compared to the average between the LD 17:7 and 20:4 points.

To test whether diapause incidences between the F2 crosses were different from each other, a generalized linear mixed model was used, assuming a binomial distribution (diapause or not), and a logit link function. Sex, Cross, and their interaction were used as explanatory variables, while Family and Cage number were added as random factors. To further test the mode of inheritance of diapause induction in the F2 crosses, Fisher’s Exact tests were used to compare the observed diapause incidences to expected diapause incidences derived from the simultaneously reared pure populations. Again, we tested any deviations from expectation of a completely Z-linked, as well as a completely additive autosomal inheritance pattern. The expected incidence was derived from the observed diapause incidence in the pure populations that were reared at the same time. For the females, who only have one copy of the Z-chromosome, an individual with a Southern Z chromosome was expected to show 100% direct development, while a female with a Northern Z chromosome was expected to yield a 90% probability to go into diapause. The males have two copies of the Z chromosome, and if individuals had two southern copies of the Z-chromosome the expectation was 100% direct development. When males had two copies of the northern Z-chromosome, they were expected to have an 87% probability of entering diapause. Males that were heterozygous for the Z chromosome were expected to show a diapause development probability of \((87+0)/2=43.5\%\), when the effect is additive, and no dominance is assumed. Numbers were rounded up to whole integers for the chi-square test. The autosomal background in the crosses is expected on average to be 75% northern, and 25% southern. Under the assumption that the southern genotype leads to direct development, and the northern to 87%-90% diapause incidence, the expected diapause incidence is 67.5% in the females, and 65% diapause for the males, and no difference in proportion between the crosses. The different reciprocal F2 backcrosses will give further insight into sex-linkage, as the average autosomal backgrounds of the F2 backcrosses...
are identical, but interact with different proportions of northern and southern sex-chromosomes.

RESULTS
In order to understand the inheritance of diapause, we started by quantifying the natural variation of photoperiodic induction in two natural populations of *P. napi*. Diapause incidence was characterized for six different photoperiods at 20°C, in which the northern population (Abisko, Sweden) showed a >90% incidence of diapause in all of the photoperiods examined (Figure 1A). Diapause incidences for the southern population (Barcelona, Spain) differed depending on photoperiod, showing an 82% diapause incidence at LD 8:16, with a steep decrease until LD 14:10 where there was >85% direct development (Figure 1A). This was corroborated by a GLMM, showing a significant effect of Population, Photoperiod, and their interaction. (Full Model: $\chi^2=328.34$, DF=3, p>0.001; Population: $\chi^2=36.83$, DF=1, p<0.001; Photoperiod: $\chi^2=242.51$, DF=1, p<0.001; Population*Photoperiod: $\chi^2=10.51$, DF=1, p=0.001).

Inheritance of diapause induction in F1 hybrid crosses
We examined the inheritance of diapause induction of F1 hybrids in four different conditions (Figure 1B). F1 crosses reared in LD 8:16, 17°C showed high diapause incidences (NxS 100%, SxN >98%), as well as in LD 12:12, 17°C (NxS >99%, SxN 100%). At LD 18:6, 23°C the NxS and SxN males exhibited an intermediate diapause induction at 40-60%. Females of the NxS cross showed 8.3% diapause and females of the SxN cross showed 100% diapause. At LD 23:1, 20°C both the NxS and SxN crosses exhibited 0% diapause incidence.

We further examined the mode of inheritance of diapause induction in the F1 crosses reared at LD 18:6 using Fisher’s Exact Tests. Expectations of Z-linked diapause frequencies and expected diapause incidences under autosomal control were calculated based on the combined diapause incidences of the pure populations reared at LD 17:7, and LD 20:4. Diapause incidences for the F1 females could not be distinguished from an expected Z-linked diapause incidence (NxS p=0.7101, SxN p=0.239), and were significantly different from an additive autosomal expectation (NxS p<0.001, SxN p=0.001). Observed male diapause incidences could neither be distinguished from Z-linked nor autosomally controlled induction of diapause (NxS p=0.37, SxN p=0.41), as the expected diapause incidences were identical between the two modes of inheritance.

Inheritance of diapause induction in the F2 hybrid crosses
We also examined the mode of inheritance using F2 backcrosses, by crossing the reciprocal F1 hybrids back to the northern population. There was a large difference between the four reciprocal F2 backcrosses, both within and between the sexes (Table 2). In the GLMM on the backcrosses there was a highly significant effect of Cross and the interaction between Cross and Sex (Full Model: $\chi^2=135.31$, DF=7, p>0.001; Cross: $\chi^2=76.93$, DF=3, p<0.001; Sex: $\chi^2=1.67$, DF=1, p=0.196; Cross*Sex: $\chi^2=40.53$, DF=3, p<0.001).

Expectations of Z-linked, as well as expected diapause frequencies under additive autosomal control were calculated using the diapause incidences of the pure controls reared in the same conditions. Diapause incidence was 0% in the pure southern cross, and 90% for females and 87% for males in the northern cross (Table 2). For the backcrosses, in females the diapause incidences adhered to the expected Z-linked distribution in three crosses (Table 3, Figure 2A). However, in the NxNS cross the proportion of diapausing animals was significantly different from a completely Z-linked inheritance and instead exhibited a higher diapause incidence close to the autosomal expectation (Table 3). Diapause incidences in males followed the expectation of a Z-linked inheritance for the SNxN cross (Table 3, Figure 2B). The NxSN cross adhered to the expected autosomal and Z-linked distributions, which were identical and could not be disentangled. The NxNS cross exhibited neither an autosomal nor a Z-linked expectation, and the NSxN cross adhered to the autosomal expectation. Crosses not showing Z-linked inheritances in general exhibited an excess of diapausing individuals.

DISCUSSION

Here the photoperiodic induction of diapause in two natural populations was investigated, along with their F1 and F2 hybrid backcrosses. We found a difference in the incidence of diapause of the natural populations that is dependent on the photoperiod experienced by the insects. The southern population (Barcelona, Spain) showed a highly dynamic response to photoperiod, while the northern population (Abisko, Sweden) showed a strong propensity to enter diapause that was independent of the photoperiod in which the population was reared. F1 hybrids showed a strong response to photoperiod and indicated a highly sex-linked inheritance of diapause induction. F2 backcrosses confirmed the pattern of sex-linked inheritance, and revealed that a single locus, or several tightly linked loci, on the Z-chromosome could explain the inheritance pattern of most of the crosses. We discuss the general findings in more detail below.

The dynamic response to photoperiod of the southern population was consistent with the two to three generations per year observed in the field, where there is strong selection pressure to
enter the correct developmental pathway over the course of the year as conditions change. The northern population occurs above the Arctic Circle and is univoltine in the field, as the season length generally does not permit a second generation. This lack of expression of the direct developmental pathway creates the possibility for relaxed selection on the photoperiodic threshold, and may eventually lead to the evolution of obligate diapause (Van Dyken & Wade 2010; Aalberg Haugen & Gotthard 2015). However, the fact that there is a subset of individuals in the northern population developing directly does show that the capacity to do so is not lost in this population, which may reflect the likely colonization history of this high latitude in the early Holocene.

In the F1 hybrid crosses at short day lengths all individuals entered diapause, and under long day lengths all F1 hybrids developed directly, regardless of the direction of the cross. Interestingly, there is evidence of strong sex-linked inheritance of diapause under the more intermediate photoperiod LD 18:6. The differences in the photoperiodic response of the F1 hybrids suggest a strong gene by environment interaction, where the diapause decision is highly dependent on the photoperiod. At LD 18:6 more than 90% of females of the NxS cross, which carry a southern Z chromosome, developed directly, and SxN females that instead carry a northern Z chromosome entered the diapause pathway. Males, which are homogametic and heterozygous for fixed differences between the populations at the Z chromosome, exhibited a more intermediate diapause incidence with a tendency to follow the paternal phenotype. This paternal effect has been observed in several other insect species, and it is as of yet unknown what the reason is (Fu et al. 2015; Lehmann et al. 2016).

Diapause incidence in the F2 crosses was strongly affected by the direction of the cross. There is a strong sex-linked component, as in a majority of the crosses diapause incidence could be explained by a single Z-linked factor. This is in concordance with the patterns observed in the F1 hybrids. As there is variation in diapause incidence between families and crosses that carry the same overall proportion of northern and southern alleles in their autosomal and Z chromosome background, it is likely that there are more genes involved, and there is genetic variation in these genes. It appears that neither in the F1 nor in the F2 crosses there is evidence of a major effect linked to the mitochondrial makeup of individuals, as then diapause would have been expected to be inherited maternally, with no difference between sex in the offspring.

Studies that have investigated the inheritance of diapause have found vastly different patterns of inheritance, and inferred characteristics such as dominance and preliminary expectations about the architecture of the trait, although most often these studies only investigate one
particular photoperiod. It has become clear from recent studies (Fu et al. 2015; Xiao et al. 2015),
and the results of the F1 hybrids presented here that the entire reaction norm should be taken
into account. Considering only the initial results found in the long day photoperiods would have
lead us to incorrectly conclude that direct development is dominant over diapause, and
conversely that diapause is dominant over direct development had we only considered the
short-day photoperiods. Rather, the inheritance of diapause as affected by hybridization
appears to influence the position of the photoperiodic threshold. The combination of the
northern and southern alleles in the F1 have moved the critical photoperiod, which in the
southern population occurs around LD12:12, to an intermediate point at LD18:6, in between the
reaction norms of the original populations. The autosomal background appears to be largely
additive, as it represents an intermediate response, corroborated by the diapause incidence in
the male hybrids. In the females, there is a very strong added effect of the Z-chromosome
around the critical photoperiod. This indicates that the Z-chromosome contains a genetic factor
with a major effect on diapause that, when homozygous, is dominant over the autosomal
background. There appears to be a disproportionately large number of Z-linked linked traits in
Lepidoptera, ranging from wing colouration to egg weight (Sperling 1994). Furthermore several
other cases of Z-linked diapause induction have been discovered (Rockey et al. 1987; Hagen &
Scriber 1989; Chen et al. 2014). Other diapause traits, such as termination and post-diapause
development and post-diapause development have also been associated with the Z-
chromosome (Levy et al. 2015). It remains unknown why there is such a strong tendency of
these traits, which have a major impact on the life cycle, to be sex-linked. It has been speculated
that this pattern might relate to sexual conflict over optimal life cycles {Nylin:1994ww,
Soderlind:2011iq}, but it could instead, or also, be a result of the more rapid fixation of alleles
on sex chromosomes when populations adapt to local conditions and diverge
{Charlesworth:1987kq, Meisel:2013bk}.

Overall, our results demonstrate that there are one or several loci on the Z chromosome that
influence the tendency to enter diapause in P. napi. These loci show a strong genotype by
environment interaction in the F1 crosses and it seems likely that they influence the position of
the entire photoperiodic threshold for diapause induction. In addition, the results suggest that
there are also effects of loci on the autosomes as in several of the crosses we see significant
deviations from a pure sex-linked inheritance pattern. Performing population crosses in Pieris
napi is the first step in unravelling the mechanism underlying photoperiodic induction in this
species, as well as improving the understanding of this phenotype as a whole. Future genomic
analyses on these crosses will be able to link genetic variation in specific genes to the phenotype
studied here.
FUNDING

This work was supported by Knut and Alice Wallenberg Foundation (grant number 2012.0058), Swedish Research Council, VR to (CWW) 621-2012-4001 and to (SN) 2012-3715.

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Figure 1 – (A): Diapause incidence for the pure Spanish and Swedish populations measured in six different photoperiods at 20°C. Solid line and squares represent the mean diapause incidences and logistic fit for the SxS (Barcelona, Spain) population. Dotted line and open squares represent the northern population (Abisko, Sweden). Lines represent the GLMM model fit. (B): Diapause incidences for the F1 hybrid crosses. Open squares and solid line represent SxN females, and open triangles and dotted line represent SxN males. Solid squares and solid line represent NxS females, and filled triangles and dotted line represent NxS males. At the lower photoperiods, all crosses entered diapause regardless of direction, and at the highest photoperiod measured all crosses developed directly. However, at LD 18:6 strong sex-linked induction was exhibited in the females, while the males showed an intermediate phenotype with a slight skew depending on paternal phenotype. Note that for the F1 hybrids LD 8:16 and LD 12:12 crosses were reared at 17°C, LD 18:6 at 23°C and LD 23:1 at 20°C.
Figure 2 – Diapause incidence in the F2 backcrosses for females (A), and males (B). Light grey bars and lines represent family Means ±1 Standard Deviation for each cross. Black bars represent observed total incidences for each cross, dark grey bars represent the expected incidences for Z-linked diapause induction, and the hollow bars represent diapause incidence under expected autosomal control. The stars denote significance by Fisher’s Exact Test, as reported in Table 2. Non-significant differences indicate the likely mode of inheritance, whereas significant differences indicate a non-likely mode of inheritance.
Table 1 – Diapause incidence of the pure populations for each photoperiod. Percentage of diapasing individuals, the total number of individuals, as well as the number of families in each cross are given.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diapause (%)</th>
<th>Total number</th>
<th>Number of families</th>
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<tr>
<td>S x S</td>
<td>82.1</td>
<td>39</td>
<td>8</td>
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<tr>
<td>N x N</td>
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<td>58</td>
<td>13</td>
</tr>
<tr>
<td>S x S</td>
<td>58.1</td>
<td>31</td>
<td>8</td>
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<tr>
<td>N x N</td>
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<td>44</td>
<td>13</td>
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<td>7</td>
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<tr>
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Table 2 – Diapause incidence for each hybrid cross. Percentage of diapausing individuals is split by sex. The total number of individuals in each cross is given, as well as the number of families in each cross.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diapause incidence (%)</th>
<th>Total number</th>
<th>Number of families</th>
</tr>
</thead>
<tbody>
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Table 3 - Expected vs observed frequencies of diapause incidence for females under complete Z-linked induction and no dominance. Based on the frequencies of the pure populations, expected diapause incidences were calculated and compared to the observed values by Fisher’s exact test.

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<th>♂ Fisher’s exact two-tailed P</th>
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<tr>
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<td>0.0050</td>
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