Divergence in photoperiod responses of a classical biological control agent *Galerucella calmariensis* (Coleoptera: Chrysomelidae) across a climatic and latitudinal gradient

Tyson Wepprich and Fritzi S. Grevstad
Department of Botany and Plant Pathology, Oregon State University
2082 Cordley Hall, Corvallis, OR 97331-2902

Abstract

A key question in classical biological control is to what extent insect agents evolve to novel environment conditions in the introduced range. The introduction of biological control agents to new photoperiod regimes and climates may disrupt the coordination between growing season length and diapause timing that evolved in the native range. We tested whether populations of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) have responded to the potential mismatch of their diapause timing through evolution since their intentional introduction to the United States from Germany in the 1990s. Colonies from populations spanning 9.4° of latitude in the western USA were reared in common garden experiments conducted in growth chambers with photoperiod treatments. Across all populations, shorter day lengths increase the proportion that induce diapause over reproduction. Populations have genetically diverged in their photoperiod thresholds for diapause and, to a lesser extent, development time. The critical photoperiods, or the day length at which half of a population diapauses, generally decrease with both longer growing seasons and shorter day lengths at lower latitudes. These results show that there was sufficient genetic variation from the two German source populations to evolve different photoperiod responses across a range of environmental conditions. This study adds to the examples of introduced insects rapidly evolving in response to novel exposure to day length cues, which may allow for diapause timing and voltinism to fit different season lengths.

Keywords: Classical biological control, critical photoperiod, diapause, rapid evolution, voltinism

Introduction

One of the key questions in classical biological control is to what extent insect agents evolve to novel environment conditions in the introduced range (Roderick et al. 2012). Insect lifecycles in temperate biomes are constrained by extrinsic factors like the length of the growing season and intrinsic traits such as temperature-dependent development time (Danilevskii 1965, Tauber et al. 1986). Multivoltine insects, which could potentially fit more than one generation in a year, have evolved genetic-based responses to environmental cues that determine when populations switch developmental pathways from reproduction to diapause (Tauber et al. 1986, Denlinger et al. 2017). Day length, which varies seasonally and across latitudes, is the principal cue insects in temperate zones use to initiate diapause development before the end of the growing season (Tauber et al. 1986, Bradshaw and Holzapfel 2007). The ubiquity of observed latitudinal clines in voltinism and photoperiod responses in native and long established insects (review by Masaki 1961, Danilevskii 1965, Tauber et al. 1986) suggests that similar selective pressures will drive geographic divergence in these seasonal adaptations in introduced insects.

In this study, we experimentally compared the diapause response and development rates in 6 populations of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae), a leaf beetle introduced across North American as a biological control agent for the wetland weed purple loosestrife (*Lythrum salicaria* L.). Our study is preceded by a phenology model applied to this insect (Grevstad and Coop 2015) that demonstrates how the introduction of insects to new photoperiod regimes and climates may disrupt the coordination between growing season length and diapause timing that evolved in the native range. Newly introduced insects can experience mismatches between the number of generations *attempted* based on photoperiod-induced diapause and the *potential* number of generations that would be available with a well-adapted

photoperiodic response (Bean et al. 2007, Grevstad and Coop 2015). Attempting reproduction too late in the season could lead to high mortality as life stages unable to diapause confront winter (Van Dyck et al. 2015, Kerr et al. 2020). Entering diapause too early in the growing season can lead to greater overwintering mortality because of the extended duration of diapause (Bean et al. 2007). These mismatches set up selective pressure for the photoperiod response and development time to adapt.

Biological control introductions draw from limited source populations in the agent's native range so that prerelease host range tests accurately assess the risk of nontarget attacks (Hinz et al. 2014). Constrained genetic variation, due to small numbers from a few sources, may limit the success of agents released across a wide range of environmental conditions (Roderick et al. 2012). While often assumed that newly introduced biocontrol agents undergo a period of adaptation following introduction, seldom are lifecycle changes documented. Examples include changes in springtime phenology in the ragwort flea beetle *Longitarsus jacobaeae* (Szűcs et al. 2012), divergence in developmental rates between alpine and valley populations of the cinnebar moth *Tyria jacobaeae* (McEvoy et al. 2012), and a shortening of the critical photoperiod that cues diapause in the tamarisk leaf beetle *Diorhabda carinulata* (Bean et al. 2012). In the case of the *D. carinulata*, the evolution in photoperiod response allowed populations to increase voltinism and extend geographic range boundaries beyond what was predicted based on the photoperiod response of the original introductions (Bean et al. 2007, 2012).

In this study, *G. calmariensis* were reared in controlled environmental chambers set with different artificial day lengths to compare thresholds for diapause induction and development rates. We show that within 27 years post-introduction, these two traits have diverged across

populations in a manner that corresponds to the variation in growing season length and day length exposure at sites spanning 9.4° of latitude in the Western USA.

Materials and Methods

Study system

Galerucella calmariensis was first introduced in 1992 across the United States and Canada as a classical biological control agent for purple loosestrife (*L. salicaria*), a noxious invasive weed common in wetlands (Hight et al. 1995). It established populations at sites with different climate regimes and photoperiod exposure (36-50°N) compared to its two source populations in Germany (50 and 54°N) (Hight et al. 1995). *Galerucella calmariensis* was introduced alongside its congener *Galerucella pusilla* Duftschmidt, which coexists in the same habitat, or even on the same plant, without apparent differences in life history or ecological niche (Blossey 1995). We focus on *G. calmariensis* in this study, although *G. pusilla* was also present at some of the sites.

Galerucella calmariensis has a facultative multivoltine lifecycle. Adults overwinter and emerge as reproductive regardless of photoperiod exposure in spring (Blossey 1995, Bartelt et al. 2008). Newly eclosed (teneral) adults from subsequent generations are photoperiod sensitive and will enter diapause in response to short days (Velarde et al. 2002). Source populations in Germany have been reported as univoltine, with a small amount of oviposition from the summer generation before entering diapause (Blossey 1995). The critical photoperiod (=critical day length), the exposure at which half of beetles would enter diapause and half would reproduce, was estimated between 15-15.5 daylight hours in the introduced beetles based on conditions observed during lab rearing (Grevstad 1999, Bartelt et al. 2008). A lifecycle model of *G.*

calmariensis predicts potential voltinism of 1-4 generations in the western USA corresponding to the degree-days available for development (Grevstad and Coop 2015).

We studied six beetle populations that established in the states of California, Oregon, and Washington after their introduction in the 1990s. Some of these populations were started with German beetle stocks (Hight et al. 1995) and others were established by redistributing from successfully established populations years later (Pitcairn personal communication). These populations span a wide range of environmental conditions across 9.4° latitude (Table 1). For each location, we summarized the average annual weather since beetle introduction (1994-2018) using Daymet daily gridded interpolations of weather station observations (Thornton et al. 1997, 2017). Grevstad and Coop's (2015) degree-day model was used to predict phenology and the sensitive stage's exposure to day length that varies with latitude and the day of year (Forsythe et al. 1995).

Growth chamber experiments

We reared populations in a common environment to attribute phenotypic variation to genetic differences between populations (Merilä and Hendry 2014). We carried out two similar experiments (in 2014 and 2019) to compare the photoperiod diapause response among *G. calmariensis* populations and one experiment (in 2018) to compare their rates of development from egg to adult. All experiments were performed in SG3-22 controlled environmental chambers (Hoffman Manufacturing, Inc.) equipped with six 32-watt 5000K tube fluorescent lights on the walls of each chamber. At least one generation of rearing in a common environment preceded each experiment to control for maternal effects. We grew *L. salicaria* host plants from seeds collected in Portland, OR (45.471, -122.657). The experiments differed slightly in the method details as described below.

Photoperiod-based diapause: 2014

Galerucella calmariensis eggs and early instar larvae were collected from established field populations in Palermo, California and Sutherlin, Oregon in early May and adults were collected near Ephrata and Bellingham, Washington in late May. We reared them in a greenhouse inside 13.5 x 13.5 x 24 inch mesh cages with potted plants until the next generation's adults emerged. After the new adults started mating, we obtained same-age cohorts of eggs from each population by placing 3 adult pairs on each of 12 caged potted plants (15-20 cm tall). This resulted in roughly 90 eggs per plant. When larvae started to hatch, whole plants were placed into growth chamber treatments (3 plants per population per photoperiod treatment) and beetles developed to adults. The photoperiod treatments in chambers were 14.75/9.25, 15.25/8.75, 15.75/8.25, and 16.25/7.75 hours of light/dark (L/D) per day. To provide additional light for plant growth, two 24-watt 6500K tube fluorescent tube lights were suspended above the plants as a supplement to the built-in lights on the walls of the chamber. These were set to go on 15 minutes after and off 15 minutes before the built-in lights to emulate twilight. Chambers were maintained at 23°C constant temperature for day and night. We assessed the developmental status (reproductive versus diapausing) in adult females by putting them individually into petri dishes with fresh leaves for 24 hours and observing if feeding and oviposition occurred.

Development time: 2018

We intended our 2018 experiment to measure both diapause response and development timing. However, the range of photoperiod treatments used (from 14.75/9.25 to 16.75/7.25) combined with the cooler 21°C temperature (compared to 2014) induced a high diapause rate across photoperiods so we were unable to obtain comparable diapause response curves.

Therefore, we present it only as a test comparing development times among populations and photoperiod treatments.

We collected 50-100 adult beetles from four sites (Bellingham, Washington, Yakima Training Center, Washington, Rickreall, Oregon, and Sutherlin, Oregon) between April 26 and May 8, 2018. Beetles from the two sites in California (McArthur and Palermo) were collected a month later. After rearing for a generation in a greenhouse, we moved reproductive adults onto caged potted plants in growth chambers on July 3, 2018 at 17.0/8.0 L/D hours and 21°C constant temperature.

We placed 30-35 beetles on potted plants in tubes to lay eggs over 24 hours so we could track age to the nearest day. After removing the adults, the plants with eggs were placed into photoperiod treatments of 14.75/9.25, 15.75/8.25, 16.25/7.75, and 16.75/7.25 L/D hours at 21°C. We moved adults onto a new plant to have 2-3 replicates for each population spaced a day apart. Shortly before hatching, eggs were transferred onto fresh leaves in 150mm-diameter petri dishes lined with moist filter paper at a density of 30 to 50 eggs per dish and returned to the growth chambers. We added fresh leaves as needed and 10 grams of potting soil to each dish when the beetles reached the 3rd instar. We removed new adults every day starting several days before we predicted pupation would end based on the lifecycle degree-day requirements (McAvoy and Kok 2004). We used days from eggs laid to adult eclosion for each individual as the dependent variable in models.

<u>Photoperiod-based diapause: 2019</u>

Diapausing beetles from the two shortest day length treatments in the 2018 experiment, where all beetles entered diapause, were stored outdoors in mesh cages with leaf litter from November 2018 through April 2019 in Corvallis, Oregon. These beetles emerged naturally and

their offspring entered this experiment. This experiment differed from the one carried out in 2014 in that larvae were reared in petri dishes (approximately 30 individuals per dish), no supplemental lights were added to the chambers (used just built in lights), and both males and females were assessed for diapause. The same rearing methods as in 2018 described above were repeated but using a higher constant temperature of 23°C. We assigned two dishes per population to six photoperiod treatments at 14.5/9.5, 15.0/9.0, 15.5/8.5, 16.0/8.0, 16.5/7.5, and 17.0/7.0 L/D hours. As they emerged each day, we moved teneral adults into group trays with fresh leaves for the pre-oviposition or pre-diapause development period. About 12-14 days after the last adult emerged, adults were moved to individual petri dishes with fresh leaves for a 48-hour diapause assessment. Each beetle was scored as a reproductive female if eggs were present, a reproductive male if feeding was present without eggs, and diapause (sex not determined) if no feeding or eggs were present. The proportion reproductive for each rearing dish was the dependent variable.

Statistical analysis

We performed all analysis and visualization with R 3.6.2 (R Core Team 2019). Each year's data (2014 and 2019) were analyzed separately for photoperiod-induced diapause rates with generalized linear mixed effects models with a binomial error distribution and logit link using the *lme4* package (Bates et al. 2015). The main effects of population (categorical covariate) and photoperiod treatment (scaled continuous covariate) and their interaction predicted the proportion reproductive in each rearing dish (weighted by the total number of beetles in each dish). As rearing dishes contained groups of beetles from the same population and treatment, they were assigned random intercepts to account for non-independence. We removed the interaction term if non-significant ($p > 0.05$) by a Chi-square test. Population differences in responses (estimated marginal means) were compared using the *emmeans* package with post-hoc

pairwise comparisons while adjusting for multiple comparisons using Tukey's method as well as linear contrasts between groups of populations (Lenth 2020). Populations' critical photoperiods (=critical day lengths), the hours of light exposure at which half of the beetles are predicted to diapause, were calculated with 95% confidence intervals using inverse predictions from the generalized linear mixed models (Venables and Ripley 2002).

We tested if development time was affected by population and photoperiod exposure with a generalized linear mixed effects model with a gamma error distribution and log link using the *lme4* package (Bates et al. 2015). The main effects of population (categorical covariate) and photoperiod treatment (scaled continuous covariate) and their interaction predicted the days from oviposition to adult eclosion for each beetle. Rearing dishes, with beetles from the same population and oviposition day, were assigned random intercepts to account for their non-independence. We removed the interaction term if non-significant ($p > 0.05$) by a Chi-square test. Population differences in responses (estimated marginal means) were compared using the *emmeans* package with post-hoc pairwise comparisons while adjusting for multiple comparisons using Tukey's method as well as linear contrasts between groups of populations (Lenth 2020).

Results

Photoperiod responses

All populations exhibited a short-day diapause response where shorter photoperiod treatments induced diapause and longer photoperiod treatments resulted in reproduction (Table 2, Table 3, Figure 1, Figure 2). Populations diverged in their critical photoperiods, defined as the day length at which half of the population reproduces, by up to 0.7 hours in the 2014 experiment and 2.3 hours in the 2019 experiment (Table 2). In the 2014 experiment, the two southern populations together are 19 times (odds ratio, SE = 11.5, $P < 0.0001$) more likely to reproduce

across day length treatments as the two northern populations together (Figure 1). In the 2019 experiment, the six populations follows a latitudinal gradient where the three southern populations together are 8.2 times (odds ratio, SE = 2.54, $P < 0.0001$) more likely to reproduce at the same day length exposure as the three northern populations together (Figure 2).

However, the six populations tested in 2019 do not have a monotonic decrease in critical photoperiod with latitude (Figure 3, Table 2). For example, the two central populations (Rickreall and Sutherlin, Oregon) have the longest (16.8, CI 16.3-17.3) and shortest (14.5, CI 14.0-15.0) critical photoperiods, respectively (3.87 difference, SE = 0.551, $P < 0.001$ on logit scale). Populations from McArthur, California and Yakima Training Center, Washington have similar critical photoperiods in spite of their separation of 5.7° latitude (0.00679 difference, SE = 0.502, $P > 0.999$).

There is no interaction between population and photoperiod response in either experiment, meaning that the slopes of the photoperiod response curves do not significantly differ across populations (Table 3). Comparing the slopes between our separate experiments, the photoperiod response curves have gentler slopes across populations in the 2019 experiment (with both males and females) compared to the 2014 experiment (only females). This indicates that rearing dishes from 2019 have a greater mixture of individuals choosing diapause and reproductive pathways in response to the same photoperiod treatments (Figure 2 compared to Figure 1).

Development time

Development time averaged 33.5 days and did not change with photoperiod treatments (Table 3, Figure 4). Populations had similar development times with the exception that the Rickreall, Oregon population required an additional day of development compared to the

Sutherlin, Oregon population (0.0419 difference on log scale, SE = 0.0127, P = 0.0122) and the Yakima Training Center, Washington population (0.0364 difference on log scale, SE = 0.0127, P = 0.0471). There was no interaction between population and photoperiod for development time (Table 3).

Discussion

In a common environment, populations of the introduced leaf beetle *Galercuella calmariensis* have genetically diverged in their diapause response to photoperiod across 9.4° of latitude. The measured critical photoperiods, where 50% of the population enters diapause, ranged from 14.5 to 16.8 hours of daylight and correspond with latitude with notable exceptions explored below (Figure 3, Table 2). Although the critical photoperiods of the two German source populations were not experimentally measured prior to the agent's introduction to the western USA in 1990s, a critical photoperiod of between 15 and 15.5 day hours was reported in earlier studies (Grevstad 1999, Bartelt et al. 2008). Mismatched photoperiod responses may explain some of the variation in initial establishment and performance of these beetles (Grevstad and Coop 2015). However, our results suggest that *G. calmariensis* has rapidly evolved photoperiod responses that resemble the adaptive latitudinal gradients in native insects.

Not all genetically-based phenotypic differences are necessarily adaptive (Kawecki and Ebert 2004, Merilä and Hendry 2014) and our experiments were not designed to confirm that these population differences are local adaptations to seasonal conditions. Nonetheless, it is reasonable to assume that natural selection is one cause for divergence in photoperiod responses across latitudes as in other temperate organisms (Bradshaw and Holzapfel 2007). Photoperiod responses in native insects provide a baseline expectation for how locally-adapted populations vary across latitudes, with shorter critical photoperiods at warmer latitudes that induce diapause

later in the year (Masaki 1961, Danilevskii 1965). Introduced insects have rapidly evolved latitudinal clines in photoperiod responses in the eastern United States, where there is a clear correlations between latitude (with corresponding day length cues) and growing season length (Urbanski et al. 2012, Levy et al. 2015). Populations of *G. calmariensis* do not follow a strictly monotonic latitudinal gradient in photoperiod responses in the more heterogeneous climate variation in the western United States (Figure 3). Higher elevation (McArthur, California) and coastal influence (Bellingham, Washington and Rickreall, Oregon) shorten the season length in terms of degree-days available for development (Table 1). Critical photoperiods from these three populations, when compared to summer day length, would lead to fewer attempted generations per year compared with the three populations at warmer sites within their respective states (Figure 3).

The critical photoperiods for many of the sampled populations appear to allow an appropriate number of generations for the season duration in degree-days, with exceptions of Palermo, California and Rickreall, Oregon. The population at Palermo, CA has a relatively short critical photoperiod of around 15.3 day hours (average of two experiments), corresponding with its southern latitude. However, this critical photoperiod is still longer than the day length when adult eclosion is predicted to occur in mid-May (14.4 day hours) and thus would tend to induce diapause after just one generation. A possible explanation for why the critical photoperiod for this population has not evolved to be even shorter, which would allow multiple generations through the summer, is that the *L. salicaria* host plants at this site regularly dry up completely by early July. If the population did evolve a shorter critical photoperiod it could add one more successful generation before plant senescence, but then it would also attempt an unsuccessful third generation (absent green host plants) because the day lengths would not fall below the

critical photoperiod again until late summer. Given this developmental conundrum, it is not surprising that the beetles were initially difficult to establish and have had little impact on the target weed at this site (Pitcairn personal communication). They also have failed to establish at California locations further to the south (Pitcairn 2018).

The population at Rickreall, Oregon has a long critical photoperiod, at 16.8 hours, which would limit it to one generation, given that the longest day length at the summer solstice is 15.8 hours. Field observations from recent years confirm a single generation at this site, whereas the Sutherlin, Oregon population just 1.6° latitude to the south and unsampled populations within 0.5° latitude to the north have 2 generations (F. Grevstad personal observations). It is unclear why this population should be limited to one generation given sufficient degree days for two generations and plants that remain green until early fall. Interestingly, we found that the Rickreall population developed slower than the other populations, albeit only by a day. Relaxation of selection for faster development may be a result of the predominantly univoltine lifecycle at this site. Other insects have evolved “sawtooth” clines in development times across latitudes in addition to locally-adapted photoperiod responses, which has the added effect of limiting attempted voltinism in places where season length is only marginally supportive of adding another generation (Levy et al. 2015). In contrast, we did not find support for photoperiod-modified development rates (Table 3), with faster growth at shorter day lengths, another trait which would improve the fit of voltinism to the season length (Lindestad et al. 2019).

Because the photoperiod response experiments used different methods, we did not carry out a detailed comparison between the photoperiod responses measured in 2014 and those measured in 2019 from the same populations collected four years apart. However, it is certainly

possible that evolution in the photoperiod response could occur in a time frame of years (Bean et al. 2012, Urbanski et al. 2012) or even annually with strong fluctuating selection (Bell 2010). For example, if an early freeze in a given year meant that all of the individuals whose parents had a shorter critical photoperiod (i.e. reproduced late) were killed, that could shift the critical photoperiod shorter in the subsequent year if heritability were high (Taylor and Spalding 1989). A surprising difference in the photoperiod response curves between our two experiments is the more gradual slopes and smaller critical photoperiods in 2019 (Table 2, Figure 3). It may be that the differences in rearing method or light intensity between the two experiments affected the response to photoperiod. It is also possible that males, which were included in the sample in 2019 but not 2014, may have a greater tendency to remain reproductive at lower photoperiods leading to a shorter critical photoperiod (Kerr et al. 2020).

Based on this study, there was sufficient genetic variation from two source populations to evolve divergent photoperiod responses across a range of environmental conditions within 27 years. We do not know if these populations are adapted to the long-term variability in season lengths at their respective sites or if critical photoperiods will continue to change. The genetic evolution of *G. calmariensis*, as well as other biological control agents (Bean et al. 2012, McEvoy et al. 2012, Szűcs et al. 2012), demonstrates the importance of season length as a selective force over ecological timescales. Screening for intrinsic traits or environmental factors that predict successful establishment and population growth of biological control agents would advance our ability to manage introductions (Zalucki and van Klinken 2006, Abram and Moffat 2018). More targeted matching of observed photoperiod responses in the native range combined with lifecycle simulations of growing season lengths in the introduced range may reduce the

length of time or number of introductions needed to establish robust populations (Grevstad and Coop 2015, Pitcairn 2018)..

Acknowledgments

We thank Lisa Weigel at Yakima Training Center and Mike Pitcairn and Baldo Villegas at the California Department of Food and Agriculture for support with site selection and collections. We thank Brittany Barker, Len Coop, Katarina Lunde, Monte Mattson, and Peter McEvoy for feedback on manuscript drafts. FG received funding from the DOD Strategic Environmental Research and Development Program (US Army Corps of Engineers, Contract No. W912HQ-17-C-0051) and the USDA Forest Service cooperative agreement 10-CA-11420004-255.

References

Abram, P. K., and C. E. Moffat. **2018**. Rethinking biological control programs as planned invasions. Curr. Opin. Insect Sci. 27: 9–15.

Bartelt, R. J., A. A. Cossé, B. W. Zilkowski, R. N. Wiedenmann, and S. Raghu. **2008**. Early-summer pheromone biology of *Galerucella calmariensis* and relationship to dispersal and colonization. Biol. Control. 46: 409–416.

Bates, D., M. Mächler, B. Bolker, and S. Walker. **2015**. Fitting linear mixed-effects models using **lme4**. J. Stat. Softw. 67.

Bean, D. W., P. Dalin, and T. L. Dudley. **2012**. Evolution of critical day length for diapause induction enables range expansion of *Diorhabda carinulata*, a biological control agent against tamarisk (*Tamarix* spp.): Evolution and range expansion of a biocontrol agent. Evol. Appl. 5: 511–523.

Bean, D. W., T. L. Dudley, and J. C. Keller. **2007**. Seasonal timing of diapause induction limits the effective range of *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) as a biological control agent for Tamarisk (*Tamarix* spp.). Environ. Entomol. 36: 15–25.

Bell, G. 2010. Fluctuating selection: the perpetual renewal of adaptation in variable environments. Philos. Trans. R. Soc. B Biol. Sci. 365: 87–97.

Blossey, B. 1995. Coexistence of two leaf-beetles in the same fundamental niche, distribution, adult phenology and oviposition. Oikos. 74: 225.

Bradshaw, W. E., and C. M. Holzapfel. **2007**. Evolution of animal photoperiodism. Annu. Rev. Ecol. Evol. Syst. 3: 1–25.

Danilevskii, A. S. 1965. Photoperiodism and seasonal development of insects.

Denlinger, D. L., D. A. Hahn, C. Merlin, C. M. Holzapfel, and W. E. Bradshaw. **2017**. Keeping time without a spine: what can the insect clock teach us about seasonal adaptation? Philos. Trans. R. Soc. B Biol. Sci. 372: 20160257.

Forsythe, W. C., E. J. Rykiel, R. S. Stahl, H. Wu, and R. M. Schoolfield. **1995**. A model comparison for daylength as a function of latitude and day of year. Ecol. Model. 80: 87–95.

Grevstad, F. S. 1999. Experimental invasions using biological control introductions: the influence of release size on the chance of population establishment. Biol. Invasions. 1: 313–323.

Grevstad, F. S., and L. B. Coop. **2015**. The consequences of photoperiodism for organisms in new climates. Ecol. Appl. 25: 1506–1517.

Hight, S. D., B. Blossey, J. Laing, and R. Declerck-Floate. **1995**. Establishment of insect biological control agents from Europe against *Lythrum salicaria* in North America. Environ. Entomol. 24: 967–977.

Hinz, H. L., M. Schwarzländer, A. Gassmann, and R. S. Bourchier. **2014**. Successes we may not have had: A retrospective analysis of selected weed biological control agents in the United States. Invasive Plant Sci. Manag. 7: 565–579.

Kahle, D., and H. Wickham. **2013**. **ggmap**: Spatial Visualization with **ggplot2**. R J. 5: 144–161.

Kawecki, T. J., and D. Ebert. **2004**. Conceptual issues in local adaptation. Ecol. Lett. 7: 1225–1241.

Kerr, N. Z., T. Wepprich, F. S. Grevstad, E. B. Dopman, F. S. Chew, and E. E. Crone. **2020**. Developmental trap or demographic bonanza? Opposing consequences of earlier

phenology in a changing climate for a multivoltine butterfly. Glob. Change Biol. 26: 2014–2027.

Lenth, R. 2020. **emmeans**: Estimated marginal means, aka least-squares means.

Levy, R. C., G. M. Kozak, C. B. Wadsworth, B. S. Coates, and E. B. Dopman. **2015**. Explaining the sawtooth: latitudinal periodicity in a circadian gene correlates with shifts in generation number. J. Evol. Biol. 28: 40–53.

Lindestad, O., C. W. Wheat, S. Nylin, and K. Gotthard. **2019**. Local adaptation of photoperiodic plasticity maintains life cycle variation within latitudes in a butterfly. Ecology. 100: e02550.

Masaki, S. 1961. Geographic variation of diapause in insects.

McAvoy, T. J., and L. T. Kok. **2004**. Temperature dependent development and survival of two sympatric species, *Galerucella calmariensis* and *G. pusilla*, on purple loosestrife. BioControl. 49: 467–480.

McEvoy, P. B., K. M. Higgs, E. M. Coombs, E. Karaçetin, and L. Ann Starcevich. **2012**. Evolving while invading: rapid adaptive evolution in juvenile development time for a biological control organism colonizing a high-elevation environment. Evol. Appl. 5: 524–536.

Merilä, J., and A. P. Hendry. **2014**. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol. Appl. 7: 1–14.

Pitcairn, M. J. 2018. Weed biological control in California, USA: review of the past and prospects for the future. BioControl. 63: 349–359.

R Core Team. **2019**. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Roderick, G. K., R. Hufbauer, and M. Navajas. **2012**. Evolution and biological control. Evol. Appl. 5: 419–423.

Szűcs, M., U. Schaffner, W. J. Price, and M. Schwarzländer. **2012**. Post-introduction evolution in the biological control agent *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae): Rapid evolution in a biological control agent. Evol. Appl. 5: 858–868.

Tauber, M. J., C. A. Tauber, and S. Masaki. **1986**. Seasonal adaptations of insects. Oxford University Press.

Taylor, F., and J. B. Spalding. **1989**. Timing of diapause in relation to temporally variable catastrophes. J. Evol. Biol. 2: 285–297.

Thornton, P. E., S. W. Running, and M. A. White. **1997**. Generating surfaces of daily meteorological variables over large regions of complex terrain. J. Hydrol. 190: 214–251.

Thornton, P. E., M. M. Thornton, B. W. Mayer, Y. Wei, R. Devarakonda, R. S. Vose, and R. B. Cook. **2017**. Daymet: Daily surface weather data on a 1-km grid for North America, version 3. ORNL Distributed Active Archive Center.

Urbanski, J., M. Mogi, D. O'Donnell, M. DeCotiis, T. Toma, and P. Armbruster. **2012**. Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient. Am. Nat. 179: 490–500.

Van Dyck, H., D. Bonte, R. Puls, K. Gotthard, and D. Maes. **2015**. The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? Oikos. 124: 54–61.

Velarde, R. A., R. N. Wiedenmann, and D. J. Voegtlin. **2002**. Influence of photoperiod on the overwintering induction of *Galerucella calmariensis* L. BioControl. 47: 587–601.

Venables, W. N., and B. D. Ripley. **2002**. Modern applied statistics with S, Fourth. ed. Springer, New York.

Zalucki, M. P., and R. D. van Klinken. **2006**. Predicting population dynamics of weed biological control agents: science or gazing into crystal balls? Aust. J. Entomol. 45: 331–344.

Tables

Table 1: Description of sites in the western USA with introduced *Galerucella calmariensis* populations.

Site	Elevation (meters above sea level)	Max day length (hours)[a]	Avg daily max/min temp (°C)	Avg annual precipitation (cm)	Avg annual degree-days (SD)[b]	Avg frost-free span in days (SD)[c]	Avg sensitive stage first emergence[d]	Population history (year introduced)[e]
Bellingham, Washington (48.76, -122.48)	17	16.3	15.2/6.5	99.2	1067 (122)	228 (24)	July 20	From Grant County, WA (unknown)
Ephrata, Washington (47.16, -119.66)	350	16.1	17.3/4.7	22.5	1677 (127)	182 (18)	June 24	Grant County, WA: from both German sources (1992-93)
Yakima Training Center, Washington (46.76, -119.99)	204	16.0	18.4/5.1	19.6	1809 (131)	189 (20)	June 19	From Grant County, WA (unknown)
Rickreall, Oregon (44.98, -123.27)	64	15.8	17.5/6.3	134.5	1366 (114)	227 (28)	July 9	Polk County, OR: from south German source (1992)
Sutherlin, Oregon (43.39, -123.32)	153	15.6	19.0/6.6	107.7	1601 (143)	236 (34)	June 27	Douglas County, OR: from south German source (1992)
McArthur, California (41.10, -121.41)	1006	15.3	19.2/3.2	78.6	1559 (122)	146 (24)	July 1	From Polk/Marion Counties, OR (1998, 2000) & Grant County, WA (2002)
Palermo, California (39.41, -121.58)	35	15.1	24.4/10.0	73.6	2769 (175)	299 (29)	May 13	From Polk/Marion Counties, OR (1998-2001) & McArthur, CA (2006). Grant County, WA release 10km northwest (2004-05)

The average annual weather over 25 years (1994-2018) is summarized from Daymet daily gridded interpolations of weather station observations with standard deviations (SD).

[a]Maximum day length includes twilight until the sun is 1.5° below the horizon (using method of Forsithe et al. 1995).

[b]Degree-days are calculated with the triangle method with 10°C/30°C thresholds.

[c]Frost days are defined as days with minimum temperatures at or below 0°C. Span is days between last spring frost and first fall frost.

[d]Predicted sensitive stage emergence is based on 25 years (1994-2018) of results from a degree-day lifecycle model (Grevstad and Coop 2015).

[e]History is from Hight et al. (1995) for original releases in Washington and Oregon and from M. Pitcairn (personal communication) for transfers to California

Table 2: Photoperiod responses of *G. calmariensis* populations in two experiments at constant 23°C temperatures.

	2014 Photoperiod response					2019 Photoperiod response				
Predictors	Beta	Std. error	*P*	CP[a] (95% CI)	Population differences[b]	Beta	Std. error	*P*	CP (95% CI)	Population differences
Photoperiod: light hours	4.05	0.67	<0.001	-	-	1.40	0.15	<0.001	-	-
Bellingham, Washington	-2.20	0.58	<0.001	16.2 (15.9-16.4)	A	-1.45	0.39	<0.001	16.6 (16.1-17.0)	AB
Ephrata, Washington	-2.43	0.59	<0.001	16.2 (16.0-16.4)	A	-	-	-	-	-
Yakima TC, Washington	-	-	-	-	-	-0.33	0.36	0.368	15.9 (15.5-16.3)	BC
Rickreall, Oregon	-	-	-	-	-	-1.85	0.39	<0.001	16.8 (16.3-17.3)	A
Sutherlin, Oregon	0.40	0.52	0.443	15.6 (15.4-15.8)	B	2.02	0.38	<0.001	14.5 (14.0-15.0)	D
McArthur, California	-	-	-	-	-	-0.32	0.35	0.356	15.9 (15.5-16.3)	BC
Palermo, California	0.86	0.49	0.080	15.5 (15.3-15.7)	B	0.99	0.35	0.005	15.1 (14.7-15.5)	CD

The main effects of photoperiod treatment and population come from two generalized linear mixed models (logistic regression), one for each year's experiment. Figures 1 and 2 plot these model results.

[a]Critical photoperiod (CP), or the light hours at which 50% would enter diapause, was estimated for each population and year with 95% confidence intervals by inverse predictions from the models.

[b]Populations with significant differences have different letters, which come from pairwise comparison tests with Tukey's method to adjust *P* values.

Table 3: ANCOVA results for tests of photoperiod and population effects on diapause proportion and development time.

	2014 Diapause			2019 Diapause			2018 Development time		
Fixed effects	Chi square	df	*P*	Chi square	df	*P*	Chi square	df	*P*
Photoperiod (light hours)[a]	36.6	1	<0.001	88.4	1	<0.001	0.0396	1	0.842
Population	25.8	3	<0.001	74.4	5	<0.001	14.8	5	0.0113
One-way interaction	1.41	3	0.703	7.29	5	0.200	9.62	5	0.0869
	Groups	N	SD	Groups	N	SD	Groups	N	SD
Random effects[b]	16	556	0.602	71	1782	0.983	71	1808	0.0135

Type II sum of squares (Wald chi square tests) from generalized linear mixed models.
[a]Photoperiod is modeled as a continuous variable and had different growth chamber treatments in different experiments.
[b]Groups were used as random intercepts in all models based on rearing dishes. Sample size (N) refers to individuals within rearing dishes. Standard deviation (SD) of the random intercepts shows between-group variation.

Figures

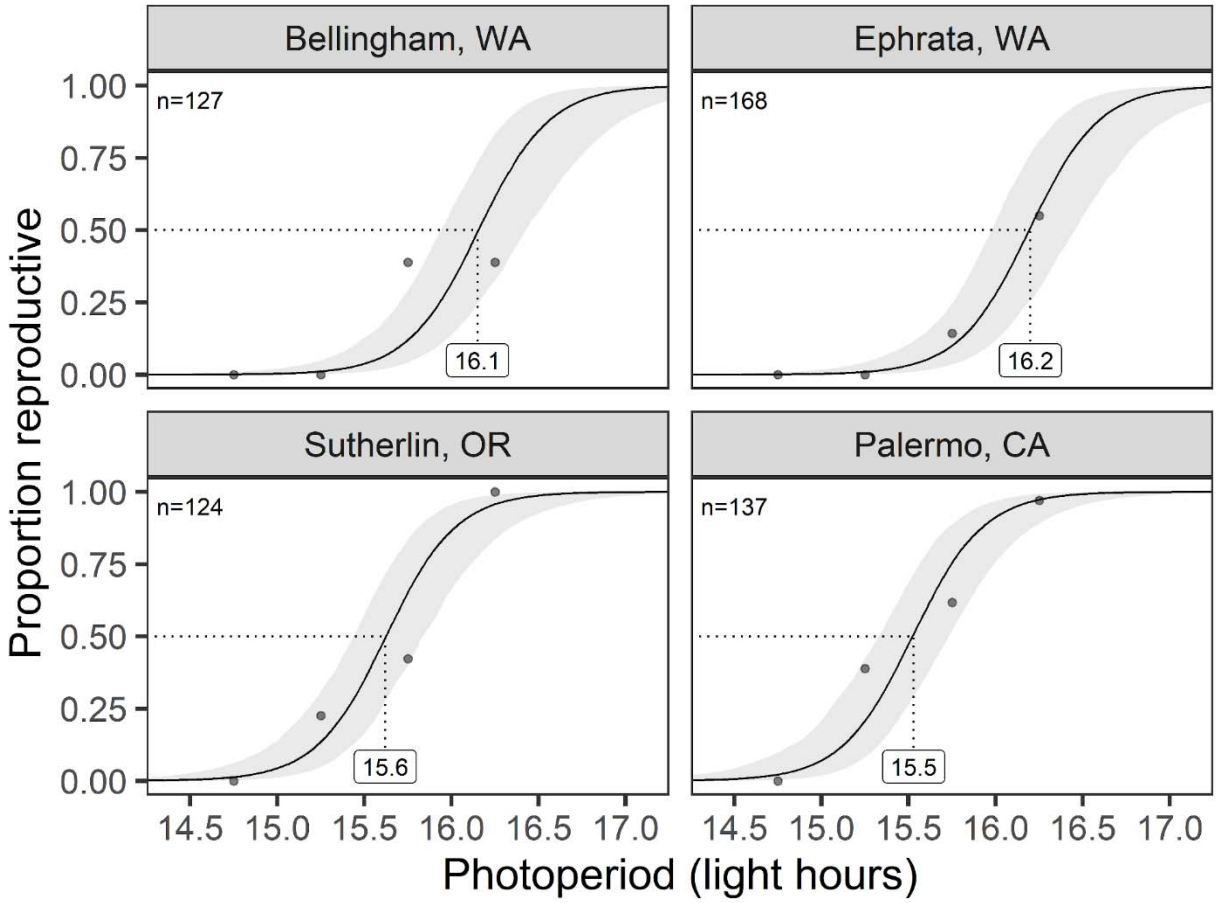

Figure 1: Photoperiod responses diverged between northern and southern populations in 2014. Each data point shows the proportion of female beetles that were reproductive for each of four populations (panels) and four photoperiod treatments (x-axis). Solid lines and 95% confidence intervals show generalized linear mixed model predictions. The critical photoperiods, at which 50% choose to diapause, are labeled below the dotted lines. Number of beetles tested (n) from each population is in each panel.

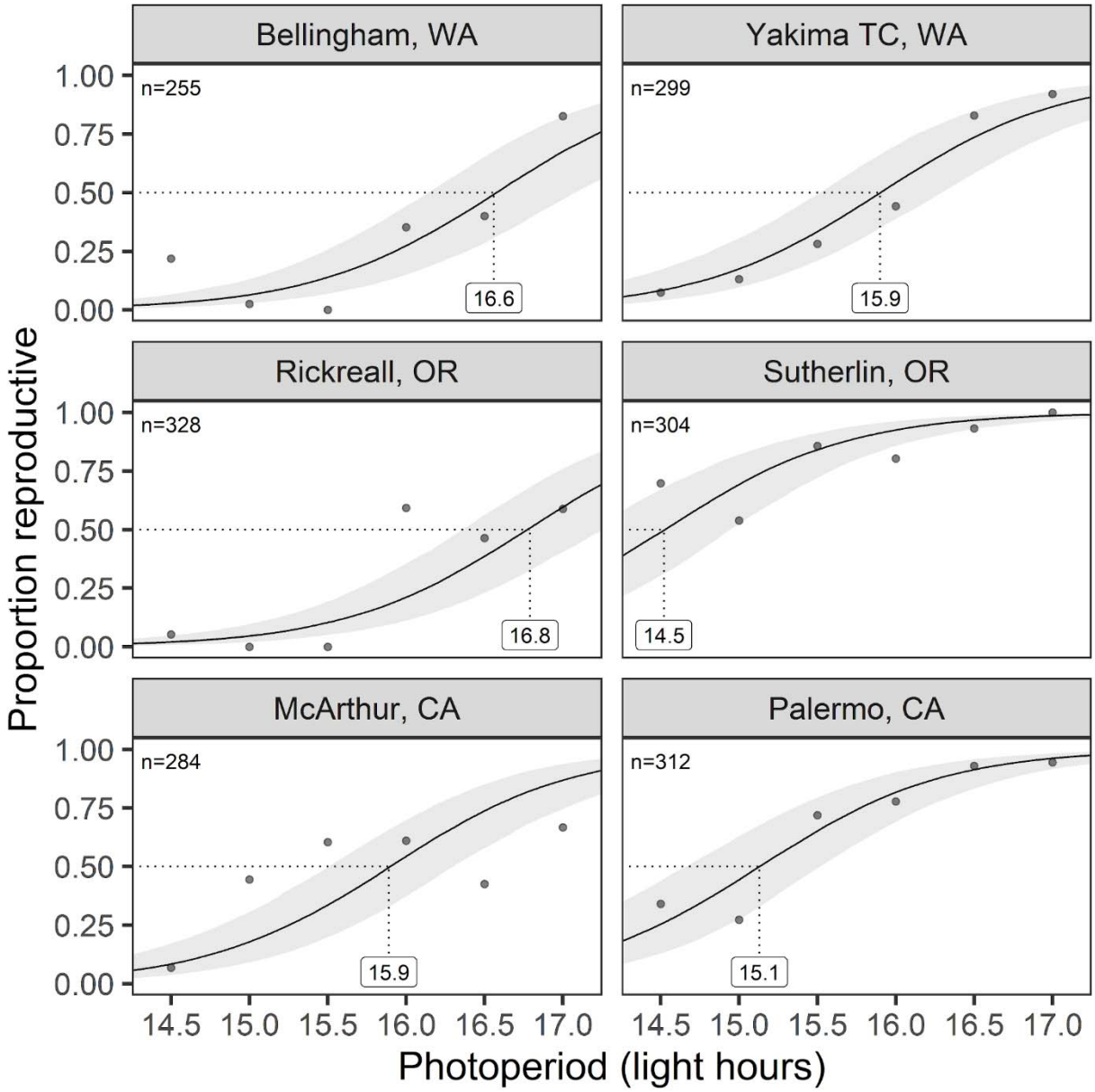

Figure 2: Photoperiod responses diverged between populations in 2019. Each data point shows the proportion of male and female beetles that were reproductive for each of six populations (panels) and six photoperiod treatments (x-axis). Solid lines and 95% confidence intervals show generalized linear mixed model predictions. The critical photoperiods, at which 50% choose to diapause, are labeled below the dotted lines. Number of beetles tested (n) from each population is in each panel.

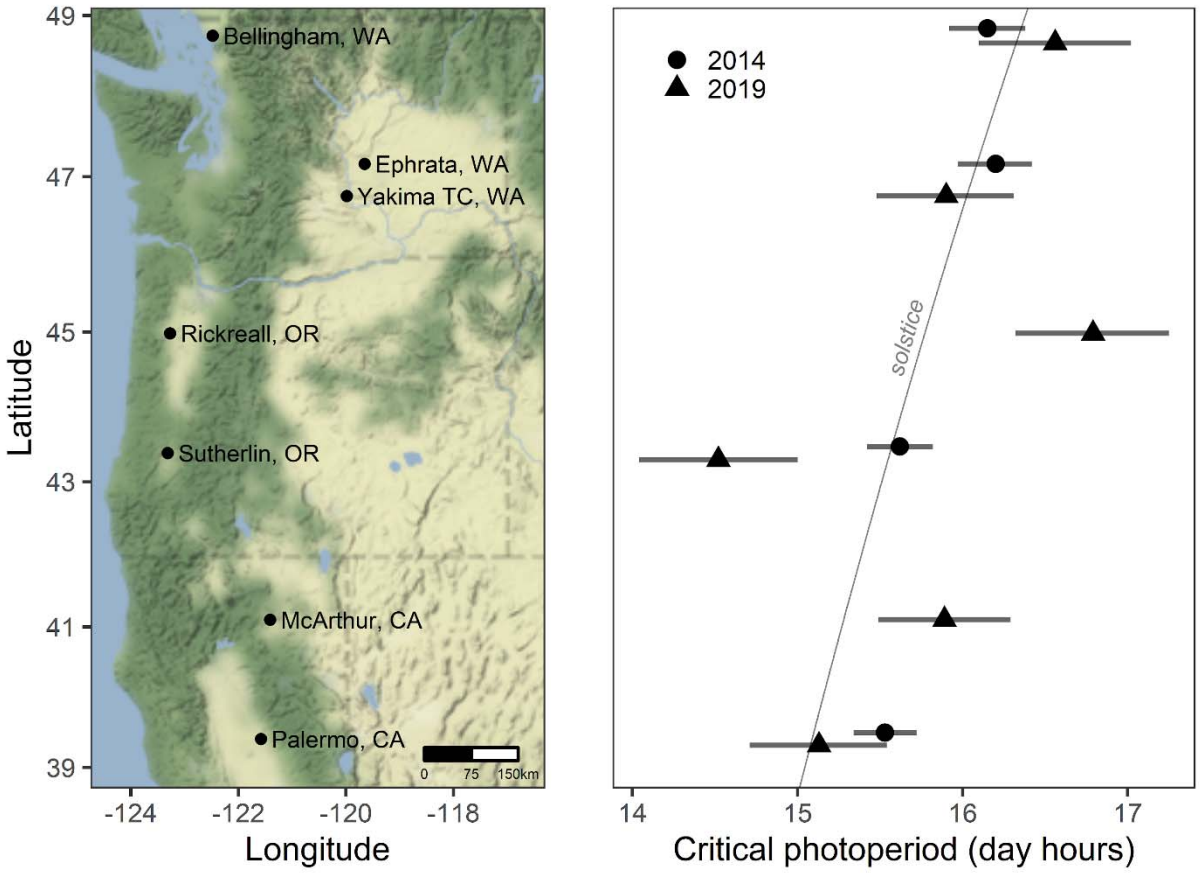

Figure 3: Latitudinal gradient in critical photoperiods of *Galerucella calmariensis* populations in the northwest USA. Critical photoperiods (with 95% confidence intervals) for populations were estimated from growth chamber experiments in 2014 and 2019 (Figures 1 and 2, respectively). The solid line shows the maximum day length at each latitude on the summer solstice for comparison (including twilight until the sun is 1.5° below the horizon). Map tiles by Stamen Design (CC BY 3.0) with data by OpenStreetMap (ODbL) are plotted with the *ggmap* R package (Kahle and Wickham 2013).

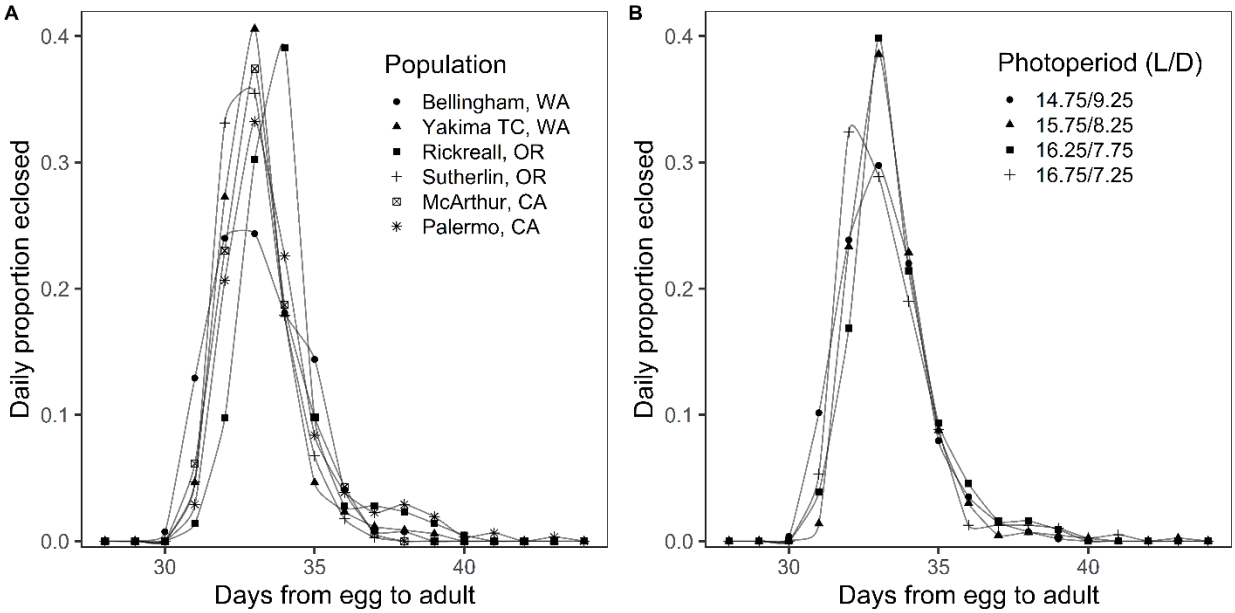

Figure 4: Main effects of population and photoperiod on development time at constant 21°C. Points represent daily observations of the proportion of the total number of beetles that eclosed in each group. Development time is similar for most populations, but the Rickreall, OR population took longer than the populations in Sutherlin, OR and Yakima, WA (A). Photoperiod treatments had no effect on development time in the four growth chambers (B).