THE ROLE OF COLD TOLERANCE IN THE GEOGRAPHIC DISTRIBUTION OF THE GIANT SWALLOWTAIL BUTTERFLY (*Papilio cresphontes)*

L'IMPORTANCE DE LA TOLÉRANCE AU FROID SUR LA DISTRIBUTION GÉOGRAPHIQUE DU PAPILLON GRAND PORTE-QUEUE (*Papilio cresphontes)*

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Abstract

Climate change is a well-known factor driving species range shifts. These shifts are often attributed to the relaxation of harsher climates at the northern range limit. Specifically, lack of cold tolerance as a constraint on range expansion at higher latitudes is one hypothesis. However, few, if any, studies have tested this hypothesis during a critical season: fall, when organisms are subjected to sporadic low temperature exposure but may not have become cold tolerant yet. In this study, I investigated the impact of low temperature on the larvae of the Giant swallowtail butterfly, *Papilio cresphontes,* at its northern range edge by integrating physiological experiments and species distribution modelling. First, the cold hardiness of the larvae was tested in a laboratory by determining their supercooling point, chill coma temperature and survival at three low temperatures. I found that the supercooling point was -6.6°C, that larvae use a freeze avoidant strategy, and that larvae enter a chill coma at 2.4°C. I also found that exposure to -2°C did not lead to a high rate of mortality, but that larval survival was impeded by temperatures below the SCP with temperatures below SCP (-8°C) produced high mortality (10-12% survival). Second, to determine the importance of low temperatures at a broad scale, I compared species distribution models of *P. cresphontes* based only on environmental data derived from other sources to models that also included the physiologically-derived parameters I generated experimentally. The modelling revealed that growing degree days and precipitation best predicted the distribution of *P. cresphontes*. The cold tolerance variables did not explain much variation in habitat suitability of *P. cresphontes* distribution. As such, the modelling results were consistent with the experimental results: low temperatures in the fall are unlikely to limit the distribution of *P. cresphontes*. Further investigation into the ecological relevance of the physiological thresholds determined here will improve our understanding of range expansion of *P. cresphontes* due to climate change. This study demonstrates that a combination of approaches provides a strong test of hypotheses related to the factors that limit species distributions.

Résumé

Les changements climatiques sont l’un des facteurs affectant les aires de répartition des espèces. Ces changements sont souvent attribués à l’adoucissement du climat dans le nord de l'aire de répartition. Une hypothèse commune quant à l’extension de l’aire de répartition aux latitudes plus élevées est reliée aux contraintes imposées par l’absence de tolérance au froid. Cependant, peu d'études ont testé cette hypothèse au cours d'une saison critique comme l'automne, lorsque les

organismes sont sujets à une exposition sporadique, à de basses températures et alors qu'ils n'ont pas encore acquis de tolérance au froid. Dans cette étude, j'ai étudié l'impact des basses températures sur les larves du *Papilio cresphonte*s dans la limite nord de son aire de répartition en intégrant des données physiologiques et une modélisation de la répartition des espèces. En premier lieu, nous avons testé la résistance au froid des larves en laboratoire en déterminant leur point de surfusion, leur température de coma au froid et leur survie à trois basses températures. Nous avons observé que le point de surfusion était de -6,6 °C, que les larves utilisaient une stratégie d'évitement du gel et qu'elles entraient dans un coma à 2,4 °C. On a également constaté que l'exposition à -2 °C n'entraînait pas un taux de mortalité élevé, alors que la survie des larves était affectée par des températures inférieures au SCP et que l'exposition à des températures inférieures à -8°C menait à une mortalité élevée (10-12% survie). En second lieu, pour tester l'importance des basses températures sur l'ensemble de l'aire de distribution, nous avons comparé les modèles de distribution des espèces de *P. cresphontes* basés uniquement sur des données environnementales avec des modèles incluant également des variables physiologiques. La modélisation a révélé que les degrés-jours et les précipitations prédisaient le mieux la distribution de *P. cresphontes*. Les variables de tolérance au froid expliquaient peu la variation de la qualité de l'habitat pour la distribution de *P. cresphontes.* Ces résultats de modélisation concordaient avec les données expérimentales. On conclut qu'il est peu probable que les basses températures à l'automne limitent la distribution de *P. cresphontes*. Une étude plus approfondie de la pertinence écologique des seuils physiologiques pourrait améliorer notre compréhension de l'expansion de l'aire de répartition de cette espèce de papillon en lien avec les changements climatiques.

Contents

List of Tables

List of figures

Introduction

Over the past few decades, range shifts have been reported in both plants and animals. Many are experiencing a poleward and/or upward shift in distribution, pushing their northern and upper range limit to higher latitudes and altitudes (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; VanDerWal et al., 2013). However, there has been substantial variation in the degree and direction of range shift across taxonomic groups (Chen et al., 2011). For example, some insect taxa (i.e., dragonflies, spiders) have shifted northwards to a much greater extent than mammals and birds, whereas other groups have expanded southwards. It remains difficult to explain this variation, suggesting that a better understanding of the factors influencing species' distributions is needed (Chen et al., 2011).

When known, these shifts have been primarily associated with climate and land use changes such as habitat loss (Hansen et al., 2001). For example, the Black-shouldered kite, *Elanus caeruleu*, has expanded its range in Europe due to the increase in area of cultivated parklands that are similar to the African savanna where it originated (Balbotin 2008). For those species with northern range edges associated with temperature clines, climatic warming has frequently resulted in the colonization of suitable areas in more northern latitudes (Parmesan, 1996; Parmesan & Yohe, 2003). For example, the northern range expansion of the deer fly (*Lipoptena cervi*) in Finland is due to warmer temperatures during the summer (Härkönen et al., 2010). However, for many other species, it remains unclear how climate change has led to range shifts (Chen 2011).

Climate change-driven range shift in insects is often attributed to the relaxation of a harsher northern climate since temperature acts as both a physiological limitation and as a phenological cue (Logan & Bentz, 1999; Paradis; Root et al., 2003; Elkinton, Hayhoe, & Buonaccorsi, 2008). More specifically, overwinter survival has been shown to be a key factor limiting the ranges of

insects (Crozier, 2003). For example, the northern range edge of the Southern pine beetle (*Dendroctonus frontalis*), is limited by temperatures below -16°C (Lesk, Coffel, D'Amato, Dodds, & Horton, 2017), and the -4°C January isotherm is the factor that limits the northern range for the Sachem skipper butterfly (*Atalopedes campestris*) (Crozier, 2003). However, low temperatures throughout the year can also constrain the ability of insects to persist at northern range limits as they can influence any life cycle stage (Ungerer, Ayres, & Lombardero, 1999). For example, low temperatures during the growing season can slow down the development of insects, impeding their metamorphosis and resource acquisition (e.g., diapause) (Ritland & Scriber, 1985).

Insects are likely to be particularly vulnerable to low temperatures during the fall since it represents a key time in their life cycle. Fall is when most insects are building up their cold tolerance and are therefore have not reached their peak cold hardiness (Tauber et.al 1984). Fall in most areas is the season in which photoperiod and temperature, the cues that trigger the build-up of cold tolerance components in insects, both decline (Kim & Song, 2000; Storey, 2004; Hugh V. Danks, 2005). For example, monarch butterflies, *Danaus plexippus,* that were not yet acclimated had a mortality rate of 60% when exposed to low temperatures in September and October compared to 20% for acclimated ones (Larsen & Lee, 1994). There is also a much higher frequency of cold temperature events in the fall than in the summer (H. V. Danks, 1978). Yet, few studies have considered low temperatures during the fall as a limiting factor on the geographic distributions of insects (Robinet & Roques, 2010).

Other than mortality, cold exposure often causes cold-related injuries (i.e., sublethal effects) in insects. Many larvae or pupae show no immediate physical effects of cold injury after exposure; however, further development can be slowed, leaving the insect in the larval or pupal stage for prolonged periods (Asahina, 1970). This leads to longer exposure to predators and parasitoids

(Culler, Ayres, & Virginia, 2015). Low temperatures can affect insect coordination and mobility (Block, Baust, Franks, Johnston, & Bale, 1990), increasing exposure and decreasing defences to predators and parasitoids. Low temperatures can also affect fecundity (Marshall & Sinclair, 2012). For example, the gall fly, *Eurosta solidaginis*, suffered from reduced egg production when it overwintered at low temperature (Irwin & Lee, 2000). Given the impact on survival and fecundity, the temperature at which larvae are unable to move or feed (i.e., chill coma) is thought to be one of the more relevant cold-related factors limiting the distribution of insects (Hazell & Bale, 2011; Overgaard et al., 2011).

Species distribution modelling is a commonly used approach to evaluate recent range shifts and forecast future shifts due to climate change. They have also been used to inform policy on land use and management ha(Rodríguez, Brotons, Bustamante, & Seoane, 2007; Zhang et al., 2012), predict invasive species' distributions (Jiménez-Valverde et al., 2011), define species' ranges and predict impacts of climate change on available habitat (Araujo, Pearson, Thuiller, & Erhard, 2005). Using correlations between georeferenced occurrence records and a set of environmental variables with geospatial data, these models predict a species' suitable habitat (Elith et al., 2011). While the approach is useful for many applications, these models often violate assumptions, such as predicting habitat suitability in novel conditions and omit key biotic variables known to influence geographic distributions such as species interactions (Zhang & Li, 2017).

One way to improve the accuracy of these models is to incorporate physiological variables (e.g., heat tolerance, metabolic needs; (Kearney & Porter, 2009). By using variables derived directly from physiological traits (e.g. upper thermal limit), the underlying processes explaining the species' distribution are thought to be better incorporated into the model (Kearney & Porter,

2009). For example, Elith et al. (2010) built a distribution model for the cane toad (*Bufo marinus*) based on the impact of topography on the above-ground activity of adults and the temperature requirement of larval growth and survival. Models produced in such a way are called mechanistic or process-based models. They calculate the likelihood of presence by eliminating regions where the conditions are detrimental to species persistence (Buckley et al., 2010; Diamond, Frame, Martin, & Buckley, 2011). These models perform similarly to correlative models for predicting future distributions but have been shown to be more accurate when modelling the fundamental niche (Peterson, 2011). The main limitation with this approach is that physiologically-derived variables can be time-consuming to develop and thus not feasible to do for large assemblages of species or over broad ranges (Peterson and al 2011). There can also be uncertainty about which traits to include in the model, so it can be difficult to find a trait that is predictive (Kearney & Porter, 2009).

Here, I use a widespread butterfly, the Giant Swallowtail, *Papilio cresphontes*, as a case study to test the hypothesis that low temperatures during the fall are the limiting factor at northern range limits. Specifically, the two main objectives are to: i) find relevant cold tolerance thresholds of *P. cresphontes*; and ii) determine the relative importance of cold-related variables on the distribution of *P. cresphontes* at the landscape level. To do so, I determine the cold tolerance strategy (Figure 1; Glossary) of larvae at the northern range limit. Determining the species' cold tolerance strategy improves our knowledge about the importance of low temperatures on *P. cresphontes* survival and can identify low temperature thresholds that could be significant in limiting the species' northern range. In this study, I focus on the life stage most likely to be vulnerable to low temperatures (i.e. larval stage), which is in contrast to most other studies which have determined the cold tolerance strategy of the life stage that overwinters (Radchuk, Turlure,

& Schtickzelle, 2013). To determine the importance of low temperatures at a broad scale, I model the geographic distribution of *P. cresphontes* with the physiologically derived parameters I generated experimentally.

P. cresphontes has undergone a rapid expansion over the past decade and now occurs as far north as Ottawa, Ontario, Canada. Finkbeiner (2011) hypothesized that the range expansion into New York state from 2000 to 2010 was due to the disappearance of frost in September. In spite of this, they still showed frost resistance in larvae and that individuals could survive exposure to -3.9°C. However, these observations were based on field observations of only a few specimens and the cold tolerance strategy was not determined. Therefore, a more in-depth study of the impact of low temperature on *P. cresphontes* is warranted.

To establish the larval cold tolerance strategy, I measured the supercooling point, the lowest temperature before the bodily fluids of insects (i.e. hemolymph) begins to freeze (see Glossary), and conducted low-temperature survival assays. The assays were used to identify a potentially relevant low temperature threshold of larvae collected at the northern range edge. To evaluate the importance of the cold tolerance strategy at the northern range limit, I modelled the geographic distribution with Maxent, a commonly used species distribution modelling technique for presence-only data (Merow, Smith, & Silander, 2013). I tested the importance of the cold tolerance strategy parameters by i) comparing model fit with and without them and ii) comparing the proportion of suitability explained among a number of climate-related factors hypothesized to constrain the range of *P. cresphontes* at the landscape scale.

Methods

1. Study system

The Giant Swallowtail (*Papilio cresphontes*) butterfly is a member of the Papilionidae family. The species' range extends from Costa Rica all throughout the North American continent. The bulk of the population is concentrated in the Eastern United States. It ranges as far north as the Ottawa region, Ontario, Canada. (Figure 2).

P. cresphontes larvae undergo five instars before entering the chrysalis stage (Bullock, 1991). In Ontario, there are two generations with flights occurring from May to July and again from late July to late September (Layberry, Hall & Lafontaine, 1998). The larval stage lasts from 3-4 weeks and pupae formed in the summer emerge after 10-12 days, but those that develop in the fall will remain as pupae until spring. The overwintering pupae undergoes winter diapause, a state of lowered metabolism, with reduced respiration, and no feeding or growth occurs (Scott, 1997). Once eclosed, adults live for 6-14 days (Layberry, Hall & Lafontaine, 1998).

P. cresphontes is known to use plants of the Rutaceae family as their primary food source in the larval stage. In Ontario, those plants consist mainly of Northern Prickly Ash (*Zanthoxylum americanum*) and Hoptree (*Ptelea trifoliata*). The adults are generalists and will gather nectar from most flowering plants, for example, goldenrod (*Solidago*), swamp milkweed (*Asclepias incarnata*) and azalea (*Rhododendron Azaleastrum*)(McAuslane, 2004.)

2. Cold tolerance experiments

i) Experimental overview

To determine the impact of low temperatures on *P. cresphontes* larval survival and developmental success, three experiments were conducted. The first experiment was done to

determine the cold tolerance strategy of the larvae by measuring the supercooling point (SCP; Glossary; Figure 1). Cold tolerance strategy is best inferred from two pieces of information gathered from the same individual during a cold exposure: (1) whether or not the insect froze, and (2) whether or not it survived (Table 1; Salt, 1961; Sinclair et al., 2015). Cold tolerance strategy can be determined with a small number of individuals that are cooled, while in contact with thermocouples, to a temperature close to the SCP. The SCP provides preliminary information on the cold tolerance strategy by setting a baseline temperature from which a larger group of individuals can be tested above and below. Typically, a single exposure is combined with multiple episodes of varying levels of intensity of cold exposure to confirm the cold tolerance strategy (e.g., lower thermal limit). (Figure 1; Glossary).

In the SCP test, if the majority of larvae die at a temperature before reaching their SCP, they are considered to be 'chill susceptible' whereas larvae that survive low temperatures (i.e., above their SCP) but die upon freezing are considered to be 'freeze avoidant.' Finally, a 'freeze tolerant' strategy is indicated by high survival post freezing (i.e., below the SCP) (Figure 1).

To help determine the cold tolerance strategy, the SCP was obtained for both generations and multiple sites. The SCP for the second generation can be used to validate if there is an active depression in the SCP. If a significant depression in SCP is found and there is mortality below SCP, then a freeze avoidant strategy is more certain for that life stage (Salt, 1961). SCP was also obtained for individuals from multiple sites (n=4) across a latitudinal gradient at the northern range limit of *P. cresphontes* (Figure 2). Latitude has the potential to affect cold tolerance due to its correlation with climate (e.g., photoperiod, temperature; Sømme, 1982; Tanaka, 1996) but see Yoshio & Ishii, 2001). Therefore, latitude could affect when and to what extent organisms are cold hardy (Glossary; Figure 1).

Additionally, since the results from the SCP experiment were not enough to clearly define the cold tolerance strategy, low-temperature assays (i.e. the ambient exposure of larvae to low temperatures for an extended period of time) were conducted. The survival rates from the first run of the SCP experiment were not different enough before and after reaching the SCP, making it difficult to confidently determine a cold tolerance strategy. Similar to the reasons mentioned above, the assays were also run for both generations and multiple sites to determine if there were differences in survival and developmental success.

The low-temperature survival assays were done at three temperatures (-2°C, -6°C and -8°C). The temperatures were chosen based on the SCP determined in the first experiment and meteorological data in the Ottawa area (Figure 1; see details for experiment 3 below). To exclude the chill susceptible hypothesis (i.e. mortality above the SCP), the first assay was done at -2°C. Survival at -2°C would confirm the ability of larvae to tolerate temperatures above their SCP, thus making them chill tolerant (i.e., not chill susceptible; Figure 1; Glossary). The second test was done at -6°C to exclude the freeze tolerant strategy since it is in the lower range of recorded frost temperatures for the region. This temperature was chosen based on conditions at the 'OTTAWA CDA RCS' meteorological station (details provided below). Given that -6°C is near the July SCP (i.e. -5.77°C) we found, survival at this temperature serves as a baseline to test whether there are differences in cold-tolerance across generations. To find a potentially ecologically relevant low temperature threshold causing high mortality (i.e. potential lower lethal limit), I also tested larval survival at the lowest recorded temperature for October at the 'OTTAWA CDA RCS' weather station from 2012-2017 (i.e. -8°C; see details below). Exposure at this temperature tests the maximum larval cold tolerance since it is lower than the SCP obtained in the previous experiment

(i.e., -5.77 °C). As such, it will be testing their resistance to freezing and could represent a potential lower lethal temperature.

The third experiment was conducted to measure the chill coma temperature: the precise point at which larvae become unreactive (i.e. immobile). Chill coma is thought to be one of the more relevant cold-related factors limiting the distribution of insects (Overgaard, Hoffmann, & Kristensen, 2011) since it represents the temperature at which larvae are unable to move or feed.

(ii) Field sampling

To capture the inter-generational variation in cold tolerance, larval sampling occurred during the two *P. cresphontes* generations: July and August of 2018. A total of 117 larvae were collected from four sites around Ottawa, Ontario, Canada (Mud Lake, Shirley's Bay, Brockville and Queens University Biological Station (QUBS) spanning a 100 km latitudinal gradient at the northern range limit of *P. cresphontes* (Figure 2). These four sites were accessible and had a high abundance of larvae.

Until the start of the experiments, captured individuals in July were provided with a fresh supply of *Z. americanum* and kept in an LTCP-19 Biochamber at the University of Ottawa, Canada (45.423325, -75.683177) on a 21°C/25°C 15h:9h L:D cycle with light intensity peaking at noon. These conditions were chosen to match the average environmental conditions in July for the Ottawa region. To replicate the conditions required for larvae to prepare to overwinter, the chamber parameters were modified weekly for the August generation to match the conditions from August to October (meteomedia.ca). Over the time spent in the chamber, peak daily temperatures reached a maximum 25 °C and a minimum of 15°C overnight at the beginning and by the end, daytime temperatures only reached 10°C and a minimum of 6°C overnight. Likewise, photoperiod fell from 14.5h to 11h. Chrysalids were moved outside once all larvae had pupated (September 28 2018).

(iii) Experimental details

Experiment 1: Supercooling point (SCP) test

To identify the cold tolerance strategy of *P. cresphontes* larvae, an SCP test was done for the first generation (i.e. the July generation; n=27 from QUBS). To further investigate their cold tolerance strategy, a second SCP test was done on the second generation (i.e. the August generation; n=29 total: n=14 from QUBS, n=10 from Brockville, n=5 from Mud Lake). The SCP test was done following the methodology of Sinclair (2015). Before being cooled, the larvae were weighed with an ultra-micro Sartorius scale and placed in individual 15 ml centrifuge tubes. Body temperatures were measured using type K thermocouples linked to a pico-tech USB 8 data logger. The vials were then placed in a cooling bath (CC-Range, Huber) filled with a mixture of ethylene glycol and water, and then cooled down at 0.1°C per minute from 21°C to -20°C. The test lasted between 200 and 300 minutes until the specimens had reached SCP, hence the total 410 minutes potential runtime was never reached. The vials containing the July individuals were removed once half of the submerged larvae showed an exothermic spike for their respective body temperature (i.e., the supercooling point). Individuals were then moved to individual containers inside the LTCP-19 Biochamber at temperature and photoperiod described above (i). Larvae were monitored daily until death or emergence. Death was acknowledged when the individuals could not maintain upright posture or did not react to physical stimuli (prodding or water spray).

To further investigate differences in the larval SCP between generations, the individuals from the August generation were subjected to the same cooling protocol as described above but

were kept submerged until all of the curves showed the exothermic spike. This was done to obtain SCP values for all individuals.

Experiment 2: Low-temperature survival assays

To identify the consequences of low temperatures likely to be experienced by the larvae before pupation, low-temperature survival assays were conducted. The duration and temperature of the assays were chosen based on meteorological data representing the developmental period before pupation. In the Ottawa region, this period corresponds to the month of October. I considered temperatures from 2012 to 2017, which correspond with the timing of the recent range expansion of *P. cresphontes* into the Ottawa area (Ontario Butterfly Atlas: www.ontarioinsects.org/atlas_online.htm) and used the 'OTTAWA CDA RCS' weather station (45.38, -75.71). For this timeframe, the average and lowest temperatures during frost events (i.e. the span of time in which the temperature is below 0°C for more than one hour) were -1.33°C and -6.3°C, respectively, and the average frost time was 6.24 hours. Consequently, and in conjunction with the SCP of -5.6°C determined in the first experiment, trials were run at -2°C, -6°C, -8°C for 7 hours each.

The low-temperature assays were done in an environmental growth chamber (Biochambers model LTCB-19) for both larval generations and across multiple sites (Table S1). The -2°C test was not repeated in August because i) there was high survival with the July individuals (see results); ii) a low number of specimens were collected in the field, and iii) this temperature was unlikely to be a limiting factor for larval survival and was not a priority to test. Larvae were weighed and measured before being placed in individual containers inside the chamber. The chamber temperature was brought down from 21°C to the predetermined test temperature at a rate

of 0.1°C per minute. Once the desired minimum temperature was reached, the temperature remained constant for 7 hours before being increased back to 21°C at the same rate. Larvae were then left to recuperate. They were allowed to feed on freshly harvested *Z. americanum* leaves during the trial and were checked daily for mortality. Death was determined when larvae could not maintain upright posture or did not react to physical stimuli (proding or water spray). All larvae that survived to pupation were kept until emergence. Surviving pupae from the second generation were moved outside to overwinter in a mesh enclosure (Appendix photo 3). Given the fragile nature of the chrysalids in the containers, the enclosure included a roof that prevented them from being completely surrounded by snow.

Experiment 3: Chill coma

To identify the temperature at which larvae become unreactive, a chill coma test was done following the Sinclair 2015 protocol. Twenty larvae were caught in July from the QUBS site, weighed and measured before being placed in 50 ml Eppendorf vials. These vials were then mounted on a metal support rack, which was submerged in a cooling bath (CC-Range, Huber) of ethylene glycol and water. The bath was cooled from 21°C to -20°C at a rate of 0.2°C per minute, and the test lasted for 185 minutes. The tubes were prodded using a plastic spoon periodically (i.e., seconds to minutes) during the cooling process. The critical thermal minimum was determined when individuals fell on their sides and were no longer able to stand. Larvae were kept in the bath until the final larva fell into a chill coma, at which point the whole rack was removed from the solution and allowed to warm back up. Larvae were then left to recuperate in a growth chamber (Biochambers model LTCB-19) at the average conditions in July, as described above. Larvae were

checked daily for mortality. The chill coma test was only done on July individuals since it required a higher number of specimens than the other tests and was not initially prioritized.

iv) Statistical analysis

To compare larval survival rates above vs. below the SCP for the July generation, a $\chi 2$ test was used. The rate above and below the SCP for the August generation could not be compared since all larvae were exposed to low temperatures until SCP was reached (i.e. no survival rate was calculated above SCP). The survival rate post SCP could not be compared across generations since the protocol was different for the two generations.

To test whether mean SCP differed across sites for the August generation, an ANOVA was run. Since there was no significant difference in mean SCP across sites (df=3, F-value=0.94 p = 0.44), site-level data was pooled. Differences in mean SCP between generations were tested with a t-test. For all tests, the assumptions of normality and homogeneity were evaluated using the Shapiro-Wilk test and the Brown-Forsythe for homogeneity of variance test, respectively.

To compare the larval survival rate and the rates of pupation and adult emergence between generations and sites for the low-temperature assays, a $\chi 2$ test was used. There was no impact of sites on survival rate or developmental success in any assay (Table S5), so site-level data were pooled. All analyses were conducted in R (version 3.4.1).

2. Distribution modelling

i) Overall approach

To determine the limiting factors of the current geographic distribution of *P. cresphontes*, I modelled its range at two different spatial extents (Figure 1) and using two different modelling approaches. To determine whether the relative importance of variables differs between the

northern range and the entire distribution, I modelled the northern range and the entire distribution. I defined the northern range as the area encompassing the upper 50th percentile of occurrences based on both latitude and longitude in the north-eastern part of the range (Figure 1).

To assess if the physiological-based cold tolerance metrics were significant predictors of *P. cresphontes'* distribution, the modelling was done using two approaches: correlative and mechanistic. The correlative approach used only environmental data derived from other sources (e.g. remote sensing, weather stations) whereas the mechanistic approach additionally included parameters derived from the cold tolerance experiments.

ii) Data

Models were built using all available occurrence records for North America from e-butterfly (www.e-butterfly.org, accessed July 2018, 2255 records), the Global Biodiversity Information Facility (GBIF; https://www.gbif.org, accessed September 2018, 3420 records) and Moths and Butterflies of North America (www.butterfliesandmoth.org, accessed October 2018, 1206 records) from 1980 to 2018. This timeframe was chosen to match the timeframe of the available environmental variables. Records that were obviously inaccurate (e.g., in the middle of the ocean) or had no geographical coordinates were removed. Duplicate records across data sources were also removed. To mitigate sampling biases, I used a low-end estimate of dispersal ability (1 km) and randomly selected a single occurrence across data sources per 1 km2. This action serves to reduce the likelihood of occurrence being based on the same individual being observed on multiple occasions. In total, 3510 and 1457 occurrences were used for the North American and the northern range extent, respectively. Occurrences were then mapped onto a North America Lambert Conical Conic projection.

Sixteen environmental variables (Table 2) were initially considered for the model building based on previous work done on butterflies (Araújo & Luoto, 2007; Finkbeiner et al., 2011; Roland & Matter, 2016). The variables included represented both the direct and indirect effects (i.e. via a species' interaction) climate can have on a species' range. Variables included low and high temperature requirements (i.e., mean temperature of the coldest month and extreme maximum temperature), precipitation, measures of heat accumulation (i.e., growing degree days), vegetation (i.e., NDVI) and frost (i.e., temperatures below 0°C for at least one hour) (Sykes, Prentice, & Cramer, 1974). As a measure of heat accumulation, GDD measures the length of the growing season, which determines the amount of time there are favourable conditions for growth and development in plants and insects (Chuine, 2010; Régnière, St-Amant, & Duval, 2012). Other variables are related to primary production and resource availability such as NDVI and precipitation. The impact of frost events were assessed since they were previously hypothesized to be a contributing factor for the range expansion of *P. cresphontes* (Finkbeiner et al., 2011). Specifically, I tested different frequencies of frost events: the number of frost-free days and duration of the frost-free period. The effect of the intensity of frost events (i.e., how cold it was during the frost event) could not be tested since the necessary climatic variables were not available.

All climatic data were downloaded at a 1 km resolution and restricted to 1980-2010. All data was downloaded at a North American extent, with the exception of Normalized Difference Vegetation Index (NDVI) which was global in extent. Variables were created for both range extents (Table 2). Growing degree days (GDD) were modelled using a 10°C base (see table 3 for calculation), which is a commonly used threshold among insects (e.g., coddling moth, *Cydia pomonella*, alfalfa weevil, *Hypera postica*, European corn borrower, *Ostrinia nubilalis*, and rootworm *Diabrotica virgifera* (Lee Townsend, Ric Bessin & Doug Johnson, 1998).

For the mechanistic models, I created variables for both range extents based on the results from the cold tolerance experiments and minimum daily temperature data from Daymet (Table 2). Three variables were built for each extent by summing the number of days below the following thresholds: -2.4°C (i.e., chill coma), -6.6°C (i.e. the SCP from the August generation) and -8°C (i.e. potential lower lethal limit) for each year of the study period 1980-2010 (Table 2). The SCP from the August generation was chosen since it is the one most likely to match when larvae are more likely to experience low temperatures.

To reduce collinearity among variables, a variance inflation factor (VIF) analysis ('car' package (John Fox)) with a threshold of 10 was conducted. This threshold was chosen instead of a more restrictive one (e.g., 3 or 5) as it provides a compromise between collinearity and model usefulness (O'brien, 2007). The test was repeated for each of the model extents and approaches (n=4). With the VIF test, the initial list of 16 variables was reduced to 8 for the Northern range and six for the full range model (Table 3). The final list of variables used for the correlative modelling approach was: extreme maximum temperature, precipitation as snow, precipitation, GDD, NDVI, and mean temperature of the coldest month. Additionally, for the mechanistic models, potential lower lethal limit and chill coma temperature were also included.

iii) Modelling

Models were built using Maxent (version 3.4.1, Philipps et al. 2006) with the BIOMOD2 package (Damien Georges) in R (version 3.41.1). Maxent uses a maximum-entropy approach to model species distributions. It relates the species' presences and pseudo-absences to the environmental variables to predict where the habitat is suitable across the mapped geographical space. Maxent is one of the more common species distribution modelling software (Elith et al.,

2011) and it performs very well compared to other modelling techniques when dealing with presence-only data (Hernandez, Graham, Master, & Albert, 2006)

To model the range, I included hinge features, which allow non-linear relationships, in the model and allowed the model the possibility of clamping (i.e., the default). Clamping serves to limit how much leniency there is in the range of values projected compared to what was observed. This action is important when predicting into novel geographic space (Stohlgren, Jarnevich, Esaias, & Morisette, 2011). However, since the training data and the projection extent were the same, no clamping was done. For each model extent and approach, a background extent was created using the minimum convex polygon function in R (Package adehabitatHR v0.4.16 by Clement Calenge; Calenge,2006). Pseudo-absences were randomly generated within the minimum convex polygons (n=10000; default value).

The models were calibrated using five-fold cross-validation (i.e., the observations were divided into k groups, or folds, of equal size). In cross-validation, the first fold is treated as a validation set, and the model is fit on the remaining k − 1 folds (James, Witten, Hastie, & Tibshirani, 2013). Iterations were limited to 5000 to leave enough time for model convergence. All other parameters were left at default values.

Models were evaluated using three metrics that assess various aspects of accuracy and discrimination (i.e., the ability of the model to distinguish between suitable and non-suitable habitat): Area Under the Curve of the receiver operating characteristics (AUC), Kappa, and True Skills Statistic (TSS). AUC characterizes the model's ability to correctly predict if a presence or absence is a true presence or absence. A value below 0.5 means that the model is no better at predicting occurrences than random, whereas a value of 1 would mean the model predicts all presences/absences perfectly (Yackulic et al., 2013). Although it is the most commonly used

metric for assessing model accuracy of species distribution models (Yackulic et al., 2013), it has been criticized for being unreliable when sample sizes are small and due to its reliance on the number of background points. As such, in these contexts, AUC should mainly be used to compare models built with the same variables and occurrences.

The discrimination of the models was further tested using Kappa and TSS. These two metrics use confusion matrices to compare models' abilities to predict occurrences correctly(Allouche, Tsoar, & Kadmon, 2006). While Kappa has been shown to be more sensitive to prevalence (i.e. the proportion of locations that are occupied), TSS provides an alternate validation metric that is independent of prevalence (Allouche, Tsoar, & Kadmon, 2006). Kappa scores between 0.4 to 0.6 indicate fair agreement, 0.6 to 0.8 indicate moderate agreement, and values greater than 0.8 indicate strong agreement. A TSS score between 0.40 and 0.75 indicates good predictive performance of the model while a TSS score above 0.75 indicates excellent performance(Allouche, Tsoar, & Kadmon, 2006).

Models were run 100 times, and the sensitivity (i.e., the proportion of correctly predicted presences) and specificity (i.e., the proportion of correctly predicted absences) were extracted from each iteration and used to calculate mean and standard error of AUC, Kappa, and TSS(Cerasoli, Iannella, D'Alessandro, & Biondi, 2017). The proportion of variation explained by the variables was also extracted from each iteration and averaged across all runs.

iv) Statistical analysis

To determine the role of low temperature in limiting the distribution of *P. cresphontes,* model accuracy was compared across the two extents and approaches using t-tests. Comparisons were based on AUC, Kappa and TSS.

Results

1. Cold tolerance experiments

Experiment 1: Supercooling point (SCP) test

The results from the July and August SCP tests provide support for a freeze avoidant strategy. July larvae had a higher survival rate above the SCP than below (above: 77% (10/13) vs. below: 43% (6/14 larvae), presenting initial evidence that larvae are not chill-susceptible. However, this difference was not statistically significant ($\chi 2$= 1.2, df = 1, p-value = 0.16) so additional evidence is required. The August larvae had a 3.5% (1/29) survival rate below SCP. Note that there is no rate above SCP for August since all larvae were exposed to low temperatures until SCP was reached. The low survival below SCP suggests that larvae use a freeze avoidant strategy. Additionally, the mean SCP for August larvae (-6.6°C (0.19SE); n=29) was significantly lower than for the July larvae (-5.77°C (0.21SE); n=14; $t_{28,4} = 2.6$, p = 0.015). An active depression of the SCP over the season indicates that the larval cold tolerance is increasing. In other words, there is an active decrease in their freezing point due to the investment of resources toward greater cold hardiness (K. B. Storey & Storey, 1989). This further supports a freeze avoidant strategy.

Experiment 2: Chill coma

The average temperature at which the larvae entered a coma was 2.14°C (0.26SE; n=20).

Experiment 3: Low-temperature survival assays

Results from the low-temperature assays confirmed that larvae are freeze avoidant. In the -2°C test, July larvae had a survival rate of 93% after 24 hours (Table 1), suggesting that exposure to average frost temperatures does not have a large impact on larval survival. This

confirmed their chill tolerance (Glossary, Figure 1). As expected, the survival rate of larvae in July was more affected by exposure to -6°C than -2°C (Table 1); however, it was still high (70%). Since larval survival was high for this test, it is likely that for most of these larvae this temperature was above their SCP (-5.77°C). This provides initial evidence that larvae are able to survive exposure near their SCP and that further testing was needed.

The improvement in larval survival rates in the August generation further supports a freeze avoidant strategy. August larval survival was significantly higher than the July rate when exposed to -6°C (100%, 17/17; χ^2 -squared = 5.1, df = 1, p-value = 0.024; Table 1). This finding provides evidence in support of a freeze avoidant strategy since the SCP for the August generation (-6.6°C) was lower than the test temperature (-6°C) and survival was still high.

In comparison, in the extreme low-temperature test (-8°C), larvae had marginal survival overall (July: 12.5% (1/8); August:10% (1/10)) and there was no improvement in survival across generations (χ^2 = 3.03e-31, df = 1, p-value = 0.99; Table 1). The high mortality in both generations further reinforces the freeze avoidant hypothesis since this temperature is below the SCP for both generations.

Overall, low temperature exposure impacted pupation and adult emergence. Of the larvae exposed to -2°C, 78.5% reached pupation and that rate fell to 52% for successfully emerged adults. After exposure to -6°C, the rate of pupation in July decreased from 60% of all individuals to 30% for adult emergence. At -8°C, the survival rate was 12.5% for all stages in July. None of the August pupae that overwintered emerged in the spring. However, since these larvae were exposed to natural ambient conditions and the experimental low temperatures before pupation, it is impossible to determine exact causes of death. Mortalities could have been from either stress from the cold tolerance assays or from the overwintering conditions. Nevertheless, these findings show that

although larval survival can be high after exposure to low temperatures, adult emergence, in particular, can still be affected by the cold exposure.

2. Distribution modelling

i) Model performance

Model predictive ability varied depending on the metric of accuracy used. All models were considered good based on TSS (i.e. between 0.4-0.75) and AUC (>0.8), whereas based on Kappa, the northern range models were weak (<0.4) and the full range models were fair (>0.5; Table S2).

The accuracy of the two modelling approaches were similar at both extents (AUC: northern range: t = 0.21, df = 999.81, p-value = 0.84; full range: t = 0.62, df = 999.56, p-value = 0.89; Figure 3). Note that these results are identical across accuracy metrics and only AUC is reported here (Table S2). Therefore, the inclusion of cold tolerance metrics does not improve the prediction of habitat suitability of *P. cresphontes*.

The accuracy of the full range models was significantly higher than the accuracy of the northern range models for all evaluation metrics and approaches (see table S3 and S4). Therefore, the habitat suitability of *P. cresphontes* is predicted more accurately across its full range than its northern range.

ii) Variable importance

Growing degree-days was the most important factor explaining the habitat suitability of *P. cresphontes* across all model extents and approaches, explaining between 27-35% of the variation in habitat suitability (Table 3). Precipitation was the second most important factor across both

extents and approaches. Across the full and northern range extents, GDD and precipitation combined explained the majority of the variation in habitat suitability (~50%; Table 3). The physiologically-derived variables included in the final model (i.e., the potential lower lethal limit and chill coma) only explained a small part of variation in habitat suitability (~7.5%) and ranked 5th and 6th in model contribution.

DISCUSSION

My experiments indicate that *P. cresphontes* larvae use a freeze avoidant strategy. The decrease in the average SCP (-5.7 to -6.6°C) between generations and the low survival rates below the SCP provides strong support that this species is unable to handle freezing before pupation. We can also be confident that temperatures above the SCP are not lethal, providing a clear picture of the lower end of thermal tolerance for this species. This means that for larval *P. cresphontes*, temperatures below the SCP will impede their survival and thus lifetime fitness. My results suggest that a single exposure to temperatures near -8°C could represent a potential lower lethal limit for *P. cresphontes* larvae and that areas with early fall temperatures that reach -8°C should be inhospitable for *P. cresphontes*. However, there are only 0-3 frost episodes on average from September to October in the Ottawa region, which is at the northern range limit, such that low temperatures (i.e. <-7°C) during the early fall are unlikely to be experienced by the larvae there or anywhere in its current range. This suggests that a single exposure to low temperatures in the early fall is unlikely to limit the range of *P. cresphontes.*

It is also unlikely that frost in the early fall is a limiting factor of *P. cresphontes'* northern range. The results from the SCP test and cold tolerance assays demonstrate that exposure to normal frost temperatures (i.e., -1°C to -2°C), which are above the SCP, did not affect larval survival. This finding is in concordance with the field observations from Finkbeiner (2011), which shows that *P. cresphontes* larvae can survive frost events. Since larvae had no problem reaching pupation after exposure to -2°C, the most common frost temperature, and the majority of larvae still successfully pupated (60%) after the low frost temperature test (i.e., -6°C), the cold tolerance of larvae at the current northern range limit seems sufficient to cope with the climate they experience. However, further testing in ambient conditions is needed to determine how significant the sub-lethal effects

of cold exposure on larvae are on adult emergence as rates of adult emergence in this experiment were less than 30%. It has been demonstrated elsewhere that the success of each life stage matters in the response of butterfly species to climate change (Radchuk et al. 2012).

The species distribution modelling results were consistent with the experimental results: cold related variables did not explain the distribution of *P. cresphontes* at a broad scale. Together, these results provide strong support that exposure to temperatures above -6.6°C during the fall does not limit the northern range of *P. cresphontes*. Instead, growing degree-days and precipitation are the most important predictors of the ones tested here, of the distribution of *P. cresphontes* at a broad scale. This suggests that *P. cresphontes* depends on specific heat accumulation and water availability to complete its life cycle. These factors have also been identified as important in predicting the range of other butterflies (Luoto, Heikkinen, Pöyry, & Saarinen, 2006; Eskildsen et al., 2013). Evidence suggests there is a strong relationship between the number of growing degree-days and the growth rates of larvae, and the foraging activities of adults(Ritland & Scriber, 1985; Kukal & Dawson, 1989; Schneider & Root, 2002). Precipitation can limit the range of insects directly due to dehydration or indirectly by imposing limitations on primary productivity, which in return transposes to higher resource availability.

The full range model was more accurate at predicting the distribution of *P. cresphontes* than the northern range model. Similar results have been found for other species, with model accuracy and performance increasing with larger study extents (VanDerWal, Shoo, Graham, & Williams, 2009; Connor et al., 2019). This is likely a result of smaller environmental gradients in the northern range, which causes the model to have a greater difficulty discerning between presences and absences (Smith, 2019). Prevalence can also decrease with larger study extents (Barbet-Massin, Jiguet, Albert, & Thuiller, 2012). However, the accuracy of the two models was

still different using TSS, a metric that is not sensitive to prevalence (Table S4; Figure 3). Nevertheless, even if model performance was lower in the northern range relative to the full range, the model was still valid and performed adequately across all metrics.

While growing degree-days and precipitation explain a lot of the variation in habitat suitability, I may have underestimated the role of cold tolerance at the northern range edge for three reasons. First, other low temperature thresholds, like the chill coma temperature, although not lethal, could still impact *P. cresphontes'* survival in natural conditions due to the enhanced risk of predation or starvation linked with the loss in mobility at temperatures below 2°C. While a possibility, this hypothesis depends on a high likelihood of predation which is unknown across *P. cresphontes'* range (Hazel, Ante, & Stringfellow, 1998; McAuslane, 2009). Second, it is also possible that prolonged or repeated exposure to low temperatures could impact *P. cresphontes* larval survival at the northern range edge because I did not test for the effects of repeated cold injury or chilling. Multiple cold events could cause an accumulation of cold injuries resulting in larval death or in sub-lethal effects such as decreased feeding, fecundity and dispersal ability (Marshall & Sinclair, 2012). For example, the impact of multiple cold exposures has been shown to result in a trade-off for increased survival at the expense of decreased fecundity for *Drosophila* species (Marshall & Sinclair, 2010). Third, the SCP estimated here is effectively a theoretical one. Since I could not collect and test the last emerging larvae of the season, they might not have reached their peak cold tolerance and therefore *in situ*, SCP could be lower (i.e., their cold hardiness may have been underestimated). On the other hand, the *in situ* SCP may be higher since environmental factors in natural conditions, such as air moisture, may further affect survival at temperatures above SCP. Nevertheless, because the low temperature thresholds derived experimentally in this study could be an underestimate, the mechanistic models may not have been

optimized perfectly, thus underestimating the role of cold tolerance metrics at a broad scale. However, since temperatures below the SCP (i.e. <-7°C) are very unlikely to occur multiple times before pupation, if at all, low temperatures during the fall are unlikely to limit the northern range of *P. cresphontes*.

Given our lack of knowledge about the overwintering ecology and physiology of this species in its northern range, pupal overwinter survival may be a limiting factor for the species' northern range limit. It is not known whether they overwinter in the leaf litter on the ground or tree branches above the ground. If on the ground, they would likely be covered in snow (average annual snowfall in Ottawa is 224 cm (Environment and Climate Change Canada) and therefore buffered against low temperatures. The temperature 30 cm below snow cover is usually -4°C (Flin 2008). This behaviour would increase their chances of surviving the winter. Indeed, West (1996) showed that in Virginia, *P. cresphontes* pupate 3-25 cm off the ground on dead branches. In the northern range, it is likely that even at these heights, chrysalids would be covered by snow. While only larvae were tested in this study, *P. cresphontes* larvae purge their food and liquids before pupation, thus they are likely to be even more cold tolerant at the pupal stage. Other studies have found ontogenetic variation in thermal tolerances (Vernon & Vannier, 1996; Rinehart et al., 2006 ;Terblanche, Klok, Krafsur, & Chown, 2006; Marais, Terblanche, & Chown, 2009; MacLean, Higgins, Buckley, & Kingsolver, 2016;but see Ouimette, 2018). Other members of *Papilio* are more cold tolerant than what I determined for the larval stage of *P. cresphontes. P.canadensis* and *P.glaucus*, are considered to be freeze tolerant at the pupal stage and have an SCP of -23°C to -27°C (Kukal, Ayres, & Scriber, 1991). *P.xuthus* is freeze avoidant with an SCP of -25°C (Shimada 1988). Therefore, the pupae of *P. cresphontes* are likely to have even greater cold hardiness than

the larvae and thus be able to survive the winter at the northern range if they pupate at similar heights as the populations in Virginia.

Conclusions

This project investigated the factors limiting the northern range of *P. cresphontes* by integrating cold tolerance lab experiments and broad-scale species distribution modelling. Such integration of approaches provides a strong test of the hypothesis that low temperatures during the fall limit species' distributions.

My results show that *P. cresphontes* larvae use a freeze avoidant strategy and that the cold tolerance of larvae is well matched to its environment in the northern part of its range. I also identified growing degree days and precipitation, rather than cold related metrics, as factors that limit the distribution of *P.cresphones*. While other factors not tested here, for example, biotic interactions, could also limit the distribution, it remains unlikely that low temperatures in the fall are limiting the northern range of *P. cresphontes* given the consistency of results across the two approaches.

The warming of fall temperatures over the past decade is unlikely to have been the factor that led to the recent range expansion of *P.cresphones*, as originally hypothesized by Finkbeiner (2011). Instead, the warming of winter temperatures could have ameliorated overwintering survival, leading to an improvement in suitability. Further study on determining the key factor(s) limiting the northern range of *P. cresphontes* should focus on defining the cold hardiness of the pupal stage as well as the overwintering behaviour. A natural history study of pupation site

preference at the northern range is needed. Determining the ecological relevance of the physiological thresholds found in this study will also help.

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Glossary

Chill coma: A reversible state when insect nerves are electrically silent with interrupted neuromuscular transmission (MacMillan, 2010). When in chill coma, insects are no longer able to feed, move or defend themselves. Chill coma is thought to be more ecologically relevant than other metrics due to its direct impact on insect behaviour (Andersen et al., 2015). More specifically, this precarious state of vulnerability is hypothesized to be one of the most important drivers of species' distribution amongst the cold tolerance metrics (Overgaard et al., 2011).

CTmin: Critical thermal minimum is the temperature at which chill coma is entered.

Cold tolerance strategy: A classification system where the effect of exposure of organisms to low temperatures is translated into a cold tolerance paradigm or strategy (see Figure 1) (Zachariassen, 1985).

Cold hardiness: The capacity to tolerate intensity and duration of cold exposure. The more cold hardy a species is, the less it will be impacted by exposure to low temperatures. Synonymous with 'cold tolerance'.

Chilling injury: Damage that occurs when there is cold exposure above the freezing point (see supercooling point) of the insect. Chilling injuries can be either direct or indirect (Quinn 1985). Although acting through different mechanisms, chilling injuries can be as lethal as freezing. A direct chill injury occurs very rapidly (i.e., minutes to hours following exposure to cold temperatures) and is thought to be the result of the cell membrane transitions from the liquid crystalline to the gel phase (Quinn, 1985; Drobnis et al., 1993). In contrast, indirect chill injury develops more slowly, over days to weeks, and is thought to reflect different mechanisms involving changes in membrane phase, a mismatch of metabolic pathways, oxidative stress and loss in ion homeostasis (Rojas and Leopold, 1996; Yocum, 2001; Kostál et al., 2004). (Figure 1)

Freezing injury: Damage that occurs when there is cold exposure below the freezing point (see supercooling point) of the insect. Freezing damages cells via physical deformation and intracellular freezing. Intracellular ice formation can cause coagulation of the cytoplasm and damage the cell structure resulting in a loss of cell function leading to mortality (Salt, 1961).

Supercooling point (SCP): The lowest temperature a solution reaches before it spontaneously freezing. It is time-, temperature-, volume-, and context-dependent for any given solution. For insects, the SCP is the lowest temperature before their bodily fluids (i.e. hemolymph) begin to freeze (Sinclair et al., 2015). When closely monitoring the body temperature of an insect being cooled, the SCP can be identified by a sudden rise in body temperature (exothermic spike) associated with the transition of water from a liquid to a solid-state which releases heat. The SCP is used as a quantitative metric to asses the cold tolerance strategy of the insect (Figure 1). For chill susceptible and freeze avoidant insects, the SCPs can also serve as an essential threshold for survival (Sinclair et al., 2015). However, SCPs are not always reliable markers of the lower thermal limits as some species can survive well below their SCP.

Chill susceptible: Insects that cannot tolerate cold temperatures above their SCP and tend to die from the direct impacts of cold exposure (i.e. chilling) rather than ice nucleation (i.e. freezing). These insects die from the consequences of low temperatures without freezing (Asahina, 1970) (Figure 1).

Chill tolerant: Insects that can tolerate cold temperatures above their SCP. Meaning the insects that die after prolonged chilling at low sub-zero temperatures This incorporates insects that are 'freeze avoidant' or 'freeze tolerant'(Sinclair,1999). (Figure 1).

Freeze avoidant: Insects that survive cold temperatures by preventing the freezing of their internal organs and fluids. They avoid freezing by using cryoprotectants (molecules such as polyols and sugars) and antifreeze proteins (these bind to small ice particles and prevent them from expanding) (Asahina, 1970) (Figure 1). However, if they cannot suppress the ice nucleation, the insect dies from the consequences of internal ice formation.

Freeze tolerant: Insects that survive below-freezing temperatures since their internal organs and fluids can tolerate ice formation (Sinclair, Coello Alvarado and Ferguson, 2015). They avoid such damage by allowing extracellular ice formation of their hemolymph (internal fluid). This causes their cells to suffer from dehydration, hence limiting intracellular ice nucleation and thus, protecting cells (Sinclair, 1999) (Figure 1).

Lower lethal temperature (LLT): The temperature that defines the survival of organisms exposed to a specific cold stress. It is expressed as a proportional lethal temperature (e.g., LT_{50} is 50% survival). The lower lethal temperature is the temperature that produces 100% mortality (LT_{100}) for a predetermined timeframe (e.g., -25°C for 30 minutes; Nedved, Lavy, & Verhoef, 1998; Sinclair et al., 2015).

Tables

Table 1: Survival rates and developmental success for the low-temperature assays. Test temperature and generation are shown. The percentage of initial larvae that survived the first 24 hours after the experiment, successfully pupated and eclosed as adults is also shown.

Test	Generation	Number of larvae	Larval survival (%)	Pupation (%)	Adult eclosion (%)
-2°C	July	23	93	78.5	52
-6°C	July	10	70	60	30
	August	17	100	100	0
-8°C	July	8	12.5	12.5	12.5
	August	10	10	10	0

Table 2. Original list of variables initially considered for the modelling and their acronyms. The data source is shown as well as the calculation used to produced the final rasters. All variables are averaged over the period from 1980-2010 and the original map projection of all variables was Lambert Conical Conic except for NDVI which was sinusoidal.

Acronym	Full name (units)	Data source	Calculation	Importance
NDVI	Normalized Difference Vegetation Index	Modis/Terra project	Averaged monthly NDVI raster's over the time frame.	Seto 2004, Pettorelli 2005
bFFP	beginning of frost free period (FFP; day of year)	Databasin	The day of the year on which FFP begins	Hayes 1982, Westwood 2010
eFFP	end of frost free period (day of year)	Databasin	The day of the year on which FFP ends	Hayes 1982, Westwood 2010
FFP	frost free period (days)	Databasin	The number of days between the last spring frost and the first fall frost	Hayes 1982, Westwood 2010
NFFD	number of frost free days	Databasin	Number of days above 0°C.	Hayes 1982, Westwood 2010
PAS	average precipitation as snow (mm)	Databasin	Accumulated snow fall averaged across the time frame	Roland 2016
Precip	average precipitation as rainfall (mm)	Databasin	Accumulated rainfall averaged across the time frame	Pearson 2008, Storch 2003
EMT	extreme minimum temperature (°C)	Databasin	Lowest temperature recorded for every given year, averaged over the timeframe.	Crozier 2003
EXT	extreme maximum temperature (°C)	Databasin	Highest temperature recorded for every given year, averaged over the timeframe.	Malcom 1987
MCMT	mean temperature of the coldest month (°C)	Databasin	Average daily temperature for the coldest month	Crozier 2004 Luoto 2006
MWMT	mean temperature of the warmest month (°C)	Databasin	Average daily temperature for the warmest month	Crozier 2004
octtre	average temperature in October (°C)	Daymet	Average daily temperature for October.	Larsen 1994
GDD	average growing degree days of base 10°C	Daymet	((Tmax + Tmin) / 2) -10	Luoto 2006
SCP	average number of days per year below -6.6°C (days)	Daymet	Based on the experimentally derived SCP; a day was counted if the average daily temperature reached -6.6°C. Days were counted and averaged across time frame	Sinclair 2012
Chill coma	average number of days per year below 2.4°C (days)	Daymet	Based on the experimentally derived chill coma; a day was counted if the average daily temperature reached 2.4°C. Days were counted and averaged across time frame	Hazell 2011
PLLT	Potential lower lethal temperature; Average number of days per year below -8°C (days)	Daymet	Based on the experimentally derived potential lower lethal temperature; a day was counted if the average daily temperature reached -8°C. Days were counted and averaged across time frame	Asahina 2005

Table 3. Contribution of the environmental variables in explaining habitat suitability across different model extents and approaches. Shown is the rank order of variable importance and mean (± S.E) proportion of variance explained. In bold is the variable that explains the most amount of variation for each model type.

Variables	North America				Northern range			
	Correlative		Mechanistic		Correlative		Mechanistic	
	Rank order	Variation explained (SE)	Rank order	Variation explained (SE)	Rank order	Variation explained (SE)	Rank order	Variation explained (SE)
Growing degree days	**1**	**31.33 (0.19)**	**1**	**27.34 (0.23)**	**1**	**37.17 (0.08)**	**1**	**35.03 (0.06)**
Precipitation	2	24.87 (0.08)	2	21.70 (0.085)	2	23.94 (0.08)	2	22.47 (0.07)
Extreme maximum temperature	3	17.54 (0.06)	3	16.22 (0.06)	6	2.38 (0.02)	7	2.41 (0.02)
Normalized Difference Vegetation Index	4	15.45 (0.10)	4	15.87 (0.11)	4	16.77 (0.11)	4	14.89 (0.11)
Mean temperature of the coldest month	NA	NA	NA	NA	3	16.9 (0.06)	3	15.57 (0.05)
Precipitation as snow	5	10.80 (0.07)	5	10.05 (0.07)	5	2.84 (0.02)	6	3.46 (0.02)
Chill coma*	NA	NA	NA	NA	NA	NA	5	5.97 (0.03)
Potential lower lethal temperature*	NA	NA	6	8.80 (0.04)	NA	NA	8	0.16 (0.004)

*mechanistic variable; derived experimentally

Figures

Figure 1. An example of a cold tolerance strategy (Glossary) with a hypothetical supercooling point (SCP; Glossary). In this example, larvae that are ‘chill susceptible’ die above the SCP from the consequences of low temperatures, whereas larvae that are ‘chill tolerant’ (top bracket) can survive cold exposure above the SCP. ‘Freeze avoidant’ and ‘freeze tolerant’ larvae would also be considered to be chill tolerant since they can survive extended exposure at low temperatures. ‘Freeze avoidant’ larvae die below the SCP from internal ice nucleation whereas ‘freeze tolerant’ larvae survive below the SCP. The type of cold exposure is shown by the outside bracket: temperatures above the SCP are considered to be chilling and below the SCP are freezing. The background colour of the text box denotes if individuals die (white) or survive (black) cold exposure. The arrow on the right shows that cold hardiness (or tolerance) increases as the survival at low temperature increases.

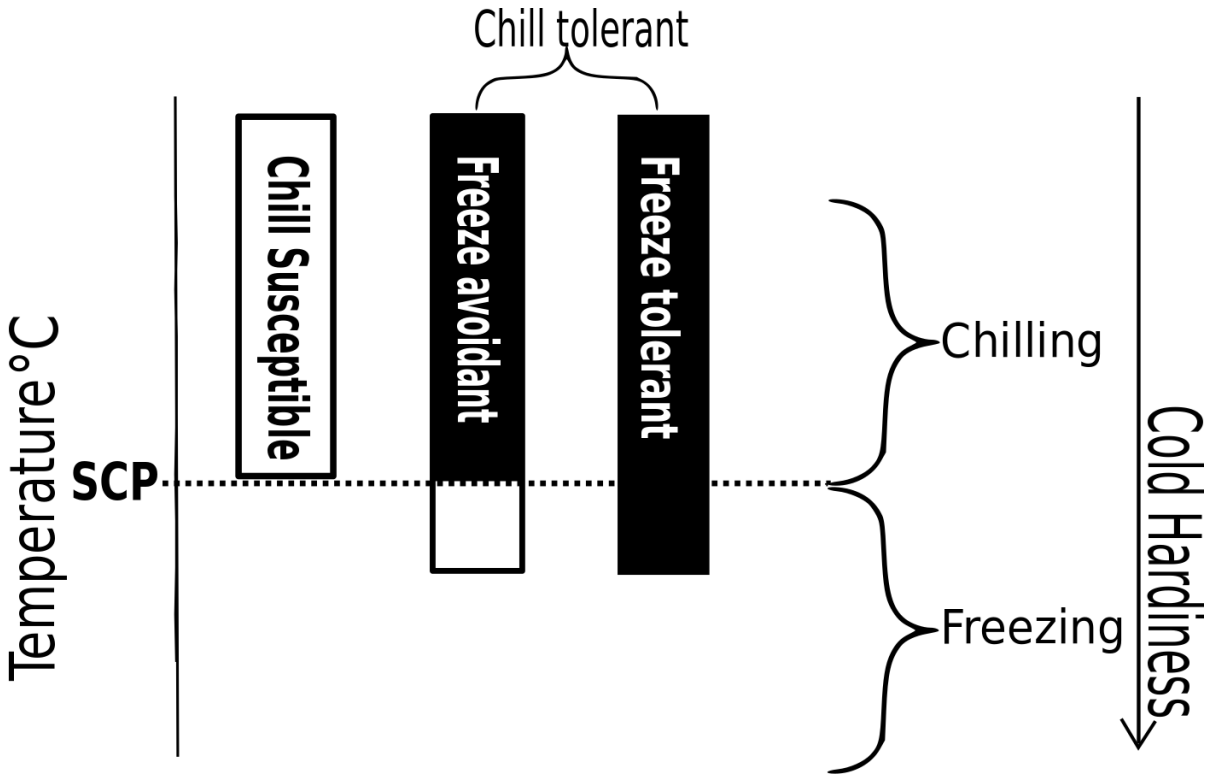


Figure 2: The geographic distribution and northern range of *Papilio cresphontes*. The first panel shows the entire range of *P. cresphontes* encompassed by the minimum convex polygon (thin black line). The second panel shows the position of the northern range, denoted by the solid blue line, relative to the entire range. The blue circle indicates the locations of the field sites. In the first two panels, the occurrence points (black) are shown against the predicted habitat suitability (red). The third panel shows the field sites where larvae were collected at the northern range limit: the blue dot indicates the Brockville site (44.84952, -75.75226), red indicates the Queen's University Biological Station site (44.56747, -76.32454), brown indicates the Mud lake site (5.37192, -75.79451) and yellow indicates the Shirley's Bay site (45.36546, -75.88302).

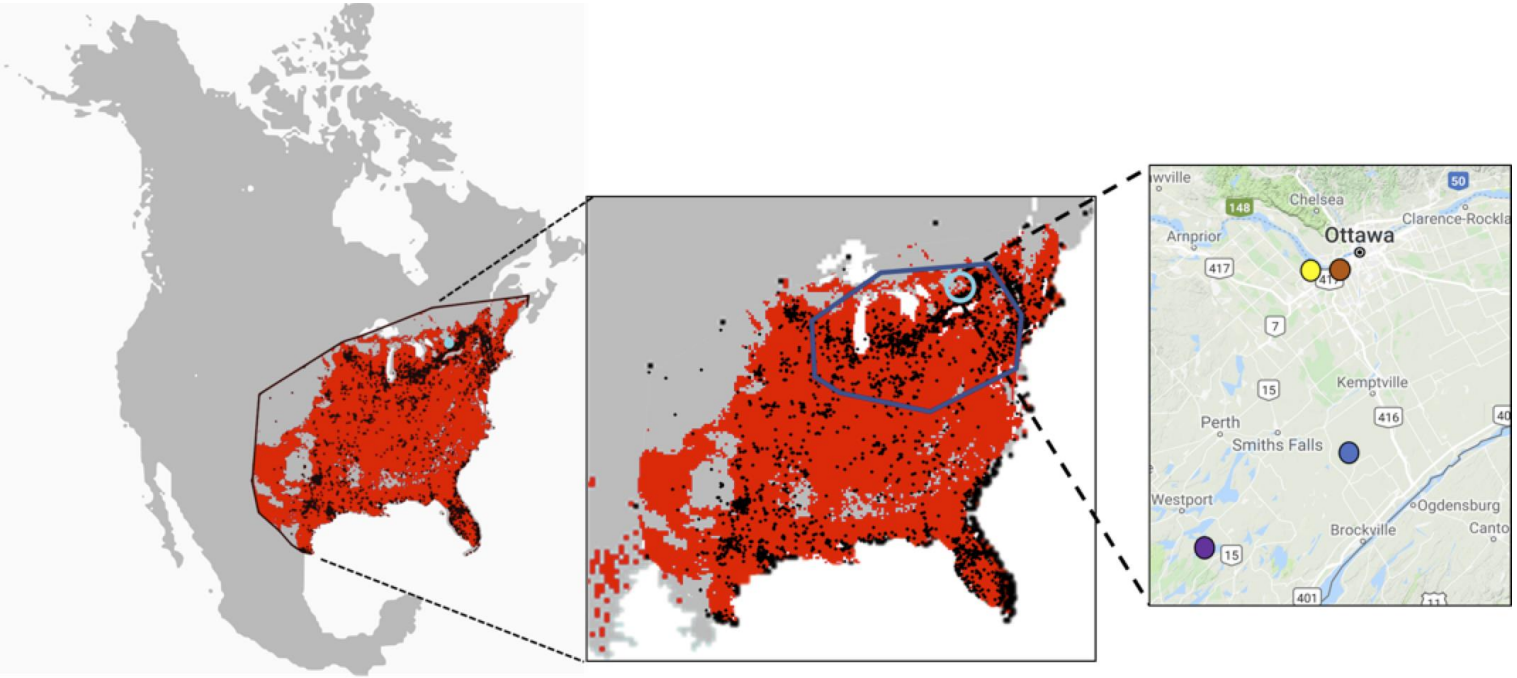
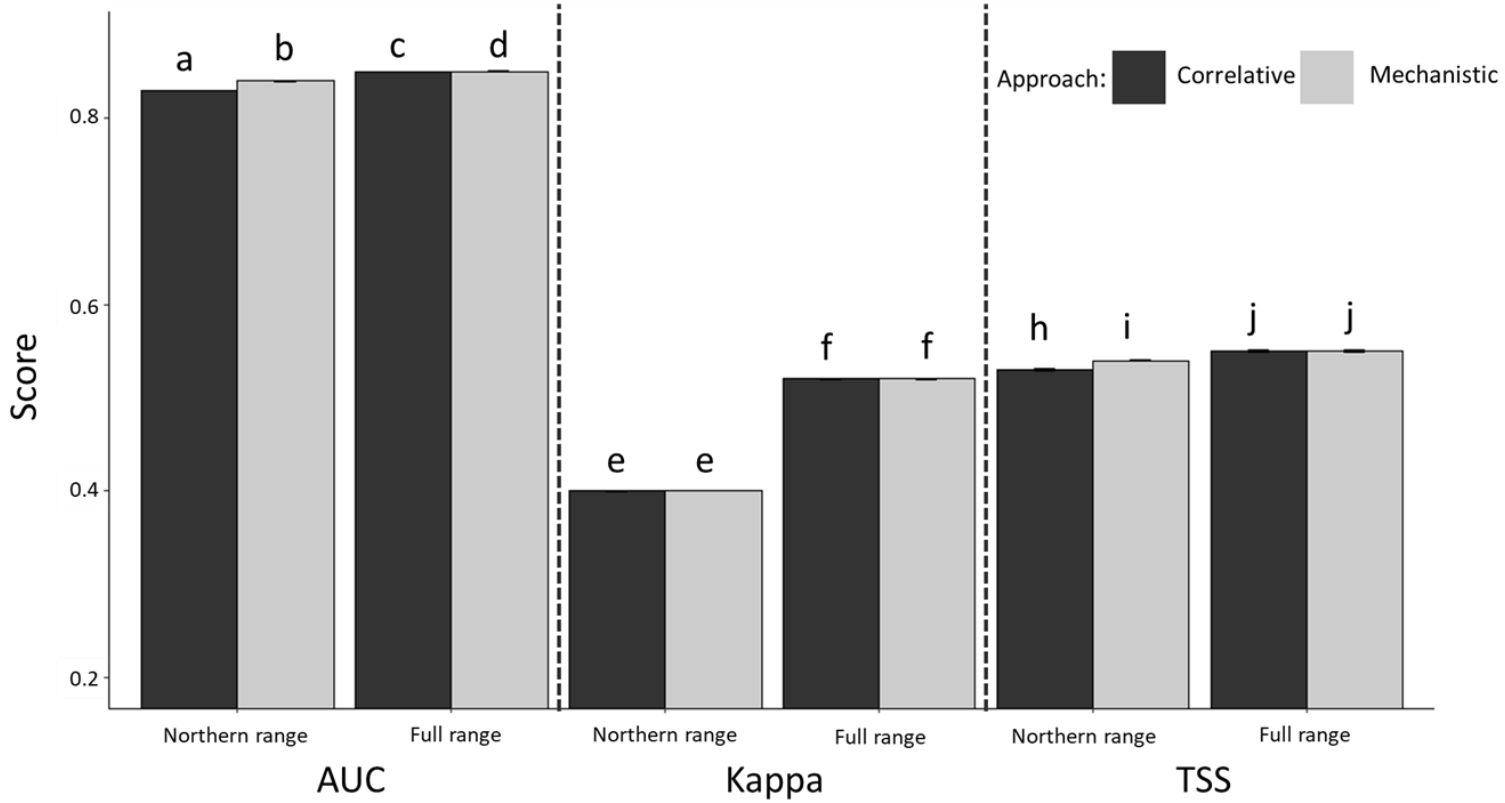


Figure 3: A comparison of model accuracy for the two model extents (northern and full range) and approaches (correlative and mechanistic) using all three evaluation metrics (area under the receiver operating characteristic curve. AUC, Kappa, true skills test (TSS). Mean accuracy scores and standard error bars across 100 iterations are shown with the y axis cropped at 0.2. Grey and black bars represent the mechanistic and correlative models, respectively. Letters indicate that mean scores are significantly different from each other ($p<0.05$).



Appendix

1. Table S1. Number of larvae among generations and sites for the low temperature assays.
2. Table S2. Results from the variance inflation factor (VIF) analysis conducted on the 16 climatic variables used in the species distribution modelling for the two different extents.
3. Table S3. Comparison of the accuracy of species distribution models with and without mechanistic variables at two extents: northern range and full range.
4. Table S4. Comparison of the accuracy of species distribution models at two extents: northern range and full range in relation to the modelling approach used mechanistic or correlative.
5. Table S5. A comparison of larval survival rate and the rates of pupation and adult emergence across sites for the low-temperature assays.
6. Picture 1. Overwintering housing for *P. cresphontes* pupae.
7. Picture 2. Healthy and dead larvae
8. Picture 3. Unemerged *P. cresphontes* pupae post-overwintering
9. Picture 4. Failed pupation attempts at QUBS

Table S1. Number of larvae among generations and sites for the low temperature assays. The sites are: Queens University biological station (QUBS), Mud lake, Shirley's bay and Brockville.

Test	Generation	QUBS	Mud lake	Shirley's bay	Brockville	Total
-2°C	July	0	15	2	6	23
-6°C	July	0	10	0	0	10
	August	4	8	5	0	17
-8°C	July	0	8	0	0	8
	August	0	2	5	3	10
Total		4	43	12	9	68

Table S2. Results from the variance inflation factor (VIF) analysis conducted on the 16 climatic variables used in the species distribution modelling for the two different extents. Shown here are the variables included in the final models based on a threshold of 10 (i.e., those with a score below 10). Variables that were 'excluded' had a VIF above 10.

Extent	Variables	VIF values
Northern range	Normalized Difference Vegetation Index	2.03
	Extreme maximum temperature (°C)	3.08
	Precipitation as snow (mm)	1.16
	Growing degree days	5.55
	Potential lower lethal temperature (days)*	1.53
	Mean temperature of the coldest month (°C)	5.59
	Precipitation (mm)	1.77
	Chill coma (days)[§]	6.95
Full range	Normalized Difference Vegetation Index	2.48
	Extreme maximum temperature (°C)	3.59
	Precipitation as snow (mm)	2.80
	Growing degree days	2.02
	Potential lower lethal temperature (days)*	1.21
	Precipitation (mm)	3.31

*Average number of days per year below -8°C

[§]Average number of days per year below 2.4

Table S3: Comparison of the mean accuracy of species distribution models with and without mechanistic variables at two spatial extents: northern range and full range. Shown are the t-test results comparing the area under the receiver operating characteristic curve. (AUC) and the true skill statistic (TSS). The comparisons in bold are statistically significant ($p<0.05$).

Extent	Metric	t-value	p-value	degrees of freedom
Northern range	**AUC**	**2.59**	**0.01**	**999.32**
	Kappa	0.21	0.84	999.81
	TSS	**4.75**	**2.4e-06**	**999.23**
Full range	**AUC**	**18.32**	**2.2e-16**	**999.95**
	Kappa	2.74	0.06	999.59
	TSS	1.58	0.12	999.41

Table S4: Comparison of the accuracy of species distribution models at two extents: northern range and full range in relation to the modelling approach used mechanistic or correlative. . Shown are the t-test results comparing the area under the receiver operating characteristic curve. (AUC) and the true skill statistic (TSS). The comparisons in bold are statistically significant ($p<0.05$).

Approach	Metric	t-value	p-value	degree of freedom
Correlative	**AUC**	**8.36**	**5.3e-16**	**957.11**
	Kappa	**109.94**	**2.2e-16**	**958.51**
	TSS	**6.61**	**6.6e-11**	**957.4**
Mechanistic	**AUC**	**24.33**	**2.2e-16**	**977.72**
	Kappa	**125.72**	**2.2e-16**	**950.57**
	TSS	**4.75**	**2.4e-06**	**958.14**

Table S5. A comparison of larval survival rate and the rates of pupation and adult emergence across sites for the low-temperature assays (i.e., -2°C, -6°C, -8°C tests). The results from $\chi 2$ goodness-of-fit tests are shown. The NAs are in cases where all individuals for a given test were from the same site or the test was not repeated for both generations. See Table S1 for the number of larvae across sites.

Test	Generation	Life stage	$\chi 2$	Degrees of freedom	p value
-2°C	July	Larval	0.40	2	0.40
		Pupal	2.41	2	0.30
		Adult	0.93	2	0.63
	August	Larval	NA	NA	NA
		Pupal	NA	NA	NA
		Adult	NA	NA	NA
-6°C	July	Larval	NA	NA	NA
		Pupal	NA	NA	NA
		Adult	NA	NA	NA
	August	Larval	1.53	2	0.47
		Pupal	NA	NA	NA
		Adult	NA	NA	NA
-8°C	July	Larval	NA	NA	NA
		Pupal	NA	NA	NA
		Adult	NA	NA	NA
	August	Larval	4.44	2	0.11
		Pupal	NA	NA	NA
		Adult	NA	NA	NA

Picture 1. Overwintering housing for *P. cresphontes* pupae.

Picture 2. The first panel shows a dead larva post a cold tolerance assay and the second panel shows a healthy larva.

Picture 3. Unemerged *P. cresphontes* pupae post-overwintering.

Picture 4. Field observations of failed pupation attempts at Queens University Biological Stations sites on October 2, 2018.