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Title: Latitudinal clines in the timing and temperature-sensitivity of photoperiodic reproductive diapause in Drosophila montana

Year: 2020

Version: Published version

Please cite the original version:

Tyukmaeva, V., Lankinen, P., Kinnunen, J., Kauranen, H., & Hoikkala, A. (2020). Latitudinal clines in the timing and temperature-sensitivity of photoperiodic reproductive diapause in Drosophila montana. Ecography, 43(5), 759-768. https://doi.org/10.1111/ecog.04892

ECOGRAPHY

Research

Latitudinal clines in the timing and temperature-sensitivity of photoperiodic reproductive diapause in *Drosophila montana*

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Reproductive diapause is a primary mechanism used by arthropods to synchronize their life cycle with seasonal changes in temperate regions. Our study species, *Drosophila montana*, represents the northern insect species where flies enter reproductive diapause under short day conditions and where the precise timing of diapause is crucial for both survival and offspring production. We have studied clinal variation in the critical day length for female diapause induction (CDL) and their overall susceptibility to enter diapause (diapause incidence), as well as the temperature sensitivity of these traits. The study was performed using multiple strains from four latitudinal clines of the species – short clines in Finland and Alaska and long clines in the Rocky Mountains and the western coast of North America – and from one population in Kamchatka, Russia. CDL showed strong latitudinal clines on both continents, decreasing by one hour per five degrees decline in latitude, on average. CDL also decreased in all populations along with an increase in fly rearing temperature postponing the diapause to later calendar time, the effects of temperature being stronger in southern than in northern population. Female diapause incidence was close to 100% under short day/low temperature conditions in all populations, but decreased below 50% even under short days in 19°C in the southern North American western coast populations and in 22°C in most populations. Comparing a diversity of climatic data for the studied populations showed that while CDL is under a tight photoperiodic regulation linked with latitude, its length depends also on climatic factors determining the growing season length. Overall, the study deepens our understanding of how spatial and environmental parameters affect the seasonal timing of an important biological event, reproductive diapause and helps to estimate the evolutionary potential of insect populations to survive in changing climatic conditions.

Keywords: adaptation, climate change, clinal variation, critical photoperiod, overwintering, phenology

Introduction

Species living in seasonally varying environments need to be able to anticipate the arrival of harsh season and have enough time to behaviorally and/or physiologically prepare themselves. In this process the 'token stimuli' that do not represent the

unfavorable conditions themselves, but are seasonally correlated with them, play an important role (Tauber et al. 1986). In the northern latitudes, seasonal variation in photoperiod is a more reliable token stimulus than temperature, as the latter may show large variation between the years. However, in the southern latitudes, where seasonal variation in the day length is small, temperature changes may have a higher predictive value than the photoperiod. In both cases, current climate change may complicate organisms' ability to adjust their life cycle according to seasonal changes, if the environmental variables that serve as token cues no longer correlate with the environment causing selection (Visser et al. 2010).

Adult reproductive diapause can be triggered by a variety of environmental cues including the photoperiod, temperature, humidity and nutrition (Saunders et al. 2002). In diapausing females the oogenesis is arrested at a pre-vitellogenic stage soon after emergence. The emerging females that enter diapause too early during the growing season may be less successful in producing progeny after overwintering, as their energy reserves are used up during diapause (Hahn and Denlinger 2007). On the other hand, the females that develop ovaries too late in the season are not able to collect energy reserves before the winter, and will either not survive over the cold period or risk producing progeny that fail to reach adulthood. Male reproductive diapause, defined as a reversible state of inability to inseminate receptive females, has been found to occur in several insect species under approximately the same environmental conditions as that of the females (Pener 1992). The physiological stage and condition of overwintering males has been studied less than that of the females, but short day length seems to affect metabolism and several physiological traits also in this sex (Pener 1992, Vesala and Hoikkala 2011). Hence, high within-population variation in the timing of reproductive diapause is likely to be beneficial for both sexes in environments, where the length of the warm period varies between years.

The timing of photoperiodic reproductive diapause has been studied mainly in females, where its stages are easier to detect than in males. Sexual maturation of females is arrested when the day length during the first days after their emergence (sensitive period for diapause induction) remains below a certain threshold (Danilevsky 1965). The critical day length for this kind of diapause (CDL; the day length where 50% of the females of a given population enters diapause) is usually estimated from the photoperiodic response curves (PPRCs), which measure female diapause incidence at different photoperiods. Alternative abbreviations often used for this photoperiod include CNL (critical night length) and CPP (critical photoperiod). In the species with a photoperiodically regulated reproductive diapause, PPRCs usually show a low diapause incidence under long summer days and close to 100% incidence during the shortening days in late summer and autumn. The timing and prevalence of reproductive diapause may also be sensitive to environmental temperature, so that low temperatures increase and high temperatures decrease female diapause incidence and/or move

the CDL towards longer or shorter day lengths, respectively (Watabe 1983, Ichijo 1986, Pittendrigh and Takamura 1987, Saunders and Gilbert 1990, Saunders 2014). Decreased diapause incidence in higher temperatures could at least partly be due to insects' faster development rate and a shorter duration of the sensitive period during which the females need to perceive a required number of Light–Dark (LD) cycles to enter diapause (Saunders and Gilbert 1990, Saunders 2014). Thus high temperatures may lead to a decoupling of synchrony between diapause-sensitive life-cycle stages and CDL (Bale and Hayward 2010).

Many insect species showing latitudinal clines in CDL have been found to possess high within-population variation in this trait, partly due to gene flow between populations and partly to the opposing selection pressures for insect reproduction and overwinter survival (Danilevsky 1965, Bradshaw and Lounibos 1977, Lankinen 1986, Lankinen et al. 2013). Species invasions into new latitudes have been found to be accompanied by changes in CDL e.g. in the green stink bug *Nezara viridula* (Musolin and Numata 2003), the fall webworm *Hyphantria cunea* (Gomi et al. 2007), a beetle *Diorhabda carinulata* (Bean et al. 2012) and a mosquito *Aedes albopictus* (Urbanski et al. 2012). Furthermore, shifts towards the southern type CDLs have been documented in the local populations of several insect species, including the pitcher plant mosquito *Wyeomyia smithii* (Bradshaw and Holzapfel 2001) and a water strider *Aquarius paludumikely* (Harada et al. 2005), likely due to global warming. This kind of shifts may help to avoid problems induced by a mismatch between the diapause-inducing token cues, environmental temperature and the length of the growing season (Visser et al. 2010).

Understanding the patterns of insects' seasonal responses, such as diapause, requires simultaneously studying different natural environments that drive these patterns (Danks 2007). There is increasing evidence that the photoperiodic timer underlying these responses is at least partly based on, or linked with, the circadian clock system (see review by Koštál et al. 2017), and that the timing and prevalence of reproductive diapause is also sensitive to environmental temperature. However, although the sensitivity of female diapause response to photoperiod and temperature, and/or its linkage with climatic factors in wild populations, have been studied in several insect species, all these aspects have rarely been taken into account in the same study. We have traced environmental factors affecting latitudinal variation in photoperiodic diapause in a truly northern fly species, *Drosophila montana*, both with the aid of laboratory studies and by analyzing a diversity of climatic data for the studied populations. Our earlier studies have shown that *D. montana* has a robust photoperiodic diapause, typical to northern insect species, and that it to possesses a clear latitudinal cline in CDL in Finland despite a high gene flow between the cline populations (Tyukmaeva et al. 2011, Lankinen et al. 2013). Here we have taken a wider view and traced clinal variation in *D. montana* females' CDL and diapause incidence, as well

as in the temperature-sensitivity of these traits, along four latitudinal clines in Europe and North-America and in one population in Asia. In addition, we have traced climatic factors linked with variation in above-mentioned traits in our study populations to identify the factors that play a major role in determining these traits in the wild. Together these studies help to predict an ability of northern insect species to meet disturbances induced by climate change.

Material and methods

Fly strains

Drosophila montana isofemale strains, used in this study, were established from the progenies of single overwintered fertilized females collected in the wild during the local spring. The strains included seven populations from a latitudinal cline in Finland, three populations from a cline in Alaska, seven populations from a cline on the western coast of Canada (British Columbia) and USA (Washington and Oregon), five populations from a cline on the Rocky Mountains in Canada (British Columbia) and USA (Wyoming and Colorado) and one population from Kamchatka (Russia, Fig. 1). The Finnish strains were established 2008–2012, the North American strains 2009–2014 and the Kamchatka strains in 2013. More information on the collecting sites and fly strains is available in Supplementary material Appendix 1 Table A1, A2, Fig. A1.

All strains were maintained in bottles containing malt medium (Lakovaara 1969) in continuous light at 19°C since their establishment. Lankinen et al (2013) and Kauranen et al. (2019) have shown that maintaining *D. montana* flies in diapause preventing conditions for years has no effect on their

CDL, therefore, the difference in strain collection year can be expected not to affect our results.

Experimental conditions

We determined the female diapause incidence (percentage of diapausing females) for all study strains in several Light:Dark (LD) cycles in 13°C, 16 or 17°C and 19°C and in one short LD in 22°C. In the four first-mentioned temperatures, PPRCs were drawn for the LDs, where female diapause incidence varied from ≤ 20% to ≥ 95%, and which the females can experience at their home site during their emergence (Supplementary material Appendix 1 Table A2). In some strains, where it was not possible to estimate CDL using the above-mentioned criteria, we measured female diapause incidence in an additional LD cycle close to the longest LD experienced in nature. The data for the CDL of most Finnish strains in 16°C have been previously published in Lankinen et al. (2013). All studies involved three strains per population (four strain from Kemi and two from Cranbrook), with 80–540 females per strain for each LD and temperature combination.

We transferred the flies into one of the experimental conditions in the malt media bottles already as pupae, and maintained the emerging females and males together until the females reached sexual maturity or entered reproductive diapause (in 13°C for 28 d, in 16°C and 17°C for 21 d, in 19°C for 18 d and in 22°C for 14 d). In *D. montana*, maintaining the two sexes together throughout the experiment does not affect female diapause incidence (Tyukmaeva et al. 2011, Lankinen et al. 2013). The same is true for *D. melanogaster* and *Drosophila simulans*, when the flies are transferred under dormancy-inducing conditions after eclosion, keeping
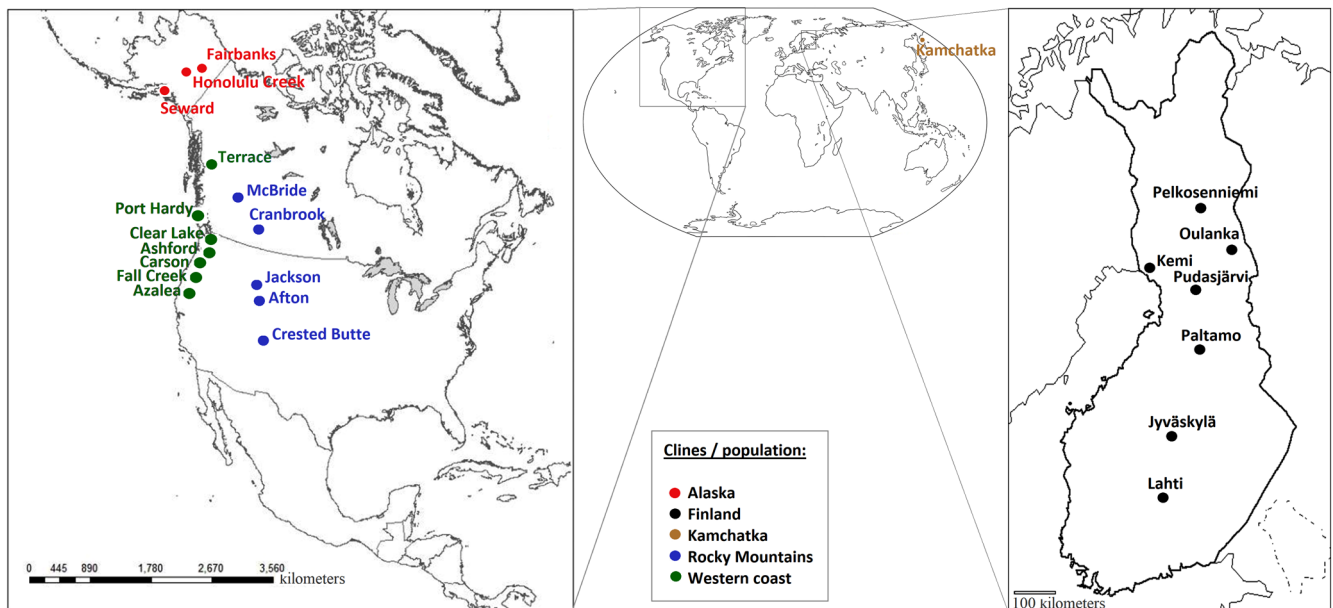

Figure 1. Map of the fly collecting sites.

both sexes together does not affect the diapause incidence (Lirakis et al. 2018).

Due to the large data sets and the need to perform the experiments simultaneously for the populations of a given cline, we performed the experiments in two laboratories at the Univ. of Jyväskylä and the Univ. of Oulu, Finland. In both laboratories, we reared the flies under different LD cycles and temperatures either in wooden, light-insulated and ventilated boxes in a temperature-controlled room, or in climate chambers (see Supplementary material Appendix 1 Table A3 for more information on the place of research and the used experimental devise). In Jyväskylä the boxes were illuminated with compact LedEnergie fluorescent lamps with light intensity of 600–1100 lux during the photophase and in Oulu with one white fluorescent lamp per chamber (9 W, Megaman, Germany) with light intensity of 300–1000 lux. The 19°C experiments were performed in Jyväskylä in the climate chambers HiPoint EH-1800 (Taiwan) and Binder FD 115 (Binder, Germany), which had similar lighting as the boxes.

We determined female reproductive state at the age of sexual maturity by dissecting their ovaries under a light microscope. Females were considered as diapausing, if their ovaries were small and transparent and/or if they contained only a little yolk and did not have any egg structures, and sexually mature, if their ovaries contained at least one fully developed egg (Tyukmaeva et al. 2011). *Drosophila montana* males enter reproductive diapause approximately under the same conditions as the females, but defining their diapause would require studies on several levels, including mating behavior, spermatogenesis and cuticular hydrocarbon profiles (Ala-Honkola et al. 2020).

Lankinen et al. (2013) have shown that the CDLs defined for the fly strains in Oulu and Jyväskylä laboratories are compatible with each other, when performed in the same temperature. In the present study, the temperature in the light-insulated boxes in temperature-controlled (16°C) room in Jyväskylä raised to 17°C during the experiment in spite of the ventilation installed in the boxes, which complicates CDL comparisons in 16–17°C between the labs.

Statistical analysis

PPRCs were estimated as a function of nonlinear regression performed with the drc package (Knezevic et al. 2007, R Core Team), which fits the generalized linear model (glm) for a given isofemale strain (or for a given population) in each temperature regime. The best fitting model was selected using mselect function. We estimated CDL from the PPRC as 50%-dose response on the interval of LD regimes that had been tested experimentally (this was possible only in cases where the female diapause incidence reached 50% threshold). Figure 2 shows the PPRCs for *D. montana* population from Pelkosenniemi (Finland). Details of the code for PPRC and CDL estimation are available in Supplementary material Appendix 2 Fig. A2.

We fitted the CDL data for each population in linear model using R software (R Core Team). In all further analyses, we considered all possible interactions and used the step function and AIC to choose the best fitting model. In the first model, we used the latitude, altitude and fly rearing temperature as continuous covariates to estimate their effect on CDL. Furthermore, we analyzed differences between the CDLs estimated at two temperatures (13°C and 19°C), where we had data points for most cline populations, to investigate variation in the magnitude of the temperature effects on CDL along the latitudinal clines. Here we fitted linear model to test how the
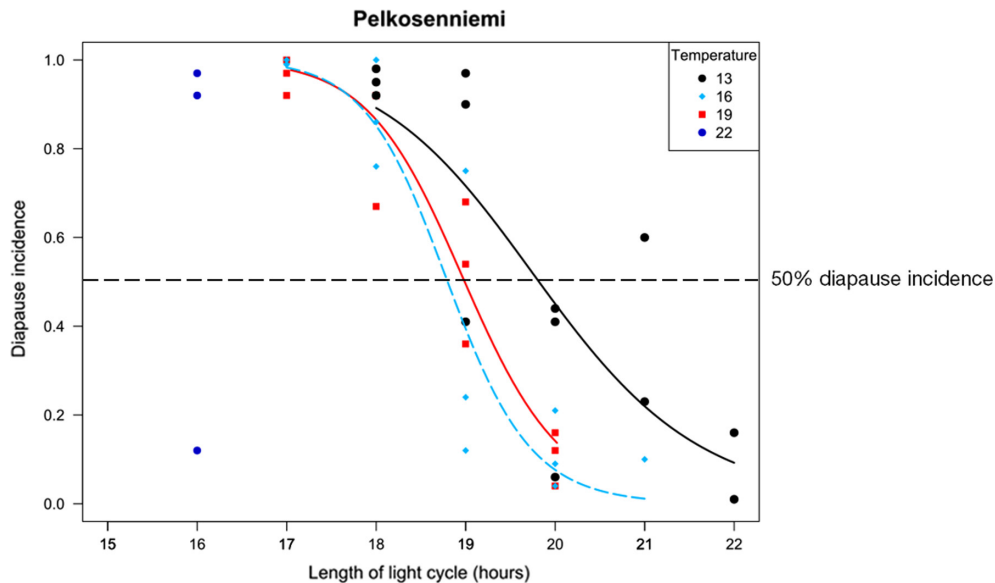

Figure 2. GLM estimates for the PPRCs of *D. montana* from Pelkosenniemi (Finland) based on female diapause incidence in different LD cycles and temperatures. LD cycle on x-axis at the point, where PPRC crosses the 50% threshold, corresponds CDL in a given temperature.

6°C difference in temperature affects the CDL, and used the latitude and altitude and their interaction as covariates.

We also obtained climatic data for all 23 populations from the WorldClimate database (<www.worldclim.org>; Fick and Hijmans 2017), using latitude and altitude coordinates. Then we extracted 19 bioclimatic variables derived from temperature and precipitation values in each locality using the 'raster' package (Hijmans 2017), reduced the number of variables using principle components analysis (PCA) in 'FactoMineR' package (Le et al. 2008) in R, and used the fly rearing temperature, latitude (as a proxy of photoperiod) and the principal component 1 (PC1) to evaluate their contribution to CDL (model 1). We also tested the effects of the fly rearing temperature, latitude and the length of the growing season on CDL (model 2). Finally, we compared these two models with ANOVA chi-test, and checked the correlation between PC1 and the length of the growing season length with Pearson's correlation test.

Results

CDL variation under different temperatures along the latitudinal clines

PPRCs drawn for different cline populations in 13°C, 16°C/17°C and 19°C were based on female diapause incidenses under several LDs, while in 22°C female diapause incidence was measured only in one LD where it had been found to be close to 100% at lower temperatures. PPRCs for each population in above-mentioned temperatures are available in Supplementary material Appendix 2 Fig. A3–A7. Information on the CDLs estimated from the PPRCs of individual strains/populations under different temperatures is available in Supplementary material Appendix 2 Table A4.

CDL showed strong latitudinal clines both in north Europe and in North America in different temperatures (the cline was not evident in 19°C in Alaska), decreasing by one hour per five degrees decline in latitude, on average (Fig. 3, Table 1). In addition, CDL showed increase in the southern high altitude populations on the Rocky Mountains (Supplementary material Appendix 1 Table A1, A2, Fig. A1) where its value depended both on the elevation and on the latitude.

In northern and high-altitude populations, an increase in fly rearing temperature to 19°C caused a small but significant decline in CDL, while in the southern western coast cline populations (Carson, Fall Creek and Azalea) it decreased female diapause incidence below 50% threshold preventing CDL estimation. At 22°C, only some strains from the Finnish and Alaskan cline populations exceeded 50% diapause incidence (Supplementary material Appendix 2 Fig. A3–A7). The CDLs for Kamchatka population (56°N), the only locality available from the northern Asian distribution of *D. montana*, were found to be unusually long (17 h 40 min at 16°C and 17 h 23 min at 19°C) and to resemble those of high-altitude populations, e.g. Lahti from Finland (61°N)
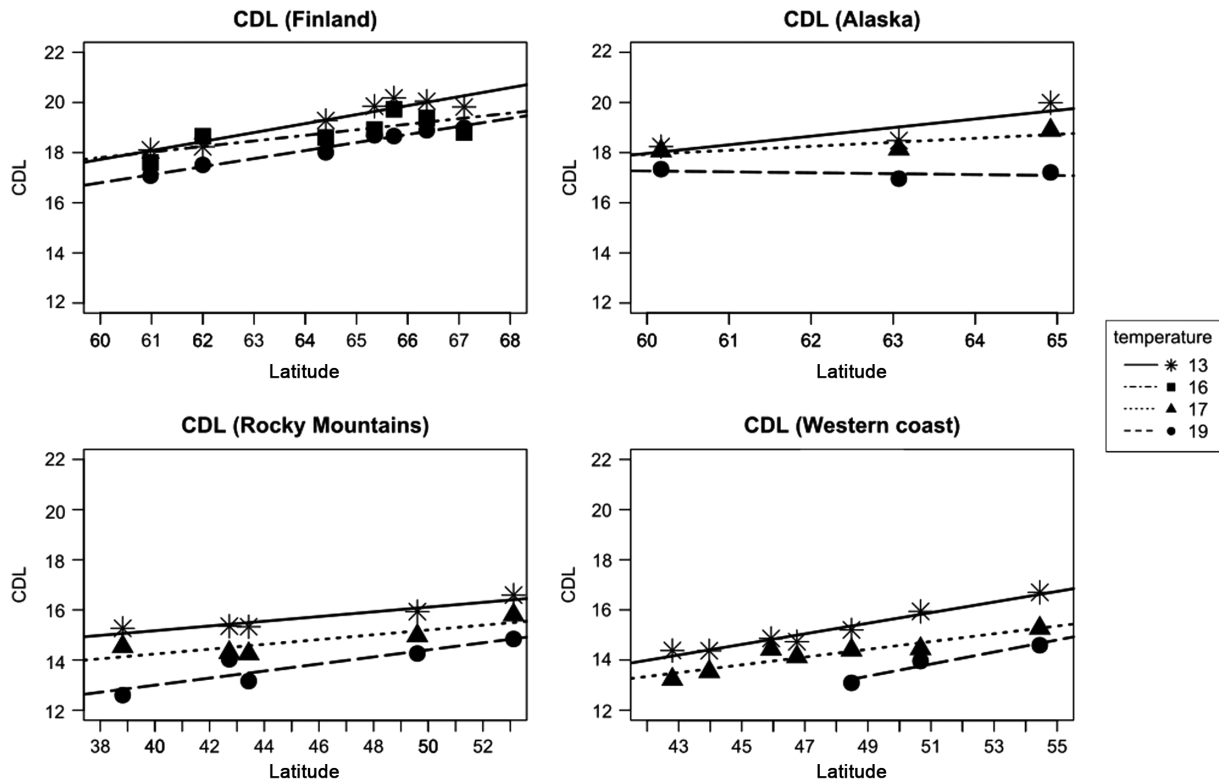

Figure 3. Effects of the latitude and temperature on the estimated CDLs along the four latitudinal clines (Finland, Alaska, Rocky Mountains, western coast).

Table 1. Summary of the best-fitted linear model for the effects of the fly rearing temperature and the latitude and altitude on CDL. Significant p values are in bold.

Coefficients	Estimate	SE	t value	p value
Intercept	9.390	2.349	3.997	**< 0.001**
Temperature	−0.491	0.146	−3.358	**< 0.01**
Latitude	0.198	0.042	4.733	**< 0.001**
Altitude	0.003	0.0007	4.634	**< 0.001**
Temperature × Latitude	0.004	0.0026	1.698	0.095
Latitude × Altitude	−0.00006	0.00002	−3.706	**< 0.001**

and Seward (60°N) from Alaska (Supplementary material Appendix 2 Table A4).

To investigate how a 6°C difference in fly rearing temperature affects CDL in populations from different latitudes, we compared the data on CDL at 13°C and 19°C in each cline population where it was possible. We fitted linear model, and after the model reduction, the best and the only predictor appeared to be the latitude (estimate = −0.035; SE = 0.014; t value = −2.523; p = 0.02; adjusted R^2 = 0.24; residual SE = 0.53 on 16 df). According to the test results, the rise in temperature had a stronger effect on CDL in the southern populations compared to the northern ones (Supplementary material Appendix 2 Fig. A8).

Effects of bioclimatic variables on CDL

Principal component analysis of bioclimatic variables (Supplementary material Appendix 3 Table A5) revealed four principal components (PCs) with eigenvalues > 1, explaining 93.5% of total variance (Supplementary material Appendix 3 Table A6). PC1 and PC2 had the highest contribution, 52.3% and 23.3% of total variance, respectively. PC1 showed a good separation of the studied populations: the western coast populations were clearly distinct from the Rocky Mountains populations, but the Finnish and Alaskan clines overlapped with the latter one in some degree (Fig. 4). PC2, on the other hand, showed some separation between the northern and southern populations within the Rocky Mountains and western coast clines, but its effects were not so clear in other clines. The contribution of different bioclimatic variables in PC1 and PC2 is presented in Supplementary material Appendix 3 Fig. A9.

We tested two models with the fly rearing temperature, latitude and either PC1 (model 1) or the growing season length (model 2) as predictors. In both models, the rearing temperature, latitude and PC1 (model 1) or growing length season
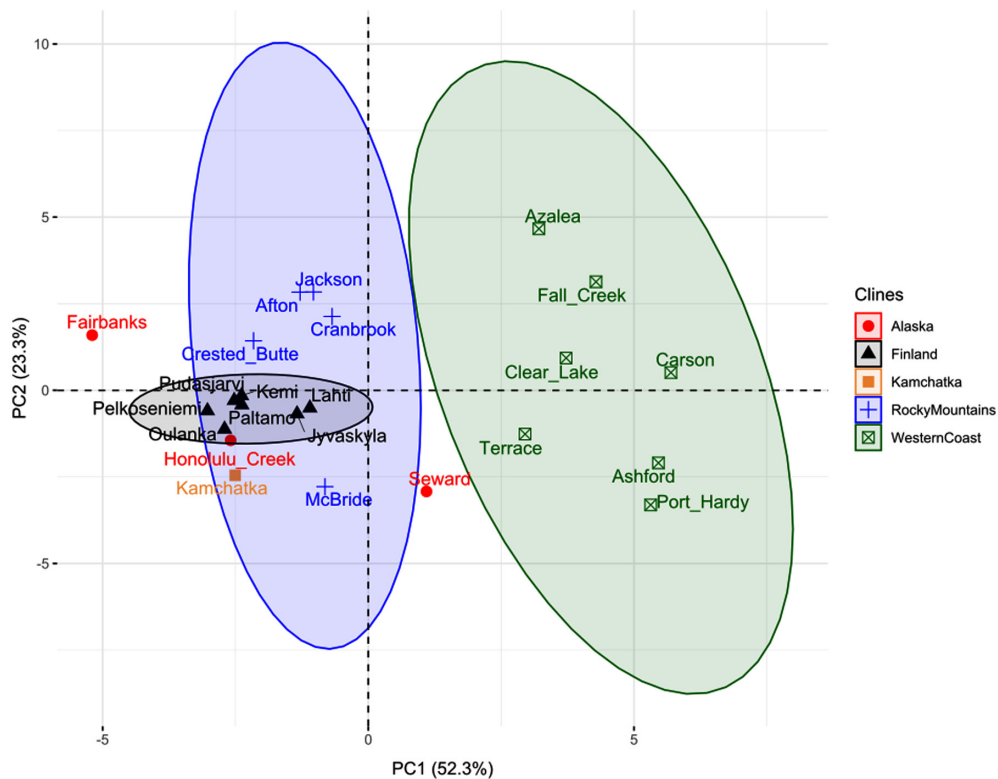

Figure 4. Results of the principal component analyses for the studied populations on the scale of PC1 and PC2 for bioclimatic variables. Alaska and Kamchatka clusters are not drawn due to too few data points. Ellipses represent 95% confidence interval of clustering.

Table 2. Summary of the best-fitted linear model on the effects of the fly rearing temperature, latitude and the PC1 (model 1) or the growing season length (model 2) on CDL. Significant p values are in bold.

	Estimate	SE	t value	p value
Model 1				
Intercept	13.565	2.657	5.106	**< 0.001**
Temperature	−0.465	0.162	−2.866	**< 0.01**
Latitude	0.127	0.048	2.660	**0.01**
PC1	−0.160	0.025	−6.348	**< 0.001**
Temperature × Latitude	0.004	0.003	1.325	0.19
Model 2				
Intercept	15.779	2.604	6.060	**< 0.001**
Temperature	−0.462	0.155	−2.991	**< 0.01**
Latitude	0.12	0.046	2.641	**0.01**
Growing season length	−0.01	0.002	−7.062	**< 0.001**
Temperature × Latitude	0.004	0.003	1.378	0.17

(model 2) had a significant effect on CDL (Table 2). ANOVA comparison with Chi-test showed no difference between the two models (RSS = 14.19 versus RSS = 12.91 for model 1 and model 2, respectively; sum of sq = 1.28). PC1 was also strongly correlated with the growing season length in the studied populations (Pearson's correlation: 0.84; t = 12.462, df = 64, p-value < 2.2e-16). These results suggest that PC1 represents bioclimatic conditions that determine the length of the growing season in the studied population, and that it serves, along with the latitude (as a proxy for photoperiod), as a good predictor of CDL. However, the effect of PC2 on CDL was not significant (Intercept: estimate = 7.96; SE = 0.79; t value = 10.075; p < 0.001. Temperature: estimate = −0.235; SE = 0.034; t value = −6.946; p < 0.001. Latitude: estimate = 0.225; SE = 0.010; t value = 21.518; p < 0.001. PC2: estimate = 0.062; SE = 0.046; t value = 1.363; p = 0.178).

Discussion

Latitudinal variation in CDL represents an adaptation to early and rapid arrival of winter at higher latitudes, and small changes in photoperiod on either side of the CDL produce a dramatic shift in diapause incidence (Bale and Hayward 2010). Accordingly, most studied insect species have been found to show latitudinal variation in this trait (Bradshaw and Lounibos 1977, Lankinen 1986, Kimura and Bessho 1993, Shimizu and Kawasaki 2001, Tyukmaeva et al. 2011, Wang et al. 2012, Lankinen et al. 2013, Paolucci et al. 2013, Lehmann et al. 2015, Yamaguchi and Goto 2019). In addition, rapid changes in CDL have been detected in several invasive species after colonization (Gomi et al. 2007, Bean et al. 2012, Urbanski et al. 2012, Tanaka et al. 2015) or as a response to climate warming (Bradshaw and Holzapfel 2001, Dalin 2011). However, the extent to which latitudinal variation and recent adaptive changes in CDL is affected photoperiod and temperature and by their interaction with bioclimatic variables determining the length of the growing period is still poorly understood.

Photoperiod-dependent responses are usually most pronounced in northern populations that have evolved to cope with highly variable thermal environments, while the higher temperature sensitivity of southern populations allows reproduction under short and warm autumn days (Bahrndorff et al. 2009, Śniegula et al. 2012, Hut et al. 2013). High temperatures have been found to suppress diapause and/or to shorten CDL e.g. in the southern populations of a mosquito *Aedes atropalpus* (Beach 1978) and a fly *Calliphora vicina* (McWatters and Saunders 1998). We studied clinal variation in *D. montana* diapause traits, CDL and diapause incidence, by defining these traits in different photoperiods and temperatures for the females of three strains per cline population. Even though variation between local strains was in some cases quite large, especially in 22°C, the general findings were clear. In all clines CDL decreased by about one hour per five degrees decline in latitude, which fits well with Danilevsky's rule 'The CDL in genetic clines lengthens 1–1 h and 30 min per 5° of latitude northwards' (Danilevsky et al. 1970). In addition, the CDLs of the southern populations showed higher temperature-sensitivity than the ones of the northern populations, and the populations that originated from higher altitudes had generally longer CDL than the ones from the lower altitudes on the same latitude.

Interpretation of phenotypic clines, and the factors inducing and maintaining them, is not always straightforward, as the studied traits may be affected by various kinds of selection pressures in the wild. In our study, *D. montana* CDL was clearly affected by the climatic conditions prevailing at different altitudes. The difference between the CDLs of two low-altitude populations from the western coast cline (Fall Creek and Azalea) and two high-altitude populations from the Rocky Mountains cline (Jackson and Afton) from similar latitudes (42–44°N) offers a good example of this. In 13°C, the CDLs of the Fall Creek and Azalea strains were about one hour shorter than the CDLs of the Jackson and Afton strains, and in 19°C the diapause incidence of the first-mentioned strains decreased below 50% preventing CDL estimation.

Diapause behaviour of *D. montana* strains from the Kamchatka peninsula population (56°N, Asia) offers an example on the effects of harsh environmental conditions and a short growing season on CDL, as the CDLs of these strains resembled the ones of the high-latitude populations

(60°C–61°N) in Finnish and Alaskan clines. The length of growing season in Kamchatka is only 120 d which is close to that of the most northern populations Pelkosenniemi and Oulanka in our study (117 and 122 d, respectively; Supplementary material Appendix 1 Table A2). This demonstrates how the local climate conditions determining the length of the growing season can affect the timing of diapause and shift the CDL towards longer values/earlier calendar time.

Even when the environmental conditions are favorable for diapause, the switch to diapause can only take place when the females are in a proper developmental and physiological stage, referred to as the sensitive period for diapause (Saunders et al. 2002). Consequently, decrease in diapause incidence in higher temperatures could be due to the faster development rate of female ovaries, combined with the facts that the switch to diapause can only take place during a few days after emergence and that the females have to experience certain number of short-day cycles during this period to enter diapause (Saunders et al. 2002). If the females pass through their sensitive stage before the CDL date, they will develop ovaries and reproduce, but if they pass through it during or after this time point, most or all of them will enter diapause. In northern *D. montana* populations, females' sensitive period lasts for 8–9 d in 16°C and 4–5 d in 19°C after emergence (Salminen and Hoikkala 2013). The females of these populations need to experience approximately three short-day cycles during their sensitive period to enter diapause, which may effectively reduce their diapause incidence at higher temperatures (Salminen and Hoikkala 2013). A dramatic decrease in female diapause incidence detected in our study in the southern western coast cline populations in 19°C could be due to latitudinal variation in the length of females sensitive period and/or in the number of short-day cycles needed for diapause induction.

Females that have entered reproductive diapause are not able to produce progeny during the ongoing season, but they gain resource allocation trade-offs in terms of higher stress resistance, slower aging and higher over-winter survival (Tatar and Yin 2001). On the other hand, late entry into diapause introduces the risk of encountering cold stress outside of diapause (Bradshaw et al. 2004, Rinehart et al. 2007, Bale and Hayward 2010, Bradshaw and Holzapfel 2010). Thus, the CDL is affected by two opposite selection forces, one favoring the females with a short CDL (reproduction in late summer) and the other one favoring the females with a long CDL (early diapause entry). Taylor and Spalding (1989) have suggested that when variation in the onset of harsh conditions is relatively low, the superior strategy for the insects is to enter diapause at a constant period before this date. In high-latitude populations with great thermal variability, natural selection favor broad 'safety margins' and initiate diapause when days are relatively long, while in the low-latitude populations strong time stress for producing several generation per year may lead to reduced sensitivity to photoperiod and postpone the diapause towards the autumn (Śniegula et al. 2012). *Drosophila montana* females clearly have broad 'safety margins', as they enter diapause already in late summer even though there might still be time to produce one more generation before the frost. In the Finnish and Alaskan populations, the females enter diapause about two months before the frost and have only one generation per year (i.e. are practically univoltine), while the more southern populations have two or even three generations per year. High altitude Rocky Mountains populations make an exception, as at least in Crested Butte (38°54′N) *D. montana* females enter diapause already at the beginning of August (Baker 1980).

According to a theoretical model developed by Cohen (1970), the optimal reproductive strategy for northern insect populations should be an extended maturation period, during which an optimal dormancy fraction is evaluated independently every day. In this model, the slow growth rate and delayed maturation during the early season will expose a larger fraction of the population to day lengths that are shorter than CDL during their sensitive period, which will lead to high diapause incidence (Cohen 1970). This will reduce time stress involved in fast maturation in univoltine populations compared to the multivoltine ones. Many temperate insect species have indeed been found to display plasticity in voltinism across latitudes (Dmitriew 2011). *Drosophila montana* has adapted to survive in seasonally varying environments also in this respect, as these flies have a long maturation period compared to most other *Drosophila* species (Salminen and Hoikkala 2013).

While stabilizing and directional selection are presumed to lead to locally optimal timing of diapause, they can also maintain high genetic variation in CDL within populations and create latitudinal clines through diversifying selection. In addition, genetic variation within cline populations can be increased by several other factors, including gene flow from other populations, temporal variation in climatic conditions and mutation and drift (Lewontin and Krakauer 1973). According to Knell and Thackeray (2016), univoltine species will always suffer the deleterious consequences of phenological mismatch, whereas multivoltine ones are likely to experience at least some relief from them in generations that occur later in the season. Conversely, univoltine species will experience continual selection to adapt to changing seasonality, whereas multivoltine species will experience reduced or no selection during the late season generations (Knell and Thackeray 2016).

Genetic variation detected in CDL within and between *D. montana* populations in the present study, and changes in this trait detected in our recent selection experiment (Kauranen et al. 2019), suggest that the CDL of *D. montana* possesses a high adaptive potential. In this species, higher temperatures during CDL will evidently postpone the diapause towards the autumn and lead to the production of additional generation(s) per year. In the northern populations, the turn from univoltine to bivoltine populations may increase the risk of encountering cold stress outside of diapause and lead to unpredictable changes in selection pressures, while in the southern low-altitude populations higher temperatures may lead to drastic decline in diapause incidence.

Conclusions

Changes in phenology, i.e. in the seasonal timing of biological events, are among the most conspicuous signs of global warming, and longer and warmer growing seasons enable many insect populations to complete more generations per year than in the past (Forest 2016). Moreover, evolutionary forces may drive insect phenologies into different directions than what is expected as a plastic response to global warming (Lindh et al. 2016). Increased temperature may, for example, modify the normal rates of development and lead to a decoupling of synchrony between diapause-sensitive life-cycle stages and CDL (Bale and Hayward 2010). Thus, it will be important to trace the environmental cues affecting the seasonal timing of biological events, like diapause, and to study their evolutionary potential in different environmental conditions to be able to predict the long-term consequences of climate change on insect phenology. Studies on the magnitude and distribution of intraspecific variation in environmental responses will also give valuable information on whether adaptation to new climatic regimes might be facilitated through natural or assisted migration (Wilczek et al. 2010).

Data availability statement

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.59zw3r241> (Tyukmaeva et al. 2020).

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Supplementary material (available online as Appendix ecog-04892 at <www.ecography.org/appendix/ecog-04892>). Appendix 1–3.