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Rearing temperature and parasitoid load determine host and parasitoid performance in *Manduca sexta* and *Cotesia congregata*

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Abstract. 1. Temperature strongly influences the rates of physiological processes in insects, including the herbivore *Manduca sexta* and its larval endoparasitoid *Cotesia congregata*. Parasitisation by *C. congregata* decreases the growth and consumption of food by larval *M. sexta*. However, the effects of temperature on parasitised caterpillars and the developing wasp larvae are largely unknown.

2. In this study, parasitised and unparasitised caterpillars were reared at three constant temperatures (20, 25 and 30 °C) throughout larval development. Caterpillar mass gain and consumption were monitored daily until wandering (unparasitised control group) or wasp emergence (parasitised group) was observed. Development time and survival to emergence were measured as metrics of parasitoid performance.

3. Parasitised *M. sexta* developed more slowly than unparasitised controls, but had similar cumulative consumption until the terminal instar. Parasitised caterpillars with relatively large parasitoid loads had higher rates of consumption and growth than those with smaller loads. Both temperature and parasitoid load strongly affected wasp success. Mean development time to wasp emergence increased with low temperatures and with large loads. The combination of warm temperature and large parasitoid loads greatly reduced wasp survival.

4. These results demonstrate the interactive effects of rearing temperature and parasitisation on host consumption and growth rates throughout larval development. In addition, wasp performance was affected by the interaction of temperature and parasitoid load size. High temperatures alter the dynamics of the interaction between the parasitoid and its caterpillar host, which could have far-reaching impacts as the global temperatures continue to rise.

Key words. Temperature, ectotherm, host–parasitoid.

Introduction

Environmental temperatures are a major determinant of the organismal performance and ecological success of insects and other ectotherms (Prosser, 1955; Huey & Kingsolver, 1989; Angilletta Angilletta Jr., 2009). Temperature impacts a range of biological processes in ectotherms, including behaviour, consumption, growth and developmental rates, digestive efficiency, immune function, fecundity and survival (Huey & Kingsolver, 1989; Atkinson, 1994; Kingsolver & Woods, 1998; Adamo & Lovett, 2011; Piyaphongkul *et al.*, 2012; Bauerfeind & Fischer, 2014).

Temperature can also affect the ecological interactions of insects with other species (Bauerfeind & Fischer, 2013; Barton & Ives, 2014; Lemoine *et al.*, 2014). Many studies on herbivorous insects have explored how temperature interacts with host plant nutrition and secondary chemistry to determine the survival, growth and development of the herbivores (Stamp *et al.*, 1994; Stamp & Osier, 1998; Diamond & Kingsolver, 2010; Bauerfeind & Fischer, 2013; Clissold *et al.*, 2013; Bauerfeind & Fischer, 2014). Temperature can alter predator–prey and host–parasitoid interactions between insects, with important implications for understanding responses to climate change

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(Barton & Ives, 2014; Furlong & Zalucki, 2017). For example, theoretical and empirical works indicate that predator, parasite and parasitoid thermal tolerances are often lower than those of their prey/hosts (Bahar *et al.*, 2012; Flores-Mejia *et al.*, 2016; Furlong & Zalucki, 2017; Agosta *et al.*, 2018). As a consequence, increasing mean temperatures may have contrasting effects on insect herbivores and their insect parasitoids, altering the outcome and dynamics of their interactions (Baffoe *et al.*, 2012; Bahar *et al.*, 2012; de Sassi & Tylianakis, 2012; Delava *et al.*, 2016; Furlong & Zalucki, 2017).

Insect endoparasitoids develop within the body of their host, and rely on the host's resources to meet their nutritional requirements (Godfray, 1994). The total number of parasitoid larvae within a host (parasitoid load) can influence parasitoid growth and survival (Beckage & Riddiford, 1978; Beckage & Riddiford, 1983; Alleyne & Beckage, 1997; Elzinga *et al.*, 2003; Gu *et al.*, 2003; Smallegange *et al.*, 2008). Large parasitoid loads lead to prolonged parasitoid development time within the host, decreased survival, and lower adult weights in the hymenopteran *Cotesia congregata* (Beckage & Riddiford, 1983). In some systems, the host insect can also be affected by large loads – heavily parasitised hosts attain greater mass by the end of parasitoid development than those with low load numbers, even after the weight of the parasitoids has been accounted for (Beckage & Riddiford, 1983). For many agricultural insect pests, the independent effects of temperature and parasitisation are well described (Stamp & Osier, 1998; Beckage & Gelman, 2004; Jaramillo *et al.*, 2009; Piyaphongkul *et al.*, 2012), but the joint effects of these factors on hosts and their larval endoparasitoids are more poorly understood (Baffoe *et al.*, 2012; Bahar *et al.*, 2012; Delava *et al.*, 2016).

The parasitoid wasp, *C. congregata*, and its most common recorded host, the tobacco hornworm (*M. sexta*), have served as a model for many aspects of host–parasitoid interactions for the past 40 years (Beckage & Riddiford, 1978; Beckage & Riddiford, 1982; Barbosa *et al.*, 1986; Kester & Barbosa, 1991a; Kester & Barbosa, 1991b; Lentz & Kester, 2008; Bredlau & Kester, 2015; Adamo *et al.*, 2016). The thermal biology of *M. sexta* has been well documented (Casey, 1976; Reynolds & Nottingham, 1985; Stamp, 1990; Kingsolver & Woods, 1997), as have the effects of parasitisation by *C. congregata*. Multiple *C. congregata* larvae develop within the body of the host, and during larval development, *C. congregata* manipulates multiple aspects of its host's physiology, including decreasing consumption, growth and locomotion prior to parasitoid emergence. These alterations are necessary for successful parasitoid emergence and pupation before the host's inevitable death. (Beckage & Templeton, 1986; Adamo *et al.*, 1997; Adamo, 1998; Adamo *et al.*, 2016). How temperature affects the consumption of food and subsequent growth of parasitised hosts, and the development and survival of the parasitoid larvae, is unknown in this system.

In this study, we took daily measurements of consumption and growth of parasitised and unparasitised *M. sexta* on artificial diet, reared at three constant temperatures (20, 25, 30 °C) known to be non-stressful for unparasitised caterpillars (Kingsolver, 2007; Kingsolver & Nagle, 2007; Kingsolver *et al.*, 2012). These treatments represent ecologically relevant temperatures that could be experienced in the field by caterpillars and parasitoids throughout the season (Diamond & Kingsolver, 2010). Wasp development time and survival to emergence were measured as metrics of parasitoid performance. We used these data to test four hypotheses: (i) parasitism of *M. sexta* by *C. congregata* will decrease the rate of host consumption and mass gain throughout host larval development; (ii) warmer rearing temperatures will increase rates of consumption, growth (mass gain) and development in both unparasitised and parasitised *M. sexta*; (iii) warmer rearing temperatures will reduce development time and survival of wasp larvae, especially at the highest rearing temperature; and (iv) parasitoid load and temperature will interact to affect the mass gain and consumption of the host, as well as the development time and survival of the wasp larvae.

Methods and materials

Study organisms

Manduca sexta caterpillars used in this experiment were obtained from the laboratory colony at the University of North Carolina-Chapel Hill (UNC-CH). This colony has been maintained under laboratory conditions at UNC-CH since the 1980s (*c*. 235 generations), with no reintroduction of wild individuals during that time. It was originally sourced from a colony in Raleigh, North Carolina, that was established in the 1960s from wild individuals collected in Clayton, North Carolina. The colony was maintained at 25 °C at all life stages. Caterpillars were reared on a standard artificial (high protein) diet modified from Kingsolver and Woods (1998). Adult moths were fed a 10% honey water solution.

Cotesia congregata wasps were obtained from a laboratory colony maintained at Virginia Commonwealth University, Richmond, Virginia. Wasps were originally sourced from *M. sexta* collected in 2005 from the Southern Piedmont Agricultural Research and Experimental Station (Blackstone, Nottoway Co. site; 37.0817°N, 77.9755°W). Wild wasps from this site were introduced annually to the laboratory colony. Wasps were shipped as cocoons to UNC-CH in October 2016. Adult wasps were fed a 60% honey-agar solution and provided with damp sponges for water. Cocoons, adult wasps, and non-experimental *M. sexta* hosts were maintained at room temperature (*c*. 25–26 °C). Upon eclosion, adult wasps were used to parasitise experimental caterpillars, as well as to perpetuate a second (overlapping) generation to ensure wasp availability throughout the study.

Experimental protocol

Our experiment used a full-factorial design, with three constant rearing temperatures that are known to be non-stressful for *M. sexta* (20, 25 and 30 °C) (Kingsolver *et al.*, 2015) and two parasitisation groups (parasitised and unparasitised controls). Caterpillar consumption, mass and development time were tracked from the start of the third instar until wasp larvae emerged or unparasitised hornworms entered the prepupal wandering stage. Caterpillar development time was measured as the number of hours from the start of the third instar to each

subsequent larval moult and either wandering or wasp emergence. With regard to wasp development, we will refer to terminology defined in Beckage and Riddiford (1978), wherein 'emergence' refers to second-instar wasp larvae exiting their host. Wasp development time was recorded in days from oviposition to emergence. Survival was determined as the number of larvae that successfully emerged, relative to the total number of larvae within the host (parasitoid load). Caterpillars were reared in environmental chambers (Percival Scientific 36VL, Perry, Iowa) at constant temperature and an LD 14:10 h photocycle. An open container of water was placed in each chamber and refilled every few days to maintain high RH and prevent potential desiccation of caterpillars and diet. Relative humidity was not measured, but this method has proved effective in preventing desiccation in environmental chambers.

To initiate the experiment, *M. sexta* eggs collected from the UNC colony were kept at 25 °C, and then assigned evenly to large Petri dishes in each temperature treatment. Egg dishes were checked every 24 h for newly hatched caterpillars. Hatchlings were transferred to a new Petri dish with fresh diet and were placed in an environmental chamber running the appropriate rearing temperature; hatch date was recorded for each day's group of hatchlings. Caterpillars were reared communally in shallow Petri dishes (diameter 14.5 cm, depth 1.5 cm) of 20–30 larvae through the first and second instars, provided new diet as needed, and monitored daily until they moulted to the third instar. On the morning of the first day of the third instar, each caterpillar was given a unique identification number, weighed, and assigned randomly to the control (20 °C, $N = 41$; 25 °C, $N = 39$; 30 °C, $N = 40$) or parasitised (20 °C, $N = 48$; 25 °C, $N = 46$; 30 °C, $N = 49$) treatment group.

Caterpillars in the parasitised treatment were exposed individually to adult wasps at room temperature (*c*. 25–26 °C) in the wasp colony enclosure. Once successful oviposition occurred (oviposition event lasting > 2–3 s), the caterpillar was removed from the enclosure and returned to its individual Petri dish. The number of ovipositions was recorded for each caterpillar. Because multiple ovipositions can result in very large numbers of parasitoid larvae, caterpillars that were parasitised more than twice were excluded from the study. The mean load size did not vary appreciably with temperature (20 °C, 112.6 ± 9.68; 25 °C, 120.3 ± 10.16; 30 °C, 111.7 ± 9.31), indicating that it is unlikely that female *C. congregata* oviposition decisions were influenced by caterpillar rearing temperature.

For the duration of the experiment each caterpillar was housed and monitored individually in a small Petri dish until the fifth instar, when they were moved to a larger, deep Petri dish if their movement was constrained (diameter 9 cm, depth 1.5 cm, and diameter 14.5 cm, depth 2.5 cm, respectively). Caterpillar mass was measured every 6 and 18 h for the first 6 days after the beginning of the third instar. After 6 days, mass was measured every 24 h until the caterpillar wandered or wasp larvae emerged, depending on parasitisation treatment. Caterpillar instar was recorded at each time point, and the age and mass were noted for each larval moult. Consumption was measured throughout the caterpillars' development by providing fresh, pre-weighed blocks of diet at the beginning of each time period (6, 18, 24 h). After each time period, the remaining diet not consumed by the caterpillar was weighed. The recorded wet mass of diet was converted to dry mass to account for mass change due to water loss, rather than consumption (see later). Control blocks of diet, similar in mass and dimension to those provided to the caterpillars, were placed in each chamber to account for mass change solely due to water loss. A subset of control blocks (eight to 15) from each temperature and time period were saved and frozen to create a dry mass conversion factor (see later).

At wandering, unparasitised caterpillars were weighed and euthanised. As parasitisation inevitably leads to host death under normal circumstances, caterpillars in the parasitisation treatment that displayed wandering behaviour were presumed to be the result of a failed oviposition and were excluded from all analyses (20 °C, $N = 3$; 25 °C, $N = 2$; 30 °C, $N = 1$). Similarly, 'parasitised' caterpillars that did not wander, failed to yield emergent wasps, and upon dissection did not contain wasp larvae were excluded from analyses (20 °C, $N = 0$; 25 °C, $N = 1$; 30 °C, $N = 1$). Diet was not weighed at wandering, as wanderers do not eat and often burrow into their food, making accurate measurements difficult. For parasitised caterpillars, mass was not recorded at wasp emergence to avoid disturbing the wasp larvae; instead, weights taken the day before were used in all analyses and figures. Caterpillars that died during the course of the experiment were excluded from analyses; mortality was low and did not vary much with temperature or parasitisation treatment (20 °C, $N_{\text{control}} = 2$, $N_{\text{parasitised}} = 2$; 25 °C, $N_{\text{control}} = 0$, $N_{\text{parasitised}} = 2$; 30 °C $N_{\text{control}} = 1$, $N_{\text{parasitised}} = 4$).

Wasp emergence, load, and survival

When parasitoid emergence was observed, individual caterpillars and all wasp larvae were transferred to a clean Petri dish. Caterpillars were not provided with food after wasp emergence, as feeding behaviour is suppressed 8–12 h prior to wasp emergence (Adamo *et al.*, 1997). Caterpillars and wasp larvae were left undisturbed for 2 days to allow for the completion and hardening of wasp cocoons, at which time cocoons were removed from the caterpillar and counted. The total number of wasp larvae that emerged, regardless of whether they successfully spun cocoons, was used for all analyses. Due to time constraints, wasp survival to adult eclosion was not measured. Parasitised caterpillars were frozen for later dissection to determine total parasitoid load. Frozen hosts were dissected by cutting along the dorsal midline with dissecting scissors. The cuticle was held to the sides with pins, and the gut was removed. All wasp larvae were removed from the host's body and counted to determine the number of wasp larvae that failed to emerge. Parasitoid load was calculated as the sum of the number of wasp larvae that failed to emerge plus the number of successfully emerged larvae.

Dry mass conversion

To calculate consumption during the experiment, the recorded wet mass of the artificial diet was converted to dry mass (e.g. Stamp & Horwath, 1992). We created two conversion factors to account for the difference in water loss between fresh diet and diet exposed to experimental conditions. The conversion

factors were developed by drying fresh diet blocks ($n = 100$) and a subset of control diet blocks from each temperature and time period combination ($n = 101$) to a constant weight. Dry mass was plotted against wet mass for each condition ('fresh' or 'exposed'), and the slopes of the linear regressions (intercept forced through 0) were used as conversion factors.

Analysis

A major goal of our study was to determine the relationships of caterpillar mass and consumption with age (from the start of the third instar), and how these relationships change with temperature and parasitism status. Caterpillar growth and consumption are non-linear and dependent on age and developmental stage, and we lack *a priori* hypotheses about parametric functions relating size or consumption to age. Preliminary analyses using linear mixed effects models with a polynomial term for age did not provide a good fit for our data. As an alternative, we used a general additive mixed-effects model (GAMM) with thin-plate splines to account for the non-linear relationships of our data. As we were not specifically interested in the functional form of our mass and consumption curves, a GAMM model allowed for more flexibility in describing the non-linear relationships between age and our treatment groups. Mass and consumption were log-transformed prior to analysis to achieve homogeneity of variance and normalcy of errors. We use a value of $P < 0.05$ to define significance for all analysis results. Mass and consumption were analysed separately using GAMM models with the function 'gam' in package MGCV in R (v.3.5.2), and parameters were estimated using maximum likelihood.

In our model, we used age as a smoothing term with an interaction of parasitisation treatment and temperature, and with the maximum number of knots set to 10. Temperature, parasitisation treatment and the interaction term were included as fixed effects. A smoothing term of individual was included as a random effect, allowing splines to vary by individual caterpillar. Comparison of models with and without the interaction of treatment and temperature in the smoothing term indicated that the full model was a significantly better fit for both mass and consumption. Examination of our model residuals by age indicate that the GAMM model is a good fit for mass at all ages (Fig. S2), and is an adequate fit for consumption except at very young ages (Fig. S2).

For the subset of individuals in the parasitised treatment, the effect of parasitoid load on mass and consumption was analysed using GAMM models with thin-plate splines. These models were similar to those used to analyse the full dataset (described earlier), but as load is a numeric variable, it was included in the smoothing term with age. Smoothing terms had an interaction with temperature and the maximum number of knots was not specified in the model for mass, but was specified as $k = 10$ for the consumption model. Temperature was included as a fixed effect, and individual was used as a random effect. Comparisons with models that specified age and load as separate smoothing terms were significantly worse than models with age and load in the same smoothing term. Examination of our model residuals by age indicate that this GAMM model is a good fit for mass at all ages (Fig. S3), and is an adequate fit for consumption except at very young ages (Fig. S3). We note that there is greater heterogeneity in the residuals (and poorer model fits) at very young ages because caterpillars do not eat or grow during moulting, and the timing of moult from the third to the fourth instar varies among individuals within and between treatment groups.

We also modelled survival and development of wasps for the parasitised treatment groups. Wasp survival was analysed with a generalised linear mixed-effects model using a binomial distribution with the glmer function in the LME4 package in R (v.3.4.1). Survival was defined as the number of wasp larvae that emerged from the host cuticle (success) versus the number that failed to emerge (failure). Temperature, load and the interaction term were included as fixed effects. A random intercept of individual host ID was used as a random effect. Parasitoid load was scaled from 0 to 1, and centred around the mean.

Parasitoid development time from oviposition to wasp emergence (in h) was analysed with a linear mixed-effects model using the 'lme' function in the NLME package in R (v.3.4.1). Temperature, parasitoid load, and the interaction term were used as fixed effects. A random intercept of individual host ID was used as a random effect.

Results

Effects of temperature and parasitisation on caterpillar consumption and growth

Accumulation of mass and consumption were significantly affected by both rearing temperature and parasitisation (Fig. 1; see parametric terms in Table 1). In addition, the curves relating to mass and consumption were significantly affected by temperature and parasitisation (Fig. 1; see smoothing terms in Table 1). Temperature increased the rate of mass accumulation and consumption, such that caterpillars reared at higher temperatures had, on average, accumulated more mass and eaten more diet at younger ages than those at lower temperatures (Fig. 1; Table 1). Increasing rearing temperature also increased mean development rates, resulting in shorter development times to each instar and to wandering (unparasitised treatment) or wasp emergence (parasitised treatment) (Fig. 1). The rates of development, consumption, and mass accumulation were more similar at 25 and 30 °C than at 20 °C; at the lower temperature, these rates slowed dramatically during the final two instars, resulting in a long delay in the time to wandering or wasp emergence (Fig. 1).

Parasitised caterpillars had significantly decreased rates of consumption and mass accumulation compared with controls; the mean mass and mean amount of diet consumed were lower for parasitised caterpillars at each age than for their unparasitised counterparts. (Fig. 1; Table 1). The reduction in the rates (i.e. the slope of curve with age) of consumption and mass accumulation began soon after parasitisation, and continued throughout the host's life span. During the third and fourth instars, total consumption during each instar was similar in control and parasitised caterpillars; by contrast, during the fifth (final) instar, total consumption was roughly three times lower in the parasitised treatment for all temperatures (Fig. 1). These

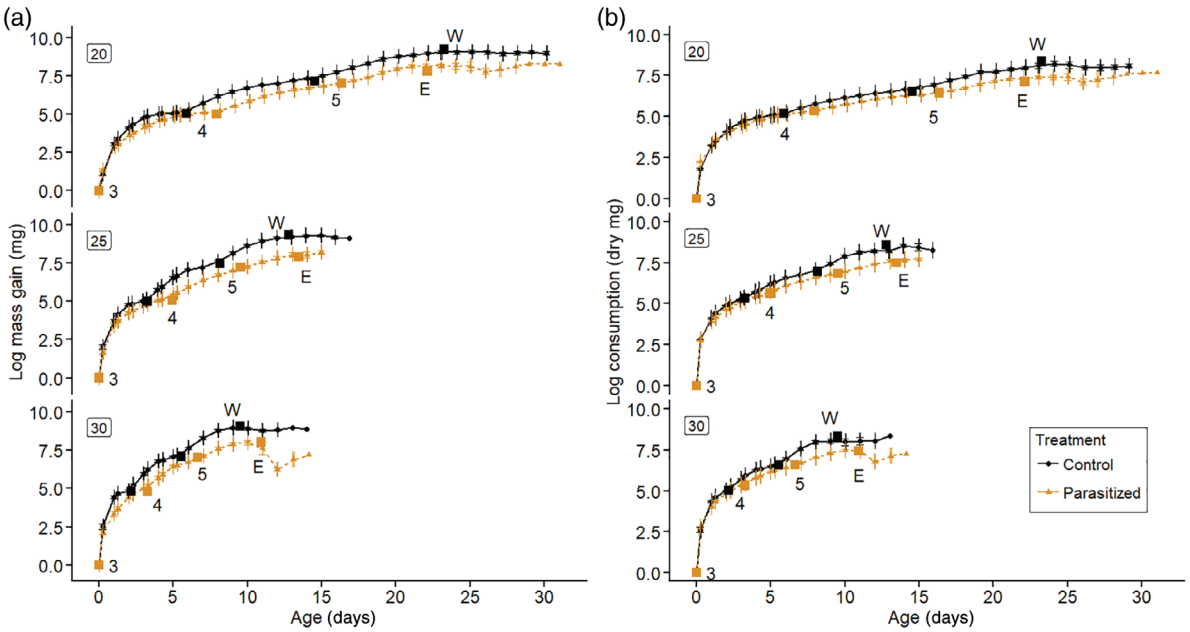

Fig. 1. Mean mass gain (a) and mean consumption (b) of unparasitised (black) and parasitised (yellow) *Manduca sexta* caterpillars at three experimental temperatures (20, 25, 30 °C). Small points (circles, unparasitised controls; triangles, parasitised) represent the average age and measurement taken at each experimental time point (every 6 and 18 h for the first 6 days after moulting to third instar, and every 24 h subsequently). Large points indicate the mean age and mass gain/consumption at each larval moult (third, fourth, fifth instars and wandering/wasp emergence). Age 0 indicates the moult to the third instar, when caterpillars entered the experiment. Mass gain and consumption were measured in mg, and displayed here on a logarithmic scale. Error bars are SEs. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 1. Results from a general additive mixed-effects model with thin plate splines on the effects of temperature and parasitisation on *Manduca sexta* mass and consumption. Age was used as a smoothing term with an interaction of temperature and parasitisation, and the maximum number of knots was set to 10. Temperature, parasitisation and their interaction were included as fixed effects. Individual was included as a smoothing term as a random effect. Parameters were estimated using maximum likelihood.

		Mass			Consumption		
		d.f.	*F*	*P*-value	d.f.	*F*	*P*-value
Parametric terms	Parasitisation	1	39.34	**< 0.0001**	1	34.32	**< 0.0001**
	Temperature	2	81.67	**< 0.0001**	2	5.90	**< 0.0001**
	Parasitasation: temperature	2	4.59	**0.0102**	2	5.60	**< 0.0001**
Smoothing terms	Age: control = 20	8.68	3622.55	**< 0.0001**	8.95	2151.87	**< 0.0001**
	Age: parasitisation = 20	8.57	3079.05	**< 0.0001**	8.94	1840.98	**< 0.0001**
	Age: control = 25	5.63	3487.56	**< 0.0001**	6.92	1895.67	**< 0.0001**
	Age: parasitisation = 25	5.42	2667.06	**< 0.0001**	6.90	1840.64	**< 0.0001**
	Age: control = 30	4.44	3120.76	**< 0.0001**	5.89	1960.03	**< 0.0001**
	Age: parasitisation = 30	4.94	2949.14	**< 0.0001**	8.63	1451.63	**< 0.0001**
	Individual	249.78	31.65	**< 0.0001**	185.19	2.22	**< 0.0001**

Bold values are significant. Defined as $P < 0.05$.

findings are consistent with previous studies reported for this host–parasitoid system (Beckage & Riddiford, 1978; Bcntz & Barbosa, 1990). There were also significant interactions between parasitisation status and rearing temperature that altered the relationship between age and consumption or mass accumulation (Table 1). For example, caterpillars ate less and accumulated less mass when parasitised, and these effects were magnified in the 25 °C treatment (Fig. 1). In addition, increasing temperature decreased the difference in development time to each moult between control and parasitised caterpillars (Fig. 1).

Total mass accumulation and final size at the end of larval development were significantly affected by both rearing temperature and parasitisation (Table 1). Mean final size decreased with increasing temperature and with parasitisation. Temperature also altered the relative timing of wandering and wasp emergence between unparasitised and parasitised caterpillars, respectively (Fig. 2). At 20 °C, wasp emergence occurred in parasitised caterpillars before wandering in the control treatment. The two warmer temperatures (25 and 30 °C) showed the opposite trend, with unparasitised caterpillars having a shorter

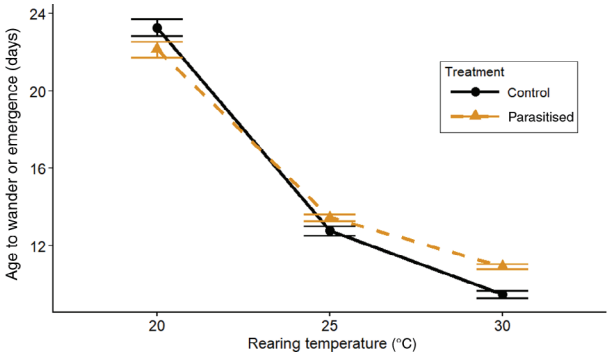

Fig. 2. Mean age (in days) from entering the experiment (moult to third instar) to either wandering (unparasitised controls,), or wasp emergence (parasitised, yellow) at three rearing temperatures (20, 25, 30). Error bars are SEs. [Colour figure can be viewed at wileyonlinelibrary.com].

development time to wandering than parasitised caterpillars to wasp emergence (Fig. 2).

Effects of temperature and parasitoid load on wasp development time and survival

Survival of *C. congregata* to emergence was significantly impacted by temperature ($F = 113.87$, d.f. $= 7$, $P < 0.0001$), parasitoid load ($F = 4.27$, d.f. $= 7$, $P < 0.0001$) and the interaction between the two ($F = 6.08$, d.f. $= 7$, $P = 0.0034$) (Fig. 3a). The mean number of emerging wasp larvae was highest in both the 20 and 25 °C rearing treatments (102.4 ± 8.8 and 109.7 ± 9.5, respectively), and was positively correlated with parasitoid load. As a result, the percentage of wasps surviving to emergence was high in both the 20 °C ($90.8 \pm 1.2\%$) and 25 °C ($90.5 \pm 1.3\%$) treatments and this was largely independent of load (Fig. S1). In contrast to this, hosts reared at 30 °C had significantly lower numbers of emerging wasps (mean $= 52.4 \pm 4.2$) and a lower percentage of wasps surviving to emergence ($52.3 \pm 3.3\%$). At 30 °C, the percentage of surviving parasitoid larvae declined strongly with increasing load (Fig. S1). As a result, the probability of survival to emergence declined with load at this temperature; no host had > 120 wasp larvae emerge, regardless of total load size (total load ranged from two to 317 across treatments) (Fig. 3a).

Increasing temperature significantly decreased *C. congregata* development time within their host ($F = 14459.94$, d.f. $= 133$, $P < 0.0001$) (Fig. 3b). The development time of wasp larvae to emergence was the longest in the 20 °C rearing treatment, and the difference in development time between temperatures was greatest between 20 °C and the two warmer treatments (25 and 30 °C) (Fig. 3b). The number of parasitoid larvae within a single host (parasitoid load) also had a significant effect on development time, increasing time to emergence at higher load numbers ($F = 47.82$, d.f. $= 133$, $P < 0.0001$) (Fig. 3b). The interaction between load and temperature significantly affected development time ($F = 14.90$, d.f. $= 133$, $P < 0.0001$); the positive correlation between load and development time was strongest at 20 °C, and weak at both 25 and 30 °C (Fig. 3b).

Consequences of parasitoid load on caterpillar consumption and mass

For parasitised caterpillars, rearing temperature significantly affected overall mass accumulation and consumption (Fig. 4; see parametric terms in Table 2), consistent with our results for both parasitised and control caterpillars (Fig. 1; ee pParametric terms in Table 1). Both age and parasitoid load significantly affected mass and consumption of parasitised caterpillars, and the effects varied across rearing temperatures (Fig. 4; see smoothing terms in Table 2). This analysis indicates that the shape of the surface relating mass (or consumption) to age and load differs across rearing temperature (Table 2). Increasing load decreased mass and consumption, primarily at later ages. However, the effects of load on mass and consumption were larger at higher rearing temperatures, and load had modest effects at the lowest temperature treatment (20 °C) (Fig. 4). The effects of load on consumption and mass accumulation were qualitatively similar (Table 2), although load had smaller effects on consumption than on mass (Fig. 4). These results from the two-dimensional smoothing of our GAMM model of age, load and temperature indicate that the effect of load size on host growth is dependent on rearing temperature (Fig. 4; Table 2). We note that parasitisation (relative to unparasitised controls) decreased mean mass accumulation and consumption at later ages (Fig. 1), whereas increasing load increased mass accumulation and consumption at later ages for parasitised caterpillars (Fig. 4).

Discussion

A primary goal of this study was to determine how parasitism by *C. congregata* affects host consumption, growth and development at different rearing temperatures. Overall, we found that temperature, parasitism and parasitoid load interacted to affect the growth of parasitised hosts, as well as the development and survival of the parasitoid wasps. Previous studies have shown that *M. sexta* caterpillars parasitised by *C. congregata* consumed less total food and gained less weight than their unparasitised counterparts (Beckage & Riddiford, 1978; Bentz & Barbosa, 1990). The present study illustrates how these effects of parasitism on consumption and growth accumulate throughout caterpillar development (Fig. 1). For caterpillars parasitised in the third instar, reductions in consumption and growth rates occur during the fourth and especially the fifth instars. Unsurprisingly, warmer temperatures increased rates of consumption and growth for both control and parasitised caterpillars; but the reduction in consumption and growth due to parasitism occurred at all temperatures (Fig. 1). The effects of parasitism on caterpillar development rates are more complex. Increasing rearing temperature decreased caterpillar development times for both control and parasitised caterpillars, but the relative effects of parasitism on development time differed across instar and temperature. Parasitised caterpillars took longer to reach the fifth instar at all temperatures, but the relative subsequent development to either wandering or emergence depended on rearing temperature (Fig. 2). As a result, temperature and parasitism interact to determine development during the fifth and final instar of *M. sexta*.

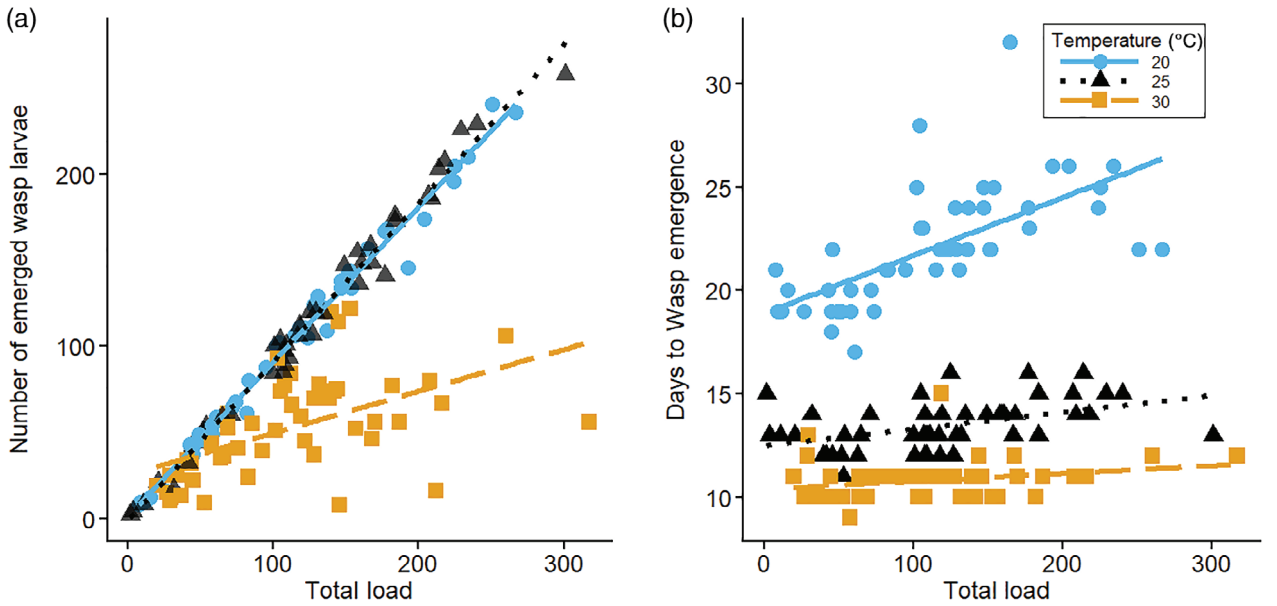

Fig. 3. *Cotesia congregata* survival (a) and development time (b) from oviposition to emergence at three rearing temperatures (20 °C, blue, solid; 25 °C, black, dotted; 30 °C, yellow, dashed). Data points indicate *Manduca sexta* hosts with wasp emergence; parasitised hosts without wasp emergence are not displayed. Number emerged includes both wasp larvae that successfully spun cocoons and those that failed to do so. Total load was determined by dissecting hosts and summing the number of unemerged wasp larvae and the number that successfully emerged. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 2. Results from a general additive mixed-effects model with thin plate splines on the effects of temperature and parasitoid load on parasitised *Manduca sexta* mass and consumption. Age and load were used as smoothing terms with an interaction of temperature. For the model of mass, the maximum number of knots was not specified; for the consumption model, the maximum number of knots was set to 10. Temperature was included as a fixed effect. Individual was included as a smoothing term as a random effect. Parameters were estimated using maximum likelihood.

		Mass			Consumption		
		d.f.	*F*	*P*-value	d.f.	*F*	*P*-value
Parametric terms	Temperature	2	95.31	**< 0.0001**	2	34.93	**< 0.0001**
Smoothing terms	(Age, load): 20	25.47	4985.08	**< 0.0001**	8.93	948.62	**< 0.0001**
	(Age, load): 25	21.17	2222.50	**< 0.0001**	8.65	704.40	**< 0.0001**
	(Age, load): 30	17.99	1990.48	**< 0.0001**	8.36	684.48	**< 0.0001**
	Individual	129.61	33.86	**< 0.0001**	47.64	0.56	**< 0.0001**

Rearing temperature also strongly affected wasp performance. Increasing temperature significantly decreased the mean development time to wasp emergence, with the fastest development rates at 30 °C. Conversely, the mean percentage surviving to emergence was significantly lower at 30 °C than at 20 or 25 °C (Fig. 3). *Mandcua sexta* caterpillars maintain high survival and development rates at constant temperatures of 30 °C, although their survival rates decline at constant temperatures > 32 °C (Petersen *et al.*, 2000; Kingsolver & Nagle, 2007; Kingsolver *et al.*, 2015). Our results are thus consistent with previous reports that endoparasitoids frequently have lower optimal and maximal temperatures than their hosts (Furlong & Zalucki, 2017). Similarly, a recent study with *C. congregata* found that adult wasps had significantly lower critical thermal maximum temperatures (CT_{max}) than did *M. sexta* caterpillars (Agosta *et al.*, 2018).

Our study demonstrates how the effects of rearing temperature on this host–parasitoid interaction are mediated by parasitoid load. Parasitoid load has been found to have strong effects in several host–parasitoid systems, affecting host growth and development as well as parasitoid development time, survival and adult mass (Beckage & Riddiford, 1978; Beckage & Riddiford, 1983; Alleyne & Beckage, 1997; Elzinga *et al.*, 2003; Gu *et al.*, 2003; Smallegange *et al.*, 2008). For gregarious endoparasitoids such as *C. congregata*, many wasp larvae develop within a single host, and rely on this limited resource pool to meet their nutritional requirements during their larval stage. High load numbers can lead to competition for resources within the host, especially in stressful conditions (Godfray, 1994). Our results reveal a potential trade-off between parasitoid development time and survival at different temperatures that could affect selection on life-history strategies or behavioural

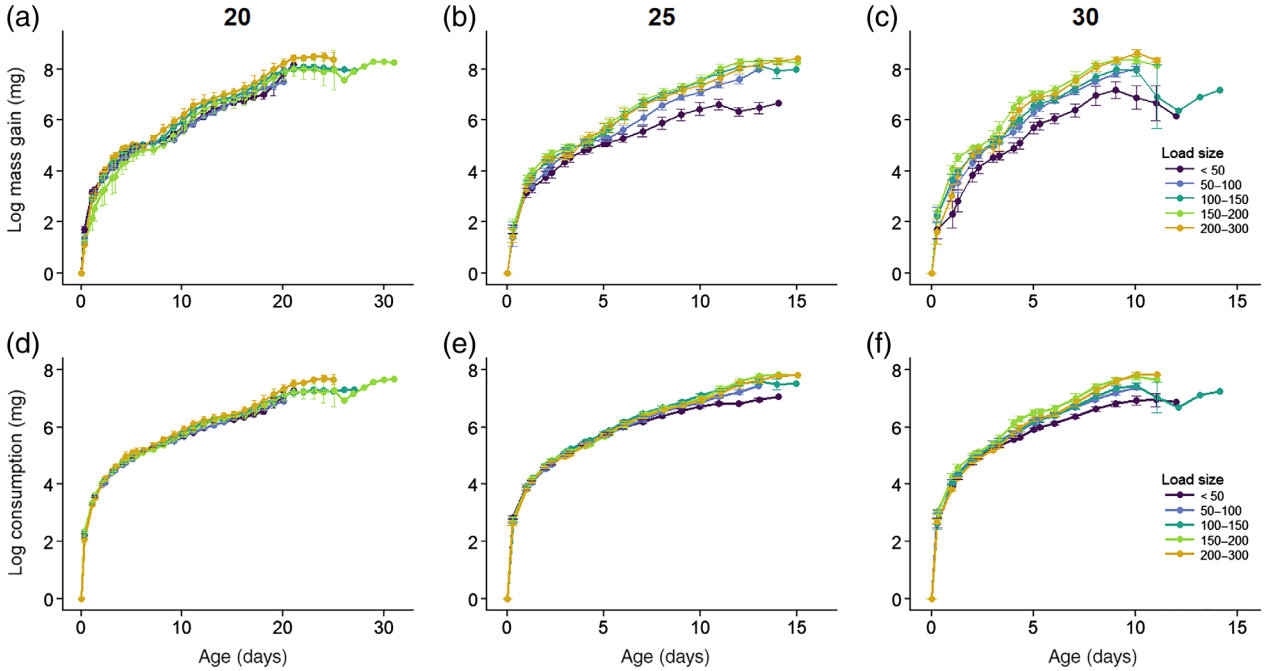

Fig. 4. The effects of parasitoid load on host mass gain (a–c) and consumption (d–f). Parasitoid load number was binned into five size ranges, and mass gain and consumption for each temperature (20, 25 and 30 °C) were averaged for each experimental time point. Load size is indicated by color. Mass gain and consumption were measured in mg, and are displayed here on a logarithmic scale. Age (in days) is plotted on the *x*-axis, and the axes were scaled individually for each temperature for visualisation purposes. Error bars are SEs. [Colour figure can be viewed at wileyonlinelibrary.com].

decisions made by the ovipositing female. One novel finding from our study is that the effects of parasitoid load on wasp development time and survival depend on rearing temperature. Large loads have a stronger effect on development time at low temperatures, and on survival at high temperatures. We see that the combination of high rearing temperature and high parasitoid load, rather than either factor individually, strongly reduces parasitoid success in this system.

Parasitoid load also interacted with temperature to affect consumption and mass in parasitised *M. sexta*. At warmer temperatures (25 and 30 °C), hosts with higher parasitoid loads had a higher rate of consumption and mass accumulation, and grew larger than those with lower parasitoid loads (Fig. 4). This corresponds with previous findings that the mass of parasitised *M. sexta* is positively correlated with parasitoid load, even when the weight of the unemerged wasp larvae is subtracted (Beckage & Riddiford, 1983). This trend was not apparent at the coolest temperature (20 °C). Wasps that developed in hosts reared at 20 °C with relatively large loads took longer to emerge than wasps from hosts with similarly sized loads at 25 and 30 °C. However, at 20 °C, hosts with large loads were not proportionately larger than hosts with small loads. This suggests that, whatever mechanism is responsible for altering host consumption and mass accumulation, be it parasitic manipulation or passive disease response, the effect is dampened or negated at low developmental temperatures.

Our findings demonstrate that temperature plays an important role in mediating the interaction between *C. congregata* and its host. In this system, reduced heat tolerance, resulting in low survival to emergence, arises from the joint effects of high temperature and parasitoid load. Dissections of parasitised caterpillars reared at 30°C following wasp emergence (see the 'Wasp emergence, load and survival' section) revealed that unemerged wasp larvae varied in size and developmental stage (M. E. Moore, unpublished). This indicates that high temperatures are increasing the variance in developmental synchrony. In *C. congregata*, the majority of wasp larvae usually emerge from the host within a few hours of each other (Beckage & Riddiford, 1978). Previous studies have shown that an ecdysone peak is elicited in the host prior to and concurrent with wasp emergence, and that this peak is necessary for wasp success (Beckage & Riddiford, 1982; Gelman *et al.*, 1998). If wasp larvae cannot continue to emerge after the initial onset of ecdysone, then anything that disrupts developmental synchrony must lead to higher mortality. Multiple different mechanisms could cause such developmental asynchrony (disruption of nutrient uptake, differential heat-tolerant phenotypes, disruption of endocrine functions, direct heat-induced mortality etc.), but with our data we were unable to determine which might be elicited by the dynamics of high temperature and large load numbers. As our hosts were frozen after emergence, we were unable to determine if the unemerged wasp larvae were alive and developmentally delayed, or had perished before becoming mature second instars.

Investigating how temperature affects the interaction between *M. sexta* and *C. congregata* is an important first step in understanding how host–parasitoid systems may respond to a changing climate. Before predictions about this or other host–parasitoid systems can be made outside of the laboratory,

temperature needs to be considered in a more realistic manner. Our experiment was conducted at constant temperatures; however, several recent studies have shown that experiments conducted at diurnally fluctuating temperatures may provide more meaningful predictions (Bozinovic *et al.*, 2011; Folguera *et al.*, 2011; Kingsolver *et al.*, 2015; Delava *et al.*, 2016). Although a few studies have investigated the effects of fluctuating rearing temperatures on the performance of *M. sexta* (Stamp, 1994; Kingsolver *et al.*, 2009, 2016), little is known about the effects of fluctuating temperature regimes on host–parasitoid interactions involving *C. congregata*. Ideally, parasitised *M. sexta*, as well as adult *C. congregata*, should be studied under a range of average temperatures and degrees of fluctuations in order to gain a better understanding of the thermal tolerance of this model host–parasitoid system.

The effects of temperature on *M. sexta* and *C. congregata*, as well as other host–parasitoid or prey–predator systems will have important ramifications for both natural and agricultural systems (Hoover & Newman, 2004; Deutsch *et al.*, 2008; de Sassi & Tylianakis, 2012; Schmitz & Barton, 2014; Harvey, 2015; Flores Mejia *et al.*, Flores-Mejia *et al.*, 2016; Stoks *et al.*, 2017). As global temperatures continue to rise, it will become increasingly imperative to understand how warming temperatures will impact the interactions between ectothermic organisms and across trophic levels (Hoover & Newman, 2004; Villalpando *et al.*, 2009; de Sassi & Tylianakis, 2012; Lemoine *et al.*, 2014; Schmitz & Barton, 2014; Harvey, 2015; Stoks *et al.*, 2017). If parasitoids have a lower thermal tolerance than their hosts, as is suggested by our data for *C. congregata* and *M. sexta*, then climate change could drastically alter the historic dynamics in host–parasitoid systems. Warming temperatures will directly affect the development and survival of parasitoids, and could select for temperature dependent trade-offs among load size, rate of reproduction and survival. Differential effects of temperature on host and parasitoid development and survival could indirectly create phenological mismatch between the parasitoid and its host, which in extreme cases could drive the extinction of the parasitoid population (Van Nouhuys & Lei, 2004; Hance *et al.*, 2007; Evans *et al.*, 2013). Reductions in parasitism in response to climate change are expected to decrease top-down control of insect herbivores, resulting in cascading effects on ecosystem processes (Stireman *et al.*, 2005; de Sassi & Tylianakis, 2012; Flores-Mejia *et al.*, 2016).

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Author contributions

MEM created the experimental design, and conducted the experiment and data analysis. KMK provided experimental animals (*Cotesia congregata*) and rearing expertise, as well as feedback on the experimental design. JGK advised on the experimental design and data analysis. All authors contributed to the manuscript.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Percentage of emerged wasp larvae by total parasitoid load for each rearing temperature (20°C, blue, solid; 25°C, black, dotted; 30°C, yellow, dashed). Data points indicate parasitised *M. sexta* with wasp emergence; parasitised hosts without wasp emergence were not included. Total load was determined by dissecting parasitised hosts after wasp emergence. Percentage of emerged larvae was calculated by the number of emerged larvae divided by the total load.

Fig. S2. GAMM model residuals for mass (a) and consumption (b) by temperature and parasitisation treatment. Residuals for unparasitised controls (black) and parasitised hosts (yellow) are plotted against hour age.

Fig. S3. GAMM model residuals for mass (a) and consumption (b) by temperature and parasitoid load for parasitised *M. sexta*. Residuals are coloured by total load and plotted against age (in h) .

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