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Upper thermal limits differ among and within component species in a tritrophic host-parasitoid-hyperparasitoid system

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Abstract

Understanding how climate change affects host-parasite systems and predicting the consequences for ecosystems, economies, and human health has emerged as an important task for science and society. Some basic insight into this complex problem can be gained by comparing the thermal physiology of interacting host and parasite species. In this study, we compared upper thermal tolerance among three component species in a natural host-parasitoid-hyperparasitoid system from Virginia, USA. To assess the ecological relevance of our results, we also examined a record of maximum daily air temperatures collected near the study site in the last 124 years. We found that the caterpillar host *Manduca sexta* had a critical thermal maximum (CT_{max}) about 4˚C higher than the parasitic wasp, *Cotesia congregata*, and the hyperparasitic wasp, *Conura* sp., had a CT_{max} about 6˚C higher than its host, *C. congregata*. We also found significant differences in CT_{max} among instars and between parasitized and non-parasitized *M. sexta*. The highest maximum daily air temperature recorded near the study in the last 124 years was 42˚C, which equals the average CT_{max} of one species (*C. congregata*) but is several degrees lower than the average CT_{max} of the other two species (*M. sexta*, *Conura* sp.) in this study. Our results combined with other studies suggest that significant differences in thermal performance within and among interacting host and parasite species are common in nature and that climate change may be largely disruptive to these systems with responses that are highly variable and complex.

Introduction

Of the many dimensions to climate change, predicting the response of host-parasite systems to warming has received considerable attention (e.g. [1–14]). Parasitism—broadly defined as including traditional parasites, many plant-feeding insects, parasitoids, and pathogens—is perhaps the most common mode of life on the planet involving interactions among a huge number and diversity of organisms [15–18]. In the terrestrial realm, one common type of host-parasite system is the interaction between herbivorous caterpillars (larval Lepidoptera) and

their wasp (Hymenoptera) and fly (Diptera) parasitoids. Unlike typical parasites, parasitoids normally kill their host after larval development [19]. Many host-parasitoid systems also involve a third level of *hyper*-parasitism, with hyperparasitoids that parasitize the original host's parasitoids.

Predicting how host-parasite systems respond to climate change, and how this may impact ecosystems, economies and human health, is a deeply complex problem [1–3, 9, 12, 14, 17, 18, 20]. At its most basic level, the problem can begin to be dissected by comparing the thermal requirements of component species to infer how changes in temperature may affect the system [1, 3, 6–8]. If, for example, hosts and parasites have similar "thermal windows" for performance, then warming may affect them similarly, and the synchrony of the system (e.g., timing of host and parasite development) may be, to some extent, preserved (e.g. [8, 14]). Alternatively, if hosts and parasites have sufficiently different thermal windows, warming may affect them differently, with the potential to disrupt synchrony and impact population dynamics and stability [10, 21–23]. Furthermore, thermal windows can vary *within* component species, which adds additional layers of complexity to the problem. For example, thermal tolerance is known to vary with age and ontogeny [24–27] and parasitization or infection status [28–32]. To the extent such age-, stage-, and state-dependent variation in thermal windows is important for describing host-parasite systems, modelling and predicting their responses to temperature changes (e.g. [4, 11]) will be all the more difficult.

In this study, we address two basic questions about the thermal biology of host-parasite systems. First, does upper thermal tolerance differ among component species in a natural host-parasitoid-hyperparasitoid (H-P-HP) system? Second, within hosts, does upper thermal tolerance differ with larval stage (instar) and parasitization status? To our knowledge, this is the first study to test for and demonstrate such differences within a natural H-P-HP system. To assess the ecological relevance of our results and the potential implications for climate change, we also examine the frequency of maximum daily air temperatures recorded near the study site in the last 124 years.

Materials and methods

Study system

The herbivore, *Manduca sexta* (L.) ("tobacco hornworm") (Lepidoptera: Sphingidae), is a specialist on solanaceous plants. Its range extends from Southern Ontario to Florida and south to Argentina [33]. Within North America, it is abundant along the Gulf Coast through the Mississippi Valley and the East Coast up to Maryland and New Jersey [34]. This species is a major defoliator of cultivated tobacco, particularly late in the growing season when populations are large [35]. In Virginia, this species has two to three generations per year. *Manduca sexta* serves as an important model organism for insect physiology and development (e.g. [36, 37]) and, in interaction with *Cotesia congregata*, as a model system for host-parasite interactions and insect immunology (e.g. [38, 39]) as well as tritrophic interactions (e.g. [40, 41]).

The parasitic wasp, *Cotesia congregata* (Say) (Hymenoptera: Braconidae), is a gregarious koinobiont and the only hymenopterous parasitoid of *M. sexta*; reportedly, it also attacks ~14 other sphingid species in North America [42]. Typically, this species attacks 2nd through early 4th instar caterpillars and can oviposit up to 300 eggs at one time [43]. Wasp larvae undergo two larval instars inside the host and molt to the final 3rd instar while egressing from the host by perforating the cuticle with their mandibles. Larvae then spin and undergo pupation within individual silken cocoons that remain attached to the caterpillar host [43].

The hyperparasitic wasp, a member of the *Conura* (formerly *Spilochalcis*) species complex (possibly, *Conura side* [Walker 1843] [Hymenoptera: Chalcididae]), is one of four common

species reported to attack the pre-pupal or pupal stages of *C. congregata* on *M. sexta*, as well as other braconids and ichnuemonids [44, 45]. This hyperparasitoid was by far the most abundant species at the study site at the time insects were collected for this study.

Field collection and laboratory rearing

Manduca sexta eggs and caterpillars, with and without *C. congregata* cocoons, were collected on 5 separate days during July-August 2015 at a privately-owned organic farm in Nottoway County near Blackstone, VA (37.01499, -78.0359), where tobacco has been grown for the past 100 years. Permission to work at the study site was granted by the owner, Mr. Johnny Bledsoe. Approximately 25–50 eggs and caterpillars were collected per field trip and then transported to the laboratory and held at ambient conditions (22 ± 2˚C, 30–50% RH). These *M. sexta* eggs and caterpillars, and *C. congregata* pupae, were the source of all individuals measured in this study, including the hyperparasitoid. Since female *M. sexta* lay eggs singly across multiple plants over a period of weeks, and since collections were made on different days over a period of a month, we assume that the stock of field-collected caterpillars and by extension parasitoid wasps used for our experiments represented a random sample of the populations at the study site during the collection period and that the probability of collecting related eggs or caterpillars was low.

To obtain non-parasitized 3rd-5th instar caterpillars for the experiment, *M. sexta* eggs were hatched in small plastic boxes (~15–20 eggs per box) lined with paper towels and provided with fresh tobacco leaf to feed neonate caterpillars. At the 2nd instar, caterpillars were transferred to individual small plastic cups and provided with fresh tobacco leaf each day. Tobacco leaves were collected from the same field site at which caterpillars were collected and stored in a refrigerator to maintain freshness.

To obtain adult parasitoids and hyperparasitoids, caterpillars with and without egressed *C. congregata*, were collected from the field. Caterpillars without cocoons were held in plastic shoe boxes (5–12 caterpillars per box) and provided with fresh tobacco leaf each day. When parasitoid egression was observed, caterpillars were moved to individual plastic cups. Likewise, caterpillars with *C. congregata* cocoons at collection were held in individual plastic cups. Each brood of emerged parasitoids, which may be the progeny of more than one female wasp, were then transferred to plastic boxes and provisioned with a wet sponge and honey agar. Wasps used in the experiments were selected haphazardly from a total of 15 broods. Cocoons that did not yield *C. congregata* were transferred to individual gelatin capsules (Capsuline, # 00) and held at room temperature until emergence of hyperparasitoids. The hyperparasitoid (*Conura* sp.) deposits a single egg in the prepupa of the primary parasitoid (*C. congregata*); in the field, a single brood of C. congregata is typically parasitized by multiple individual hyperparasitoids.

Finally, to obtain parasitized caterpillars to compare with non-parasitized caterpillars, individual 1-day old *C. congregata* females without prior ovipositional experience were presented with a 3rd instar day 1 caterpillar reared from field collected eggs and allowed a single oviposition. As for non-parasitized caterpillars, parasitized caterpillars were held in individual plastic cups and fed on tobacco leaf until reaching the desired developmental stage for this study.

Upper thermal tolerance

To quantify upper thermal tolerance, we measured the critical thermal maximum (CT_{max}) as defined by the onset of muscular spasms, which indicates a loss of voluntary muscular control [46, 47]. Like most previous studies, we used the dynamic ramping method to estimate CT_{max} [46, 48–51]. This method involves heating an organism at a constant rate until a predefined

endpoint, such as the onset of muscular spasms, is observed. Lighton and Turner [48] demonstrated the physiological basis for this method in insects by showing that the point at which the onset of spasms is observed during a temperature ramp is the same as when ants lose the ability to control respiration, presumably due to a loss of spiracle control.

Following previous studies, we used a ramping rate of 0.25˚C min^{-1} for all estimates of CT_{max}, which is generally considered an adequate rate for equilibration of body temperature with changes in water/air temperature in small ectotherms ([48–51]; and see below). CT_{max} was measured for individual 1-day old (1) non-parasitized and parasitized 3^{rd}, 4^{th}, and 5^{th} instar *M*. *sexta*; (2) adult *C*. *congregata*; and (3) adult *Conura* sp. To begin CT_{max} trials, individual caterpillars or wasps were placed inside a small glass vial and completely submerged inside a water bath (Huber CC 118A with Pilot One). We used a start temperature of 30˚C which is well within the normal physiological range of these species. Prior to ramping, the organisms were held at constant 30˚C for 15 minutes to allow body temperature to equilibrate with the temperature of the water bath and air inside the vial.

To observe the onset of spasms, each trial was recorded with a video camera (Sony HDR SR-11) positioned to focus on the organism inside the glass vial during the temperature ramp. Recordings were analyzed using Windows Live Movie Maker software; the water bath temperature when the first muscular spasm was observed was recorded as CT_{max}. Videos of each observation (N = 67) are available upon request from the authors. The final, corrected data after calibration (see below) used for analyses in this paper are given as supplementary information (S1 Appendix).

Calibration of CT_{max} measurements

The above method for estimating CT_{max} assumes the temperature of the water bath (T_{water}) at time *t* during the thermal ramp, which was measured, is equal to the air temperature (T_{air}) and body temperature of the organism (T_{body}) inside the glass vial at time *t*, which were not measured. However, due to thermal inertia, the potential for a lag between T_{body} and T_{water} or T_{air} increases with increasing body size. At the ramping rate of 0.25˚C min^{-1}, T_{body} of very small ectotherms like the wasps and 3^{rd} instar caterpillars in this study rapidly equilibrates to changes in T_{water} and T_{air} producing little to no lag [48–51]. However, for larger ectotherms like the 4^{th} and 5^{th} instar caterpillars in this study, lags become likely, which can give misleading results especially when comparing organisms of different sizes.

To address this issue, we calibrated our experiment with two ramping trials to test the assumption that $T_{water} = T_{air} = T_{body}$ using a 4^{th} and 5^{th} instar *M*. *sexta* caterpillar, chosen because they represent the two largest size classes in the experiment. Ramps began at 35˚C and ended at 50˚C at a rate of 0.25˚C min^{-1}. Prior to ramping, T_{body} was given sufficient time to equilibrate with T_{water} and T_{air}. At the onset of ramping, all three temperatures were recorded simultaneously every 2 min until the end of the ramp for n = 30 paired observations. The internal thermometer of the water bath measured T_{water}. To measure T_{air}, a bare tip T-type thermocouple probe (Cooper-Atkins model 39138-T) was inserted inside a glass vial by punching a hole through the plastic cap and sealing it with silicone to keep out water. The same technique was used to measure T_{body} except a thinner 26-gauge T-type thermocouple (Physitemp Instruments model W-TW-26) was used with the exposed tip inserted through the body wall and into the body core of a frozen-then-thawed caterpillar. Both thermocouples measuring T_{air} and T_{body}, each in separate vials, were connected to the same digital thermometer (Amprobe model TMD-52). To the extent that significant lags were detected (i.e., $T_{water} \neq T_{air} \neq T_{body}$), these data were used to calculate correction factors to provide better estimates of CT_{max} based on $T_{body,}$ rather than T_{water}.

Maximum air temperatures

To compare with CT_{max}, a record of daily maximum air temperatures near the Blackstone, VA study site was obtained from NOAA's National Centers for Environmental Information (NCEI) from the Global Historical Climate Network-Daily Summaries (GHCN-Daily) database [52]. These data are publicly available upon request from NCEI. The subset we obtained [53] spans the past 124 years (01-Jan-1893 to 19-Mar-2017; N = 103,159 days excluding 1,465 days with missing data) from the greater Richmond, VA area, which is the nearest network of GHCN-Daily stations and about 72 km northeast of the study site.

Statistical analysis

CT_{max} data approximated a normal distribution based on normal quantile plots and variances were homogeneous among species (Brown-Forsythe test, $p > 0.05$). A one-way ANOVA was used to test for differences in CT_{max} among species including non-parasitized 3^{rd}-5^{th} *M. sexta* caterpillars, adult *C. congregata* wasps, and adult *Conura* sp. wasps. A two-way ANOVA was used to test for differences within the host *M. sexta* including the effects of instar, parasitization status (parasitized vs. non-parasitized), and their interaction.

All analyses were conducted in JMP Pro version 11.1.1. All tests were considered significant at $p < 0.05$. All means are reported with ± 1 standard error.

Results

Body size of component species

The average wet mass of non-parasitized caterpillars used in the experiment was: 3rd instar = 0.080 ± 0.003 g (n = 7), 4th instar = 0.293 ± 0.042 g (n = 8), 5th instar = 2.251 ± 0.202 g (n = 8). The weights of adult *C. congregata* (n = 16) and *Conura sp*. (n = 10) wasps used in the experiment were not measured. Both species are similar in body length (~2–4 mm) and much smaller than the caterpillar host. In a laboratory colony of *C. congregata* maintained by one of the authors (KMK), the average wet mass in a sample of 1-day old adult males (n = 10) and females (n = 10) was 0.0002 g.

Calibration of CT_{max} measurements

Simultaneous measurement of T_{water}, T_{air}, and T_{body} during two separate ramping trials demonstrated that T_{body} closely matched T_{air} in the 4^{th} instar but was consistently about 1.0˚C lower in the 5^{th} instar (S2 Appendix). In addition, both data sets showed that T_{air} was consistently about 0.5˚C lower than T_{water} (S2 Appendix). Therefore, we subtracted 0.5˚C from all values (all caterpillars and wasps) and a further 1.0˚C from 5^{th} instar caterpillar values to obtain the final estimates of CT_{max}.

Differences among trophic levels

There were significant differences in CT_{max} among trophic levels ($F = 70.03$, d.f. = 2, 46, $p < 0.0001$; Fig 1). Tukey's HSD post-hoc test indicated the hyperparasitoid *Conura sp*. had the highest CT_{max} (47.9 ± 0.3˚C), the parasitoid *C. congregata* had the lowest (42.3 ± 0.2˚C), and the host *M. sexta* was intermediate (45.7 ± 0.3˚C) between the two (Fig 1).

Differences within the host *M. sexta*

There were significant differences in CT_{max} within the host *M. sexta* (Table 1, Fig 2). A two-way ANOVA found significant main effects of both instar and parasitism on CT_{max}, but no

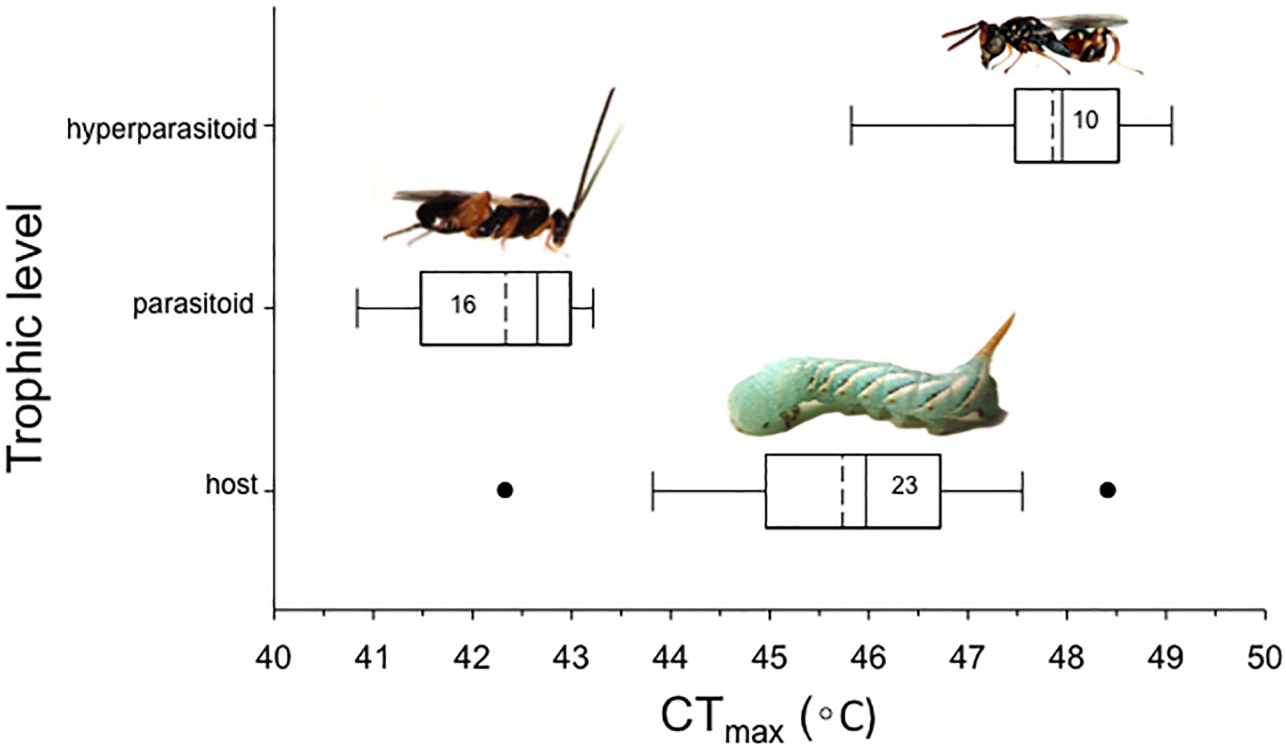

Fig 1. Box-plots of variation in upper thermal tolerance (critical thermal maximum, CT_{max}) in component species of a tri-trophic system involving a caterpillar host (*Manduca sexta*), a wasp parasitoid (*Cotesia congregata*), and a wasp hyperparasitoid (*Conura* sp.). Dashed lines = mean; solid lines = median; points = $5^{th}/95^{th}$ percentile outliers. Values inside boxes represent sample size (number of individuals). The means were different among all three species (One-way ANOVA with Tukey HSD post-hoc test; all P's < 0.05). Photo credits: Justin Bredlau.

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significant interaction (Table 1). There was no difference in CT_{max} between 3^{rd} and 4^{th} instars, but it was about 1.5˚C lower in 5^{th} instars (Tukey's HSD post-hoc test; Fig 2). In addition, parasitized caterpillars had a CT_{max} approximately 1.0˚C lower than non-parasitized caterpillars (Student's t post-hoc test; Fig 2).

Maximum daily air temperatures

The maximum daily air temperature recorded near the study site in the past 124 years was 42˚C, which was observed twice (Fig 3). The total number of days observed at or above 40˚C was 34 (0.03%; Fig 3).

Discussion

To our knowledge, this is the first study to test for differences in the upper thermal tolerance of component species in a natural H-P-HP system. Based on our estimates of CT_{max}, the upper

Table 1. Effects of ontogenetic stage (instar) and parasitism by the wasp *Cotesia congregata* on upper thermal tolerance (critical thermal maximum, CT_{max}) of *Manduca sexta* caterpillars.

Source	d.f.	Sums of squares	F-ratio	P-value
Instar	2	19.79	4.88	0.0135
Parasitism	1	9.40	4.64	0.0383
Instar x parasitism	2	2.26	0.56	0.5783
Error	35	104.46		

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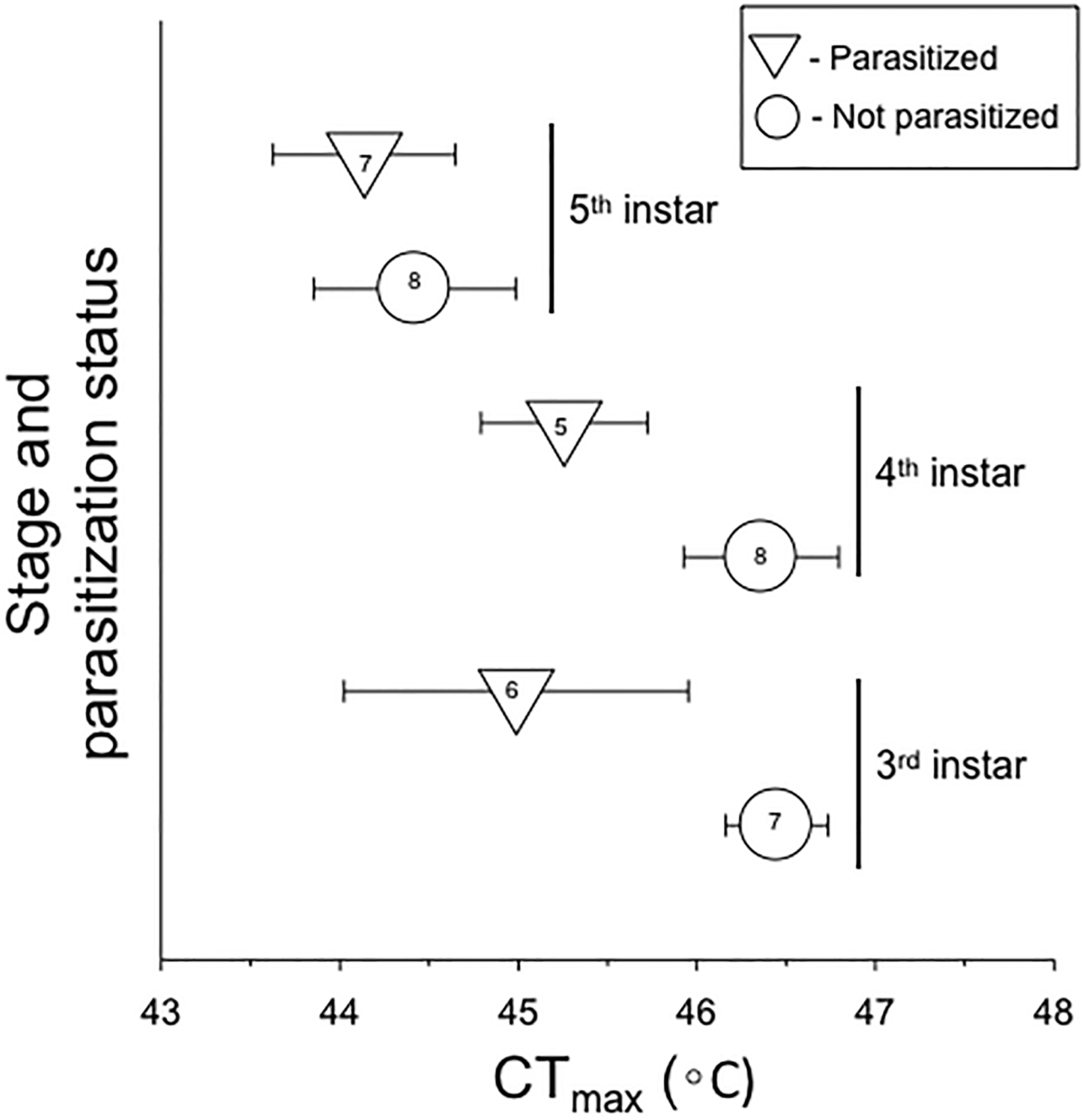

Fig 2. Variation in upper thermal tolerance (critical thermal maximum, CT_{max}) within the caterpillar host *Manduca sexta* in relation to ontogenetic stage (instar) and parasitism by the wasp *Cotesia congregata*. Data points represent the mean ± 1 standard error. Values inside data points represent sample size (number of individuals). The main effects of instar and parasitism were both significant (Two-way ANOVA; Table 1).

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limits to performance differ by several degrees between the caterpillar, *M*. *sexta*, and adults of its major parasitoid, *C*. *congregata*, and between *C*. *congregata* and adults of one of its major hyperparasitoids, *Conura* sp. The specific average values of CT_{max} that we estimated for the component species ranged from 42–48˚C, which is well within the range of previously measured values for insects [54].

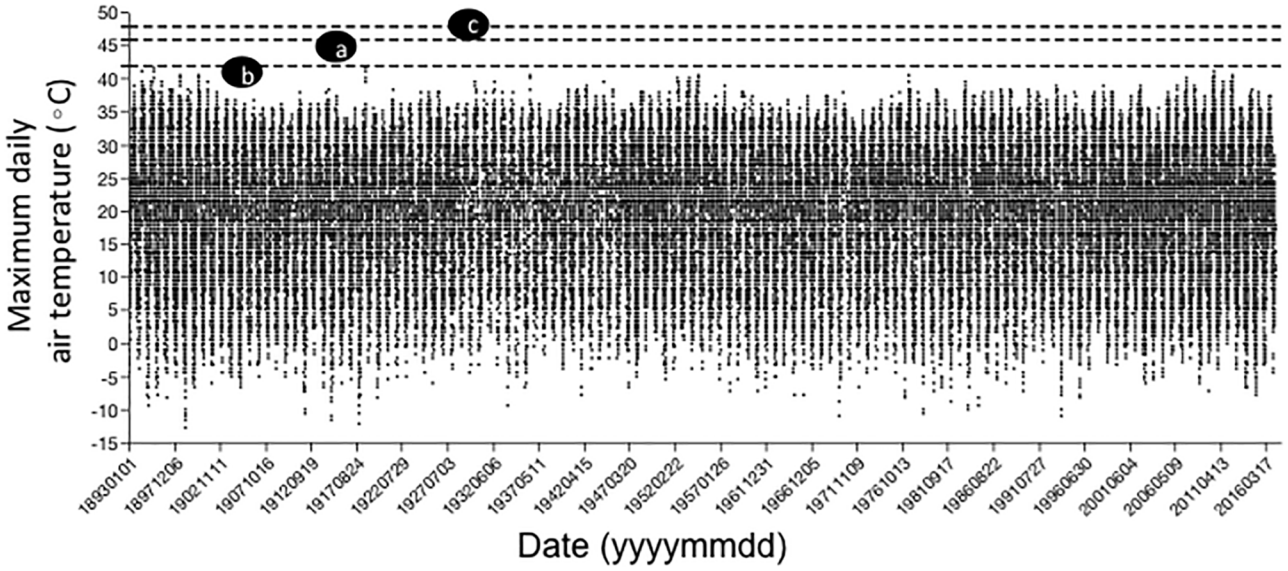

Fig 3. Maximum daily air temperatures recorded near the study site in the past 124 years compared with the average critical thermal maximum (CT_{max}) of component H-P-HP species measured in this study. Dashed lines: a = CT_{max} of host caterpillar, b = CT_{max} of parasitoid wasp, c = CT_{max} of hyperparasitoid wasp.

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Only two other studies that we are aware of have made similar comparisons in H-P-HP systems, both of which focused on cold tolerance. Campbell et al. [55] estimated lower developmental threshold temperatures in several aphid species and their parasitoids, including a H-P-HP system. In general, they found that adult parasitoids and hyperparasitoids had similar or slightly higher threshold temperatures than their hosts. Rice and Allen [56] estimated lower developmental threshold temperatures in a chrysomelid beetle, three of its parasitoids, and a hyperparasitoid of one of the parasitoids. Thresholds differed by less than 0.5°C among the parasitoid species but were about 2˚C higher in the host and about 4˚C higher in the hyperparasitoid. These quantitative differences in lower thermal tolerance among component H-P-HP species are comparable to the differences we found in upper thermal tolerance in our system.

Numerous other studies have compared various aspects of thermal performance between parasitoids and their hosts (i.e., between the first two trophic levels). Nealis et al. [57] studied lower developmental threshold temperatures in the non-native butterfly *Pieres rapae* from Canada and Australia, and three parasitoids from its native range that were introduced for biocontrol. In general, immature stages of parasitoids had similar or slightly higher threshold temperatures for development than their host. As in our study, van Baaren et al. [7] found the aphid, *Sitobion avenae*, had an upper thermal tolerance about 1–2˚C higher than adults of three parasitoid wasp species. Hughes et al. [58] found adult wasps had both higher heat tolerance and lower cold tolerance than their aphid host; although, the data derived from mass-reared laboratory populations which may not be representative of wild populations (e.g. [59, 60]). Wang et al. [61] compared entire thermal performance curves of a fruit fly introduced to California and two wasp parasitoids from its native range in Africa. One parasitoid had lower heat tolerance than the host, whereas the other had higher heat tolerance. Finally, Bahar et al. [62, 63] found several differences between the introduced caterpillar, *Plutella xylostella*, and its North American parasitoid, *Diadegma insulare*, that suggest larvae of this parasitoid species have lower heat tolerance than their recently acquired host. Beyond parasitoids, other studies

involving more traditional host-parasite systems have shown similar differences in various aspects of thermal performance [64–67].

In addition to variation among species, we found significant variation in upper thermal tolerance within the host, *M. sexta*, with respect to both instar and parasitism. Evidence for ontogenetic variation in thermal tolerance is widespread in insects [24–27]. We found that older and much larger 5th instar *M. sexta* had about 1.5˚C lower CT_{max} than 3rd and 4th instars. This result is consistent with a prior study suggesting that thermal tolerance decreases through larval ontogeny in *M. sexta*, with 5th instars being more sensitive to high temperatures than earlier instars ([68], and see [69]). We also found that caterpillars parasitized by *C. congregata* had about 1.0˚C lower CT_{max} than non-parasitized caterpillars. To our knowledge, this is the first study to show a difference in upper thermal tolerance between parasitized and non-parasitized hosts in a host-parasitoid system. Müller and Schmid-Hempel [70] demonstrated that parasitized bumblebees selected cooler climates by staying outside the nest overnight, which slowed development and reduced the success of its fly parasitoid. More generally, it is well-known that parasites and pathogens can modify host thermal tolerance (e.g. [28–32]) and host thermoregulatory behavior (e.g. [30, 31, 71, 72]).

Taken together, these results imply that significant differences in thermal performance among and within component species in host-parasite systems are common in nature. Although the specific reasons for the differences are unknown, they are presumably related to the evolutionary histories of habitat use and associated thermal environments and biophysical properties of the component species. Regardless of the reasons, these differences suggest that species may often respond differently and in complex ways to changes in thermal regimes and that large-scale climate change is likely to disrupt many existing interactions to some degree. In fact, climate change has been a driver of change in host-parasite systems throughout earth history [12, 14, 17, 73]. The current emerging infectious disease (EID) crisis is but one example [2, 18, 20, 73]. EIDs arise when parasites and pathogens encounter new, evolutionarily novel hosts. Large-scale perturbations like climate change are associated with shifting host and parasite phenologies, abundances and distributions, which increase biotic mixing, which is the source of EIDs [2, 18, 20, 73].

Relevance of CT_{max} to organisms in nature

Several simple indices to assess the vulnerability of organisms to climate change have been proposed, including "warming tolerance", sometimes defined as the difference between CT_{max} and the average habitat temperature [54, 74–76], and "thermal safety margin" or "thermal buffer", sometimes defined as the difference between CT_{max} and the maximal habitat temperature [77–79]. Whether such simple measures can capture enough inherent complexity of the problem to be useful remains to be seen. In the meantime, such proposals draw attention to the extreme limits to thermal performance and bring into question their relevance to organisms in nature. For instance, how often do organisms experience temperatures near or above their upper thermal limits? Although it may be infrequent, even occasional exposure to extreme high temperatures can have large impacts on the performance, abundance, and distribution of ectotherms [6, 11, 78, 80–85].

Ideally, information to gauge the frequency of exposure of small ectotherms to extreme high temperatures would derive from detailed studies of the microclimates and body temperatures they experience in their natural habitats [86–88]. In the absence of such information, we used maximum air temperatures recorded near the study site in the past 124 years as a crude but potentially informative tool. The highest daily air temperature recorded near the study site was 42˚C, which occurred twice, and the frequency of days with temperatures at or above

40˚C was less than a tenth of a percent. This is consistent with other data summarized for sites even closer to our study site for the period 1949–2001, when the highest recorded air temperature was 40˚C [89]. Two species (*M*. *sexta*, *Conura* sp.) we studied had average CT_{max} values that were several degrees higher than 42˚C while one species (*C*. *congregata*) had a value that was equal to 42˚C. Thus, maximal air temperatures recorded near the study site have never exceeded the CT_{max} of the three species in our study in the past 124 years. On the other hand, given the coarseness of the data, the fact that our estimated CT_{max} values were near the highest ever recorded maximum air temperatures suggests the organisms may occasionally experience body temperatures near their upper thermal limits in the field. Furthermore, climate projections predict an increase in heat waves and extreme high temperatures in the next 50–100 years in many temperate regions [90, 91]. Some models suggest this may have an especially large impact on mid-latitude species [78, 81], such as those studied here. Taken at face value, our results suggest that the parasitoid *C*. *congregata* may be the most vulnerable species in this H-P-HP system to the predicted warming because it currently has the lowest upper thermal tolerance.

Of course, organisms will begin to experience the sub-lethal effects of high temperature before reaching the extreme limits to thermal performance captured by measures such as CT_{max}. We showed recently evidence that the efficiency of mitochondria in harnessing oxygen and organic substrates into cellular energy (ATP) drops off rapidly past 35˚C in a laboratory strain of *M*. *sexta* [37]. This drop in mitochondrial efficiency is correlated with decreased larval growth rates and increased metabolic rates [36], suggesting that the "cost of living" goes up dramatically for caterpillars past 35˚C, long before reaching the estimated CT_{max} of 46˚C. The data in Fig 3 and those reported by Tilson et al. [89] suggest *M*. *sexta* may routinely experience temperatures near and above 35˚C in our study area over the course of a typical summer. In southern California, *M*. *sexta* caterpillars frequently experienced body temperatures above 36˚C during the day in July [92]. While body temperatures may rarely approach or exceed the extreme limits to performance, selection on performance at less extreme temperatures may indirectly drive their evolution and partly account for differences in CT_{max} among and within species.

Methodological issues with measuring CT_{max}

There are three issues with our methodology for estimating CT_{max} that are important to note. First, different ramping rates for the same species can give different values of CT_{max} and the same ramping rate can affect species differently [49–51]. Second, thermal history, which was unknown for our field-collected organisms, can affect CT_{max} [93]. Third, there is a question of how comparable CT_{max}—as measured by the onset of muscular spasms [46, 47]- is between organisms as different as wasps and caterpillars. We recorded videos of each individual, which allowed us to observe the temperature at the first observable muscular spasm very precisely. Were the spasms we observed representative of the same "event" in soft-bodied caterpillars and hard-bodied wasps? One way to address this question would be to use other measures of thermal tolerance (e.g., knockdown temperature, lethal temperature) to independently corroborate findings based on muscular spasms. Lighton and Turner's [48] method of thermolimit respirometry, which measures CT_{max} based on patterns of respiration as opposed to observing behavior, could be an especially useful technique to compare among different types and sizes of organisms. Due to limited samples of field-collected organisms, we were unable to address these issues in our study; however, it would be highly interesting to repeat our experiment using different ramping rates, thermal histories, and methods of estimating thermal tolerance to examine how methodology may affect the results.

Conclusions

Our study demonstrates differences in upper thermal limits among and within species in a natural tritrophic system involving parasitoids and their hosts. Other studies demonstrate similar differences in various other aspects of thermal performance between hosts and parasites. Such differences imply that climate change will be largely disruptive to these systems and that their responses will be highly varied and complex.

Supporting information

S1 Appendix. Data set analyzed in this study.
(XLSX)

S2 Appendix. Calibration of CT_{max} measurements.
(DOCX)

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References

1. Harrington R, Woiwood I, Sparks T. Climate change and trophic interactions. Trends Ecol Evol. 1999; 14: 146–150. PMID: 10322520
2. Epstein PR. Climate change and emerging infectious diseases. Microb Infect. 2001; 3: 747–754.
3. Thomas MB, Blanford S. Thermal biology in insect-parasite interactions. Trends Ecol Evol. 2003; 18: 344–350.
4. Hoover JA, Newman JA. Tritrophic interactions in the context of climate change: a model of grasses, cereal Aphids and their parasitoids. Glob Change Biol. 2004; 10: 1197–1208.

5. Brooks DR, Hoberg EP. How will global climate change affect parasite-host assemblages? Trends Parasitol. 2007; 23: 571–574. https://doi.org/10.1016/j.pt.2007.08.016 PMID: 17962073
6. Hance T, van Baaren J, Vernon P, Boivin G. Impact of extreme temperatures on parasitoids in a climate change perspective. Ann Rev Entomol. 2007; 52: 107–126.
7. van Baaren J, Le Lann C, van Alphen JJM. Consequences of climate change for aphid-based multi-trophic systems. In: Kindlmann P, Dixon AFG, Michaud JP, editors. Aphid Biodiveristy Under Environmental Change: Patterns and Processes. Springer; 2010. pp. 55–68.
8. Klapwijk MJ, Grobler C, Ward K, Wheeler D, Lewis OT. Influence of experimental warming and shading on host-parasitoid synchrony. Glob Change Biol. 2010; 16: 102–112.
9. Thompson LJ, Macfadyen S, Hoffmann AA. Predicting the effects of climate change on natural enemies of agricultural pests. Biol Control. 2010; 52: 296–306.
10. Dyer LA, Richards LA, Short SA, Dodson CD. Effects of CO_2 and temperature on tritrophic interactions. PLOS ONE. 2013; 8: e62528. https://doi.org/10.1371/journal.pone.0062528 PMID: 23638105
11. Bannerman JA, Roitberg BD. Impact of extreme and fluctuating temperatures on aphid-parasitoid dynamics. Oikos. 2014; 123: 89–98.
12. Kutz SJ, Hoberg EP, Molnar PK, Dobson A, Verocai GG. A walk on the tundra: host-parasite interactions in an extreme environment. Int J Parasitol Parasites Wildl. 2014; 3: 198–208. https://doi.org/10.1016/j.ijppaw.2014.01.002 PMID: 25180164
13. Agosta SJ, Hulshof CM, Staats EG. Organismal responses to habitat change: herbivore performance, climate and leaf traits in regenerating tropical dry forests. J Anim Ecol. 2017; 86: 590–604. https://doi.org/10.1111/1365-2656.12647 PMID: 28146325
14. Hoberg EP, Cook JA, Agosta SJ, Boeger W, Galbreath KE, Laaksonen S et al. Artic systems in the Quaternary: ecological collision, faunal mosaics and the consequences of a wobbling climate. J Helminth. 2017; 91: 409–421. https://doi.org/10.1017/S0022149X17000347 PMID: 28412980
15. Price PW. Evolutionary Biology of Parasites. Princeton University Press; 1980.
16. Brooks DR, McLennan. Parascript: Parasites and the Language of Evolution. Smithsonian Institution Press; 1993.
17. Hoberg EP, Brooks DR. A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host–parasite systems. J Biogeogr. 2008; 35: 1533–1550.
18. Agosta SJ, Janz N, Brooks DR. How specialists can be generalists: resolving the "parasite paradox" and implications for emerging infectious disease. Zoologia. 2010; 27: 151–162.
19. Godfray HCJ. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press; 1994.
20. Brooks DR, Hoberg EP. How will global climate change affect parasite-host assemblages? Trends Parasitol. 2007; 23: 571–574. https://doi.org/10.1016/j.pt.2007.08.016 PMID: 17962073
21. Godfray HCJ, Hassell MP, Holt RD. The population dynamic consequences of phenological asynchrony between parasitoids and their hosts. J Anim Ecol. 1994; 63: 1–10.
22. Hassell MP. The Spatial and Temporal Dynamics of Host-Parasitoid Interactions. Oxford University Press; 2000.
23. van Nouhuys S, Lei G. Parasitoid-host metapopulation dynamics: the causes and consequences of phenological asynchrony. J Anim Ecol. 2004; 73: 526–535.
24. Bowler K, Terblanche JS. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol Rev. 2008; 83: 339–355. PMID: 18979595
25. Kingsolver JG, Woods HA, Buckley LB, Potter KA, MacLean HJ, Higgins JK. Complex life cycles and the responses of insects to climate change. Integr Comp Biol. 2011; 51: 719–732. https://doi.org/10.1093/icb/icr015 PMID: 21724617
26. Chown SL, Duffy GA, Sorensen JG. Upper thermal tolerance in aquatic insects. Curr Opin Insect Sci. 2015; 11: 78–83. https://doi.org/10.1016/j.cois.2015.09.012 PMID: 28285762
27. Sinclair BJ, Marshall KE, Sewell MA, Levesque DL, Willett CS, Slotsbo S, et al.Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecol. Lett. 2016; 19: 1372–1385. https://doi.org/10.1111/ele.12686 PMID: 27667778
28. Vernberg WB, Vernberg FJ. Influence of parasitism on thermal resistance of the mud-flat snail, *Nassarius obsoleta* Say. Exp Parasitol. 1963; 14: 330–332. PMID: 14099845
29. Meißner K, Schaarschmidt T. Ecophysiological studies of *Corophium volutator* (Amphipoda) infested by microphallid trematodes. Mar Ecol Prog Ser. 2000; 202: 143–151.
30. Sherman E. Thermal biology of newts (*Notophthalmus viridescens*) chronically infected with a naturally occurring pathogen. J Therm Biol. 2008; 33: 27–31.

31. Bates AE, Leiterer F, Wiedeback ML, Poulin R. Parasitized snails take the heat: a case of host manipulation? Oecologia. 2011; 167: 613–621. https://doi.org/10.1007/s00442-011-2014-0 PMID: 21594622

32. Bruneaux M, Visse M, Gross R, Pukk L, Saks L, Vasemagi A. Parasite infection and decreased thermal tolerance: impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. Funct Ecol. 2017; 31: 216–226.

33. Kawahara AY, Breinholt JW, Ponce FV, Haxaire J, Xiao L, Lamarre GP, et al.Evolution of *Manduca sexta* hornworms and relatives: biogeographical analysis reveals an ancestral diversification in Central America. Mol Phylogenet Evol. 2013; 68: 381–386. https://doi.org/10.1016/j.ympev.2013.04.017 PMID: 23643972

34. Hodges RW. The Moths of America North of Mexico, Fasc. 21, Sphingoidea. E. W. Classey; 1971.

35. Madden AH, Chamberlin FS. Biology of the tobacco hornworm in the southern cigar-tobacco district. USDA Tech Bull. 1945; 896: 1–51.

36. Kingsolver JG, Woods HA. Thermal sensitivity of growth and feeding in *Manduca sexta* caterpillars. Physiol Zool. 1997; 70: 631–638. PMID: 9361137

37. Martinez E, Menze MA, Agosta SJ. 2017 Reduced mitochondrial efficiency explains mismatched growth and metabolic rate at supraoptimal temperatures. Physiol Biochem Zool. 2017; 90: 294–298. https://doi.org/10.1086/689871 PMID: 28277956

38. Beckage N, Tan F, Schleifer K, Lane R, Cherubin L. Characterization and biological effects of *Cotesia congregata* polydnavirus on host larvae of the tobacco hornworm, *Manduca sexta*. Arch Insect Biochem Physiol. 1994; 195: 165–195.

39. Beckage NE. Parasitoid polydnaviruses and insect immunity. In: Beckage NE, editor. Insect Immunology. Academic Press/Elsevier; 2008. pp. 243–270.

40. Kester KM, Barbosa P. Behavioral responses to host foodplants of two populations of the insect parasitoid *Cotesia congregata* (Say). Oecologia.1994; 99: 151–157. https://doi.org/10.1007/BF00317096 PMID: 28313961

41. Kester KM, Eldeib GM, Brown BL. Genetic differentiation of two host–foodplant complex sources of *Cotesia congregata* (Hymenoptera: Braconidae). Ann Entomol Soc Amer. 2015; 108: 1014–1025.

42. Krombein KV, Hurd PD Jr, Smith DR, Burks BD. Catalog of Hymenoptera in America North of Mexico, Vol 1. Smithsonian Institution Press; 1979.

43. Fulton BB. The hornworm parasite, *Apanteles congregatus* (Say) and the hyperparasite, *Hypopteromalus tabacum* (Fitch). Ann Entomol Soc Amer. 1940; 33: 231–244.

44. McNeil JN, Rabb RL. Life histories and seasonal biology of four hyperparasites of the tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae). Can Entomol. 1973; 105: 1041–1052.

45. Hansen JD. The life history and behavior of *Spilochalcis albifrons* (Hymenoptera: Chalcididae), a parasite of the larch casebearer, *Coleophora laricella* (Lepidoptera: Coleophoridae). J Kansas Entomol Soc. 1980; Jul 1: 553–566.

46. Lutterschmidt WI, Hutchison VH. The critical thermal maximum: data to support the onset of spasms as the definitive end point. Can J Zool. 1997; 75: 1553–1560.

47. Lutterschmidt WI, Hutchison VH. The critical thermal maximum: history and critique. Can J Zool. 1997; 75: 1561–1574.

48. Lighton JRB, Turner RJ. Thermolimit respirometry: an objective assessment of critical thermal maxima in two sympatric desert harvester ants, *Pogonomyrmex rugosus* and *P. californicus*. J Exp Biol. 2004; 207: 1903–1913. PMID: 15107444

49. Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL. Critical thermal limits depend on methodological context. Proc R Soc B Biol Sci. 2007; 274: 2935–2942.

50. Terblanche JS, Hoffmann AA, Mitchell KA, Rako L, le Roux PC, Chown SL. Ecologically relevant measures of tolerance to potentially lethal temperatures. J Exp Biol. 2011; 214: 3713–3725. https://doi.org/10.1242/jeb.061283 PMID: 22031735

51. Chown SL, Jumbam KR, Sorensen JG, Terblanche JS. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depends on methodological context. Func Ecol. 2009; 23: 133–140.

52. Menne MJ, Durre I, Vose RS, Gleason BE, Houston TG. An overview of the global historical climatology network-daily database. J Atmos Oceanic Technol. 2012; 29: 897–910.

53. Menne MJ, Durre I, Korzeniewski B, McNeal S, Thomas K, Yin X et al. Global Historical Climatology Network—Daily (GHCN-Daily), Version 3. NOAA National Climatic Data Center. 2012; Subset: CITY: US510015, 1893.01.10–2017.03.21, Accessed: 2017-03-23.

54. Hoffmann AA, Chown SL, Clusella-Trullas S. Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct Ecol. 2013; 27: 934–949.

55. Campbell A, Frazer BD, Gilbert N, Gutierrez AP, Mackauer M. Temperature requirements of some aphids and their parasites. J Appl Ecol. 1974; 11: 431–438.

56. Rice AD, Allen GR. Temperature and developmental interactions in a multitrophic parasitoid guild. Austral J Entomol. 2009; 48: 282–286.

57. Nealis VG, Jones RE, Wellington WG. Temperature and development in host-parasite relationships. Oecologia. 1984; 61: 224–229. https://doi.org/10.1007/BF00396765 PMID: 28309416

58. Hughes GE, Owen E, Sterk G, Bale JS. Thermal activity thresholds of the parasitic wasp *Lysiphlebus testaceipes* and its aphid prey: implications for the efficacy of biological control. Physiol Entomol. 2010; 35: 373–378.

59. Kingsolver JG, Nagle A. Evolutionary divergence in thermal sensitivity and diapause of field and laboratory populations of *Manduca sexta*. Physiol Biochem Zool. 2007; 80: 473–479. https://doi.org/10.1086/519962 PMID: 17717810

60. Thompson LM, Faske TM, Banahene N, Grim D, Agosta SJ, Parry D et al. Variation in growth and developmental responses to supraoptimal temperatures near latitudinal range limits of gypsy moth *Lymantria dispar* (L.), an expanding invasive species. Physiol Enotmol. 2017; 42: 181–190.

61. Wang X, Levy K, Son Y, Johnson MW, Daane KM. Comparison of the thermal performance between a population of the olive fruit fly and its co-adapted parasitoids. Biol Control. 2012; 60: 247–254.

62. Bahar MH, Soroka JJ, Dosdall LM. 2012 Constant versus fluctuating temperatures in the interactions between *Plutella xylostella* (Lepidoptera: Plutellidae) and its larval parasitoid *Diadegma insulare* (Hymenoptera: Ichnuemonidae). Environ Enotmol. 2012; 41: 1653–1661.

63. Bahar MH, Hegedus D, Soroka J, Coutu C, Bekkaoui D, Dosdall L. Survival and *Hsp70* gene expression in *Plutella xylostella* and its larval parasitoid *Diadegma insulare* varied between slowly ramping and abrupt extreme temperature regimes. PLOS ONE. 2013; 8: e73901. https://doi.org/10.1371/journal.pone.0073901 PMID: 24040110

64. Mullens BA, Paine EO, Velten RK. Temperature effects on survival and development of *Heleidomermis magnapapula* in the labatory. J Nematol. 1995; 27: 29–35. PMID: 19277258

65. Woodhams DC, Costanzo JP, Kelty JD, Lee RE Jr. Cold hardiness in two helminth parasites of the freeze-tolerant wood frog, *Rana sylvatica*. Can J Zool. 2000; 78: 1085–1091.

66. Welsh D, Clopton RE, Parris CL. Differential temperature acclimatization responses in the membrane phospholipids of *Posthodiplostomum minimum* and its second intermediate host, *Lepomis macrochirus*. J Parasitol. 2006; 92: 764–769. https://doi.org/10.1645/GE-741R.1 PMID: 16995394

67. Gsell AS, de Senerpont Domis LN, van Donk E, Ibelings BW. Temperature alters host genotype-specific susceptibility to chytrid infection. PLOS ONE. 2013; 8: e71737. https://doi.org/10.1371/journal.pone.0071737 PMID: 23990982

68. Petersen C, Woods HA, Kingsolver JG. Stage-specific effects of temperature and dietary protein on growth and survival of *Manduca sexta* caterpillars. Physiol Entomol. 2000; 25: 35–40.

69. Woods HA. Ontogenetic changes in the body temperature of an insect herbivore. Funct Ecol. 2013; 27: 1322–1331.

70. Müller CB, Schmid-Hempel P. Exploitation of cold temperatures as defence against parasitoids in bumblebees. Nature. 1993; 363: 65–67.

71. Moore J, Freehling M. Cockroach hosts in thermal gradients suppress parasite development. Oecologia. 2002; 133: 261–266. https://doi.org/10.1007/s00442-002-1030-5 PMID: 28547314

72. Paranjpe DA, Medina D, Nielsen E, Cooper RD, Paranjpe SA, Sinervo B. Does thermal ecology influence dynamics of side-blotched lizards and their micro-parasites? Integr Comp Biol. 2014; 54: 108–117. https://doi.org/10.1093/icb/icu069 PMID: 24920752

73. Brooks DR, Ferrao AI. The historical biogeography of coevolution: emerging infectious diseases are evolutionary accidents waiting to happen. J Biogeogr. 2005; 32: 1291–1299.

74. Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC et al. Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl Acad Sci USA. 2008; 105: 6668–6672. https://doi.org/10.1073/pnas.0709472105 PMID: 18458348

75. Bonebrake TC, Deutsch CA. Climate heterogeneity modulates impact of warming on tropical insects. Ecology. 2012; 93: 449–455. PMID: 22624199

76. Diamond SE, Sorger DM, Hulcr J, Pelini SL, Del Toro I, Hirsch C et al.Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. Glob Change Biol. 2012; 18: 448–456.

77. Kellerman V, Overgaard J, Hoffman AA, Flojgaard C, Svenning J, Loeschcke V. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. Proc Natl Acad Sci USA. 2012; 109: 16228–16233. https://doi.org/10.1073/pnas.1207553109 PMID: 22988106

78. Kingsolver JG, Diamond SE, Buckley LB. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct Ecol. 2013; 27: 1415–1423.

79. Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT et al. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc Natl Acad Sci USA. 2014; 111: 5610–5615. https://doi.org/10.1073/pnas.1316145111 PMID: 24616528

80. Kingsolver JG. Feeding, growth, and the thermal environment of cabbage white caterpillars, *Pieris rapae* L. Physiol Biochem Zool. 2000; 73: 621–628. https://doi.org/10.1086/317758 PMID: 11073798

81. Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS et al. Increased temperature variation poses a greater risk to species than climate warming. Proc R Soc B. 2014; 281: 20132612. https://doi.org/10.1098/rspb.2013.2612 PMID: 24478296

82. Buckley LB, Huey RB. How extreme temperatures impact organisms and the evolution of their thermal tolerance. Integr Comp Biol. 2016; 56: 98–109. https://doi.org/10.1093/icb/icw004 PMID: 27126981

83. Buckley LB, Huey RB. Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerability. Glob Change Biol. 2016; 22: 3829–3842.

84. Roitberg BD, Mangel M. Cold snaps, heatwaves, and arthropod growth. Ecol Entomol. 2016; 41: 653–659.

85. Williams CM, Buckley LB, Sheldon KS, Vickers M, Portner HO, Dowd WW et al. Biological impacts of thermal extremes: mechanisms and costs of functional responses matter. Integr Comp Biol. 2016; 56: 73–84. https://doi.org/10.1093/icb/icw013 PMID: 27252194

86. Willmer PG. Microclimate and the environmental physiology of insects. Adv Insect Physiol. 1982; 16: 1–57.

87. Pincebourde S, Woods HA. Climate uncertainty on leaf surfaces: the biophysics of leaf microclimates and their consequences for leaf-dwelling organisms. Funct Ecol. 2012; 26: 844–852.

88. Bakken GS, Angilletta MJ. How to avoid errors when quantifying thermal environments. Funct Ecol. 2014; 28: 96–107.

89. Tilson WM, Teutsch CD, Wilkinson WB III. Weather data compiled for Blackstone, Virginia: 1949–2001. Virginia Agricultural Experiment Station. 2002; Information Series 02–2.

90. Meehl GA, Tebaldi C. More intense, more frequent, and longer lasting heat waves in the 21st century. Science. 2004; 305: 994–997. https://doi.org/10.1126/science.1098704 PMID: 15310900

91. Battisti DS, Naylor R. Historical warnings of future food insecurity with unprecedented seasonal heat. Science. 2009; 323: 240–244. https://doi.org/10.1126/science.1164363 PMID: 19131626

92. Casey TM. Activity patterns, body temperature and thermal ecology in two desert caterpillars (Lepidoptera: Sphingidae). Ecology. 1976; 57: 485–497.

93. Kellermann V, van Heerwaarden B, Sgro CM. 2017 How important is thermal history? Evidence for lasting effects of developmental temperature on upper thermal limits in *Drosophila melanogaster*. Proc R Soc B. 2017; 284: 20170447.