

Physiological Ecology

Effects of Temperature on *Anoplophora chinensis* (Coleoptera: Cerambycidae) Larvae and Pupae

Melody A. Keena^{1,*}, and Jessica Y. Richards

Northern Research Station, Northeastern Center for Forest Health Research, USDA Forest Service, 51 Mill Pond Road, Hamden, CT 06514, United States, and ¹Corresponding author, e-mail: melody.keena@usda.gov

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Abstract

Anoplophora chinensis (Forster) is a xylophagous invasive cerambycid whose larvae feed on the lower bole and exposed roots of many tree species in orchard, urban, and forested habitats. Larval survival and development of *A. chinensis* from Italy and China were evaluated at eight constant temperatures (5, 10, 15, 20, 25, 30, 35, and 40°C). Development was slow or did not occur at temperatures ≤ 10 and $>35^\circ\text{C}$. The T_{Min} for the first six instars and the pupa was $<10^\circ\text{C}$, while T_{Min} for the higher instars was closer to 12°C . The ultimate instar for both populations was insensitive to temperature. When the T_{Max} thresholds could be estimated they were between 31 and 41°C . Temperature also influenced larval weight gain; larvae held at $25\text{--}30^\circ\text{C}$ generally weighed the most from the fourth instar on. The number of degree days for 50% of the population to molt to the next instar increased with increasing instar for both populations up through the sixth instar. *Anoplophora chinensis* may use wood moisture content in conjunction with temperature as a cue as to when to pupate. These responses of *A. chinensis* to temperature can be used for developing phenological models to predict timing of stages for management or eradication efforts.

Key words: *Anoplophora chinensis*, temperature, survival, development, phenology

As ectotherms, insects' timing of molting to different life stages and their survival is dependent on the environmental temperatures to which they are exposed. However, individuals within and between populations of the same species may respond differently to temperatures. To estimate potential geographic ranges and develop phenology models for invasive insects we must first understand how variable these responses to temperature are, both within and between populations.

A phenology model has already been developed for *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) that makes it possible to predict the timing of life stages that are critical to deploying survey and management options (Trotter and Keena 2016). *Anoplophora chinensis* (Forster) (Coleoptera: Cerambycidae) is a congeneric of *A. glabripennis* that is sympatric over part of its native range and for which no phenology model has been developed. Both have become invasive outside their native range after being transported through international trade, *A. chinensis* primarily in live plants and *A. glabripennis* in solid wood packaging materials

(Haack et al. 2010). The *A. glabripennis* phenology model could be adapted for use with *A. chinensis* but modifications are likely needed for several reasons.

First, although the native ranges of both species include most of China and Korea, *A. glabripennis*'s native range extends further north and *A. chinensis*'s range extends further south beyond where they are sympatric (Huang et al. 2003). The native range of *A. chinensis* is also more extensive, including most of Japan, and it is occasionally present in Vietnam, Malaysia, Indonesia, and the Philippines (Lingafelter and Hoebeke 2002, Haack et al. 2010). Second, females of both species lay their eggs in sites they chew in the bark of trees but *A. glabripennis* females oviposit primarily in the lower crown and trunk while *A. chinensis* females oviposit primarily in the lower bole and exposed roots (Haack et al. 2010). Temperatures, both under the bark where early instars tunnel and in the xylem where later instars feed, likely differ greatly throughout the year between these two locations in the tree. During the winter, tree branches receive solar radiation resulting in strong temperature gradients, while

in the summer the foliage helps maintain more consistent temperatures. In contrast, the lower tree bole and roots are generally more insulated which may reduce temperature fluctuations in the summer and prevent freezing in the winter.

Third, *A. chinensis* is composed of what are likely two subspecies: the original species *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) which is found in Japan and *A. chinensis* which is found in China and other areas which were recently synonymized (Lingafelter and Hoebeke 2002). These two original species can be differentiated based on the presence or absence of two white spots on the pronotum and by their mitochondrial COI haplotypes (Ohbayashi et al. 2009, Iwazumi et al. 2014). The invasive populations that have established in Europe originated from areas where both putative subspecies exist; e.g., the Italian populations in the Lazio and Lombardy regions have haplotypes similar to Japanese populations while those from Tuscany have haplotypes closer to Chinese populations (Strangi et al. 2017). These different subspecies may very well have different larval and pupal responses to temperature since this has already been demonstrated in adults and eggs (Keena et al. 2021). Since countries are attempting to eradicate all invasive infestations of *A. chinensis*, regardless of origin, it is critical to determine if there are differences between these original species. For simplicity, *A. malasiaca* will be referred to as *A. chinensis* from Japan.

Pupal and larval developmental rates, time in instar, and survival for each instar of *A. chinensis* from Japan on citrus twigs have previously been evaluated at three constant temperatures (20, 25, and 30°C) and a diel fluctuating-temperature regime (Adachi 1994). Under the fluctuating-temperature regime 70% of the larvae pupated, but some larvae required two years and they all spent a several-month period without feeding prior to pupation (Adachi 1994). Only 57% of the larvae held at 20°C and none held at the 25 or 30°C constant temperature completed development (Adachi 1994). The durations and survival of the larval and pupal stages of *A. chinensis* from China have also been assessed on artificial diet containing *Casuarina equisetifolia* L. (Fagales: Casuarinaceae) sawdust under two constant temperatures (25 and 28°C) and a fluctuating-temperature regime (Cai et al. 2016). Under the fluctuating-temperature regime 90% of the larvae pupated in an average of 37 weeks, while 84% pupated in 42 weeks at 25°C, and only 50% pupated in 51 weeks at 28°C (Cai et al. 2016). These two studies suggest that these subspecies may differ in their larval responses to temperature and their ability to pupate at constant temperatures $\geq 25^{\circ}\text{C}$. There have been no side-by-side comparisons of the two subspecies under the same rearing conditions.

In this study, we compared the temperature responses of *A. chinensis* larvae and pupae from both subspecies at a range of constant temperatures on the same artificial diet. We determined time in instar and developmental rates, estimated upper, and lower temperature thresholds for development based on the fitted mathematical relationships between temperature and developmental rate, and assessed survival for each instar. The differences between the *A. chinensis* subspecies were determined and compared with those already available for these subspecies. These data will facilitate the modification of the *A. glabripennis* phenology model for use with *A. chinensis*. We also explore how the *A. chinensis* results differ from those for *A. glabripennis* and the potential impacts for management and eradication programs.

Materials and Methods

Populations and Temperatures

The sources of the two *A. chinensis* populations used in this study were Lombardy, Italy (45.47° N and 9.83° E) and Yancun,

Guangdong Province, China (23.43° N and 113.55° E) (Keena et al. 2021, same populations but coordinates corrected here for the Chinese population). The two populations were imported as larvae in diet in 2018 under permit from USDA Agricultural Research Service (ARS), European Biological Control Laboratory in Montferrier-sur-Lez, France to the USDA Forest Service quarantine facility in Ansonia, CT. The Italian individuals used were from the 16th, 17th, or 18th laboratory generations and the Chinese individuals were from the 6th or 7th laboratory generations. Molecular analysis of the invasive Italian population indicated its origin was Japan and that it is part of what was called *A. malasiaca* prior to the revision of this genus (Lingafelter and Hoebeke 2002, Strangi et al. 2013). Specimens from both populations were deposited at the Yale Peabody Museum of Natural History, New Haven, CT.

Both populations were subjected to eight constant temperatures between 5 and 40°C in five-degree increments, all with a photoperiod of 16:8 (L:D) h. Temperature fluctuations within the environmental chambers that were used typically remained within 1°C of the set value. Humidity was passively maintained by placing open buckets (with 896 cm² of surface area) of water in the bottom of each chamber. Three chambers (15, 20, and 25°C), which had full humidity controls, did not require the open humidity source. Humidity in the 5–25°C chambers averaged $\geq 60\%$ RH and chambers in the 30–40°C range averaged 45–68% RH (for exact values see Keena et al. 2021). Actual humidity in the diet-filled petri dishes was likely much higher than in the chamber because the diet was moist and the petri dishes were held in lidded boxes. There were not enough larvae to set up all eight temperature treatments at one time, so the studies were set up in three phases. In the first phase, larvae from both populations were set up at 5, 10, 15, 30, 35, and 40°C. In the second phase, Chinese larvae were set up at 20 and 25°C and Italian larvae were set up at all temperatures. In the third phase, Italian larvae were set up at 20, 25, and 30°C. Additionally, during the second phase some Chinese larvae were reared at 25°C to the third or fifth instar and then moved to 5 or 40°C to get an estimate of developmental time for these instars at temperatures near the lower or upper developmental thresholds. This method of moving larvae to assess development was previously used in studies on *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Liu et al. 2002) and *A. glabripennis* (Keena and Moore 2010).

Obtaining Newly Hatched Larvae

The adults that laid the eggs used in these studies were part of a previously published study (Keena et al. 2021). Adults were held as single mating pairs in 3.8-liter glass jars with *Acer rubrum* L. (Sapindales: Sapindaceae, red maple) twigs with leaves removed as a food source and provided an *A. rubrum* bolt (4–8 cm diameter and 20 cm long) with both ends waxed as an oviposition substrate. Twigs and bolts were cut fresh every 2 wk and were stored at 4°C and $\geq 80\%$ RH until use. Twigs were held in plastic bags.

Both twigs and bolts were changed weekly until a female died. After removal from the mating jars, the bolts were labeled with the female's individual number and the date of removal. Eggs were obtained as previously described (Keena et al. 2021). Eggs removed from under the bark were placed individually in the wells of a 24-well tissue culture plate and held in a water box at the temperature at which the egg was laid (15–30°C). Eggs were checked daily, and larvae were inserted into diet within 24 h of hatch.

Larval Set up and Monitoring

The artificial diet used in these studies was the *A. glabripennis* modification AG2 (Keena 2005). For the six temperature treatments

during the first phase (5, 10, 15, 30, 35, or 40°C), 1–4 larvae from each of 14 Italian (15–40 total larvae per temperature, 183 total larvae across all temperatures), and 23 Chinese female parents (50–51 total larvae per temperature, 301 total larvae across all temperatures) were used. For the 20 and 25°C temperature treatments in the second phase, 1–4 larvae from each of 9 Italian (20 total larvae per temperature), and 1–3 larvae from each of 20 Chinese female adults (50 total larvae per temperature) were used. In addition, Italian larvae were put into the 15°C (10 female adults, 1–4 larvae each, 19 total larvae) and 30°C (5 female adults, 1–4 larvae each, 13 total larvae) treatments. For 5, 10, 35, and 40°C, 3–4 larvae from a single Italian female adult were used in each treatment (14 total larvae). In total, 10 Italian female adults were used to set up 86 larvae across all temperatures for the second phase. In the third phase, 1–4 larvae from each of 5 Italian female adults (4–13 total larvae per temperature, 29 total larvae across all temperatures) were used. Across all three phases, a total of 298 larvae from 29 Italian female adults and 401 larvae from 40 Chinese female adults were used. Larvae were set up over 60, 138, and 40 d in the first, second, and third phases, respectively. During the second phase, larvae from 18 of the same Chinese female adults as used for the 20 and 25°C treatments were used to set up an additional four sets of 1–2 larvae from each female (30 total larvae per set, 120 larvae overall) at 25°C. Each set was reared to either the third or fifth instar, and then moved to either 5 or 40°C. If a larva died before reaching the appropriate instar, it was replaced with another larva from the same female adult. These larvae were set up over a period of 74 d. The numbers of larvae used from each female and the number of females per population for all parts of the study were based on availability.

Newly hatched larvae from each mating pair were individually weighed and assigned on a rotating basis to a temperature treatment to keep equal numbers per treatment. Each first instar larva was set up individually in a 35 × 10 mm petri dish (Corning Inc., Corning, NY, Falcon product #351008), filled with diet. A 1-cm² chunk of diet was cut from the center of the dish and a hollow was cut out that the larva was placed into, then the chunk was replaced with the larva next to the bottom of the dish. Petri dishes were kept in a 45.7 × 27.9 × 15.2-cm opaque plastic box (US Plastic Corp., Lima, OH, product #52730) with an overlapping lid (US Plastic Corp., product #52762) and held at the assigned temperature. These boxes maintained the larvae in the dark except when the lid was opened to mimic the conditions inside the tree. The diet was changed after each larval molt or when the diet was unusable due to tunneling, microbial growth, or desiccation. The diet was changed at a minimum every 7, 10, 14, 20, 30, and 30 d at 40, 35, 25, 20, 15, and 10°C, respectively. For larvae held at 5°C, the diet was changed when the larvae were weighed at 200 d. Dishes of diet were stored at 5°C until they were needed and were brought to room temperature before use. All petri dishes used were vented to prevent condensation and allow air exchange. Second instar larvae were also held in 35 × 10 mm petri dishes, third instars in 60 × 15 mm dishes (Corning Inc., Falcon product #351007), and fourth instars and up in 100 × 15 mm dishes (Corning Inc., Falcon product #351029). Once larvae reached a weight ≥0.7g, they were moved to 150 × 15 mm dishes (Corning Inc., Falcon product #351058), and larvae ≥3.5g were moved to 150 × 25 mm dishes (Corning Inc., Falcon product #353025). Second-through fourth-instar larvae were inserted in the diet the same way as the first instars, using larger chunks of diet with larger hollows created in the center; later instars were placed in canoe-shaped openings cut in the middle of the diet.

All larvae held at ≥15°C were observed daily to check for molts, death, or need for a diet change. Larvae at 5°C were checked every

10 d, while larvae at 10°C were checked weekly until first molt, then second instars were checked twice per week until molting. After 30 d at third instar, larvae were checked three times per week. The time that the larvae were at room temperature was kept to a maximum of 15 min to prevent the diet temperature from changing significantly and affecting the developmental rate. Larvae were weighed after each molt, and the shed head capsule from each larva was saved in a separate labeled 1.5-ml microcentrifuge tube. Occasionally a head capsule was not found but the molt was confirmed because the new larval head capsule was not yet fully sclerotized and sufficient time had elapsed since the last molt. Larvae that appeared to be dead were observed (date recorded) for two additional days to ensure they were not just close to a molt and inactive. Any larvae that were killed by human error were censored from the study and replaced, if possible, with a new larva from the same female adult.

First instars held at 5 or 10°C that had not molted after 200 d were weighed and moved to 20°C. Larvae were checked daily until first molt, then held in a 4- or 8-ounce cup of diet (depending on larval size) until pupation or death in order to record sex or mortality. Second or higher instar larvae held at 10°C that did not molt for 150 d were moved to 20°C and held until pupation or death. At 15°C, half of all larvae that reached 300 d without pupating and were ≥0.75 g were moved to 25°C. The rest remained at 15°C for an additional 100 d, then were moved to 25°C. Larvae that were moved from 15 to 25°C that did not pupate within 60 d were chilled at 10°C for 84 d, then returned to 25°C. After chill, if a larva did not pupate within 150 d it received a second chill.

For the 20–40°C treatments, half of the larvae were chilled at 10°C for 84 d once they reached the seventh instar. If a larva was chilled and did not pupate within 150 d, it received a second chill. Any larvae that spent 80 d in an instar without molting were chilled. Larvae that reached 400 d without being chilled or pupating were also chilled. After chill, larvae were returned to their original temperature treatment.

These protocols were put in place because larvae apparently require a chill period before they will progress to pupation, and larvae held at low temperatures may never molt or pupate without a period at a higher temperature. For larvae that did not pupate, observations were ended either 150 d after a second chill or 730 d (two years) from setup, whichever came first.

Any larva that shrank in length, became flaccid, and had a noticeable lighter color just behind the head was considered to be a prepupa and moved to a 50-ml centrifuge tube with a 2-cm-wide piece of damp paper towel. The tubes were held at temperature in the same type of box as the larvae but with a piece of plastic egg crating in the bottom over water to increase the humidity level. Pupae were weighed within the first 24 h, then returned to the 50-ml tube. Once the adult emerged, the tube was moved to a dry box and held for 4 d. The adult was then sexed, weighed, and given twigs to feed on. Adults were used for other studies as needed.

Statistical Analyses

The number of days spent in each instar or stage was calculated and the time spent in chill was subtracted from any instar in which it occurred because it was below the T_{Min} for all instars in which pupation occurred, with the exception of the sixth instar. The days spent in the ultimate instar were assessed separately, regardless of the actual instar in which pupation occurred. A Keyence Corp. of America (Itasca, IL) VHX-200E digital microscope was used to photograph the head capsules and then the internal software was used to measure the width (mm) of each head capsule. The following dependent variables

were analyzed in PROC GLIMMIX (SAS Institute, 2015): days in each instar and stage, larval weights at the beginning of each instar, pupal weight within 24 h of discovery, adult weight 4 d post eclosion, and head capsule widths. The data were checked for normality using the Shapiro–Wilk test and when not normally distributed PROC UNIVARIATE was used to assess the fit of the data to a gamma distribution which can handle data with long right tails. The time in instar/stage was normally distributed, except for the seventh through twelfth instars for which a gamma distribution with a log link was used. The hatch weights were normally distributed, but the remainder of the weight data was not so and thus was fit to a gamma distribution, as was all the head capsule width data. An analysis of the residuals using Levene's test indicated that the days in instar/stage and weight data had unequal variances between temperatures, so the model was modified to correct for this by adding group = temperature to the random statement. Temperature, population (China or Italy), and the interaction between the two were used as the fixed effects in the model for time in instar, larval weights, and head capsule widths. The models for time as a prepupa and a pupa, and total days to adult included the same fixed effects plus the additional fixed effects of sex and the following interaction effects: temperature*population*sex, population*sex, and temperature*sex. The models for pupal and adult weights included the same base fixed effects plus sex and temperature*population*sex. The Tukey–Kramer test with $\alpha = 0.05$ was used to assess differences among means (SAS Institute, 2015).

The relationship between temperature (t) in °C and developmental rate ($y = 1/\text{time (days)}$) spent in each instar or stage) was fit to one or more of the following equations. Before choosing these specific models, multiple other models were evaluated

(including those listed in Rebaudo et al. 2018) and the models with the best fit based on adjusted r^2 values were ultimately used. The nonlinear relationships were estimated using the Marquardt convergence method (PROC NLIN (SAS Institute, 2015)).

$$y = bt + a \quad (1)$$

A linear model where the lower threshold for development (T_{Min}) was calculated as a/b (intercept divided by slope).

$$y = \begin{cases} (t - T_{\text{max}})(T_{\text{min}} - t)^{1/b} & \text{for } T_{\text{max}} \leq t \leq T_{\text{min}} \\ \text{otherwise} & 0 \end{cases} \quad (2)$$

A Briere equation where a and b are empirical constants, and T_{max} and T_{min} are the upper and lower temperature thresholds, respectively (Briere et al. 1999). The optimum was calculated by equating the first derivative to zero and solving for t .

$$y = dt^3ct^2 + bt + a \quad (3)$$

A third-degree polynomial where a , b , and c are empirical constants. The T_{min} was calculated by equating y to zero and choosing the solution for t that fell within the 0–30°C range.

The cumulative number of individuals (P) (across all temperatures) completing each instar and stage over the accumulated degrees days (DD) above the appropriate T_{Min} was described using a Gompertz function:

$$P = \exp[-\exp(-bDD + a)], \quad (4)$$

The a and b in the equation are the lag and the rate of increase, respectively (Brown and Mayer 1988). The nonlinear relationships were estimated using the Marquardt convergence method (PROC NLIN (SAS Institute 2015)).

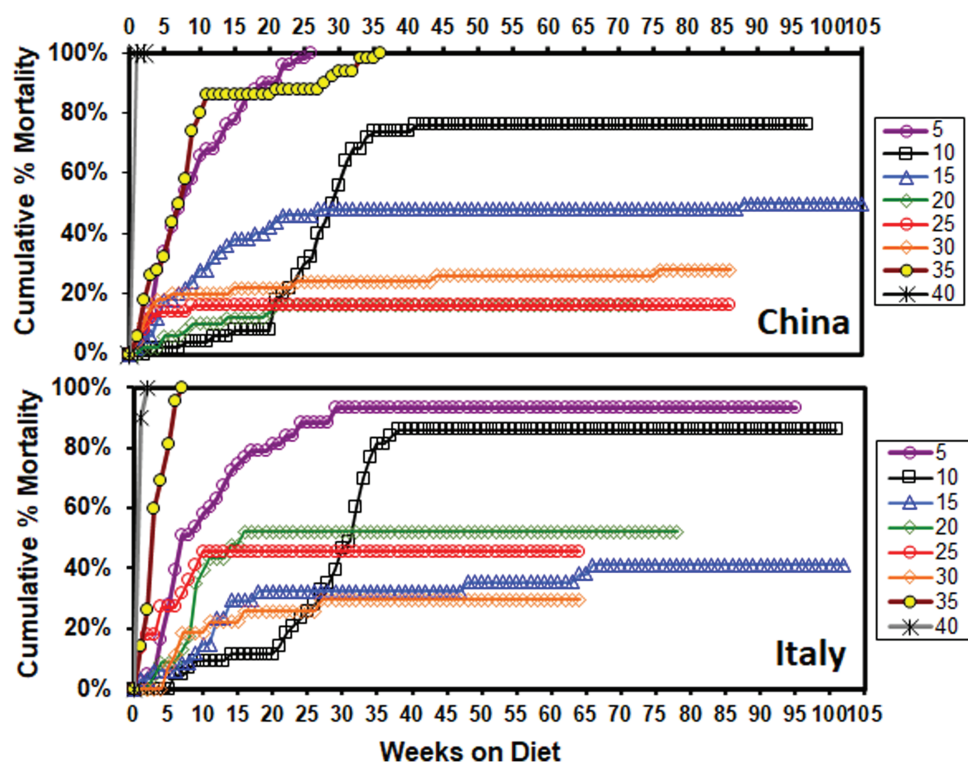


Fig. 1. Weekly cumulative mortality curves for *A. chinensis* larvae held at eight constant temperatures from two populations. The line for each temperature ends at the week that all larvae were dead. Larvae at temperatures $\leq 15^\circ\text{C}$ were moved as indicated in the methods to higher temperatures to complete their development.

Table 1. Developmental time (d) of immature stages of *Anoplophora chinensis* at six constant temperatures (mean $\pm$ SE (n))

Source	Instar/stage	Temperature (°C)						F-statistics
		10	15	20	25	30	35	
Italy	1st	129 $\pm$ 3.4a (5)	16.8 $\pm$ 1.5c (26)	9.6 $\pm$ 2cd (24)	5.5 $\pm$ 2.1d (22)	5.6 $\pm$ 1.5d (27)	4.7 $\pm$ 1.5d (25)	Pop.*Temp.: $F = 88.18$; $df = 5, 295$; $P < 0.0001$
China		46.7 $\pm$ 1.7b (20)	14.4 $\pm$ 1.3c (31)	7.9 $\pm$ 1.2d (46)	5.5 $\pm$ 1.3d (43)	4.5 $\pm$ 1.1d (44)	4.3 $\pm$ 1.2d (40)	
Italy	2nd	63.4 $\pm$ 6.8a (4)	19.1 $\pm$ 1.2bc (25)	12.9 $\pm$ 1.5cd (23)	7.5 $\pm$ 1.5de (22)	5.8 $\pm$ 1.1e (27)	5.2 $\pm$ 1.1e (23)	Temp: $F = 69.67$; $df = 5, 10$; $P < 0.0001$
China		65.4 $\pm$ 6.3a (10)	20.7 $\pm$ 1b (30)	11.9 $\pm$ 0.9d (45)	6.9 $\pm$ 0.9e (42)	5.2 $\pm$ 0.8e (44)	4.8 $\pm$ 0.9e (38)	
Italy	3rd	115 $\pm$ 7a (4)	42.9 $\pm$ 2b (23)	14.5 $\pm$ 1.8cde (22)	9.5 $\pm$ 1.8de (22)	7.2 $\pm$ 1.3e (27)	9.5 $\pm$ 2.6de (6)	Pop.*Temp.: $F = 49.01$; $df = 5, 252$; $P < 0.0001$
China		53.4 $\pm$ 6.3b (10)	20.9 $\pm$ 1.7c (30)	13.8 $\pm$ 1.1d (44)	8 $\pm$ 1.1e (42)	6.3 $\pm$ 1e (42)	6.9 $\pm$ 1.1e (37)	
Italy	4th	81 $\pm$ 10.1a (1)	40.2 $\pm$ 5.9b (23)	18.5 $\pm$ 2.8cde (21)	13.2 $\pm$ 2.8de (22)	10.5 $\pm$ 2de (27)	NA	Temp: $F = 44.50$; $df = 5, 10$; $P < 0.0001$
China		73.5 $\pm$ 3.2a (10)	33.9 $\pm$ 5.7bc (28)	18.7 $\pm$ 1.7cd (44)	11 $\pm$ 1.7e (42)	9.6 $\pm$ 1.6e (40)	9.2 $\pm$ 1.9e (29)	
Italy	5th	NA	38 $\pm$ 2.8c (23)	23.1 $\pm$ 3.5de (21)	19.5 $\pm$ 3.5de (22)	18.2 $\pm$ 2.5e (26)	NA	Pop.*Temp.: $F = 3.31$; $df = 3, 220$; $P = 0.0210$
China		95.3 $\pm$ 4a (10)	50.3 $\pm$ 2.5b (26)	28.9 $\pm$ 2.1cd (44)	18.7 $\pm$ 2.1e (42)	16.2 $\pm$ 2e (39)	12 $\pm$ 2.8e (21)	
Italy	6th	NA	42.3 $\pm$ 2.6c (23)	31.8 $\pm$ 3.9cd (12)	23.6 $\pm$ 3.1de (22)	20.6 $\pm$ 2.4de (24)	NA	Temp: $F = 51.91$; $df = 5, 9$; $P < 0.0001$
China		95.5 $\pm$ 8a (2)	55.7 $\pm$ 2.3b (26)	39.2 $\pm$ 2.1e (43)	26.7 $\pm$ 1.9d (42)	24.5 $\pm$ 1.8d (39)	12.1 $\pm$ 3e (14)	
Italy	7th	NA	64.8 $\pm$ 12.3a (21)	32.8 $\pm$ 6.6ab (7)	28 $\pm$ 3.4b (13)	23.9 $\pm$ 4.4b (11)	NA	Temp: $F = 39.94$; $df = 4, 8$; $P < 0.0001$
China		NA	58.6 $\pm$ 10.5a (22)	42.2 $\pm$ 7.3ab (15)	30.4 $\pm$ 2.4b (19)	23.1 $\pm$ 3.9b (27)	6.6 $\pm$ 0.8c (7)	
Italy	8th	NA	58 $\pm$ 4.7a (12)	35.8 $\pm$ 4.1bcd (7)	33.5 $\pm$ 4.7bcd (7)	23.2 $\pm$ 4.4d (3)	NA	Temp: $F = 41.55$; $df = 4, 7$; $P < 0.0001$
China		NA	56 $\pm$ 4.8ab (11)	51.6 $\pm$ 6.5abc (6)	42.3 $\pm$ 3.3abcd (16)	33 $\pm$ 3.5cd (24)	11.3 $\pm$ 1.3e (6)	
Italy	9th	NA	65.7 $\pm$ 9.9a (3)	44.2 $\pm$ 5.1ab (5)	42.9 $\pm$ 11.8ab (2)	21.1 $\pm$ 5.8bc (1)	NA	Temp: $F = 31.32$; $df = 4, 7$; $P = 0.0001$
China		NA	65 $\pm$ 8.5a (4)	60.8 $\pm$ 7.9a (4)	52.7 $\pm$ 6ab (9)	34.1 $\pm$ 3.2b (18)	15 $\pm$ 1.6c (6)	
Italy	10th	NA	NA	39.5 $\pm$ 7.5a (2)	NA	NA	NA	Temp: $F = 19.89$; $df = 4, 4$; $P = 0.0067$
China		NA	67 $\pm$ 18.1a (1)	54.5 $\pm$ 10.4a (2)	49 $\pm$ 7.6a (4)	38.9 $\pm$ 4a (12)	14.3 $\pm$ 1.6b (6)	
Italy	11th	NA	NA	32 $\pm$ 6.7a (2)	NA	NA	NA	Temp: $F = 24.55$; $df = 2, 1$; $P = 0.1413$
China		NA	NA	54 $\pm$ 11.1a (2)	39.4 $\pm$ 4.1a (8)	14 $\pm$ 1.8b (5)	NA	
Italy	12th	NA	NA	25 $\pm$ 9.8ab (1)	NA	NA	NA	Temp: $F = 8.3$; $df = 2, 1$; $P = 0.2384$
China		NA	NA	57 $\pm$ 15.9ab (2)	51.8 $\pm$ 8.3a (6)	15 $\pm$ 4.2b (2)	NA	
Italy	13th	NA	NA	27 $\pm$ 6.1a (1)	NA	NA	NA	Temp: $F = 8.73$; $df = 1, 2$; $P = 0.0980$
China		NA	NA	NA	NA	58 $\pm$ 7.5a (3)	NA	
Italy	Ult.	NA	NA	103.7 $\pm$ 15.9a (19)	110.6 $\pm$ 16.8a (19)	135.5 $\pm$ 37.8a (20)	NA	Temp: $F = 0.86$; $df = 2, 4$; $P = 0.4892$
China		NA	NA	74.3 $\pm$ 8.6a (40)	80.2 $\pm$ 9.2a (37)	140.1 $\pm$ 37.7a (29)	NA	
Italy	PP	NA	NA	10.4 $\pm$ 0.4a (19)	6.1 $\pm$ 0.4b (19)	6.9 $\pm$ 0.3b (19)	NA	Temp: $F = 90.22$; $df = 2, 131$; $P < 0.0001$
China		NA	NA	10.4 $\pm$ 0.2a (40)	6.8 $\pm$ 0.2b (37)	6.5 $\pm$ 0.3b (29)	NA	
Italy	Female pupa	NA	NA	25.8 $\pm$ 0.5ab (14)	16.3 $\pm$ 0.8cde (7)	13.9 $\pm$ 0.7cde (9)	NA	Temp: $F = 366.16$; $df = 2, 148$; $P < 0.0001$
China		NA	NA	28.3 $\pm$ 0.5a (18)	17.1 $\pm$ 0.4c (28)	15.5 $\pm$ 0.5cde (16)	NA	
Italy	Male pupa	NA	NA	25 $\pm$ 0.9ab (5)	15.7 $\pm$ 0.6cde (12)	13 $\pm$ 0.7e (9)	NA	
China		NA	NA	25.6 $\pm$ 0.4b (22)	16.7 $\pm$ 0.7cd (9)	14.8 $\pm$ 0.6cde (12)	NA	

Within instars (across all temperatures and for both populations), means followed by the same letter are not significantly different based on a Tukey test with $\alpha = 0.05$ (SAS Institute, 2015). The days spent in chill at 10°C were subtracted from the total for the instar in which they occurred. NA = parameter not applicable since there were no larvae or no molts at these temperatures for these instars, NS = no significant findings, Pop. = population, Temp. = temperature, Ult. = ultimate, PP = prepupa.

Results

Mortality and Developmental Time

Development was slow or did not occur at temperatures $<10^{\circ}\text{C}$, and mortality was variable (Fig. 1). At 5°C , no first-instar larvae molted after being held for 200 d, but three of five Italian larvae that were still alive molted to the second instar in an average of 21 d after being moved to 20°C . This is longer than it would normally take at 20°C , even though the larvae did gain weight at 5°C as evidenced by the 200-day weights (6.1 ± 1.2 mg, see Supp Table 1 [online only] for average hatch weights).

At 10°C , 11.6% of the Italian and 40.0% of the Chinese larvae were able to molt to the second instar within 200 d. Larvae that had molted to at least the third instar by 200 d were held for 400 d and some were able to molt up to five times (Table 1). Although 58.0% of the larvae from both populations survived to the point at which they were moved to 20°C , fewer Italian (7.0%) than Chinese (22.0%) larvae survived to complete development.

More than half of the larvae held at constant 15°C (67.7% Italian and 56.0% Chinese) survived to the move to 25°C (at 300 or 400 d). After being moved the Italian larvae molted 0–4 times and the Chinese larvae molted 0–6 times then stopped feeding. Even after experiencing 1–2 chills several 15°C larvae did not proceed to pupation during the allotted timeframes (7 Italian and 9 Chinese larvae), often feeding very little and not molting for several hundred days.

At 35°C , no Italian first-instar larvae survived to molt to the second instar and the seven Chinese larvae that survived and molted were smaller and more translucent than those at 25°C . All the first-instar larvae held at 40°C died without molting, only surviving 4.53 ± 0.32 and 4.16 ± 0.17 d for the Italian and Chinese populations, respectively (Fig. 1).

All the third and fifth instar Chinese larvae that were moved to 5 or 40°C died without molting. Third instar larvae moved to 5°C survived an average of 76.3 ± 6.1 d while fifth instars survived 104.5 ± 5.3 d. At 40°C , third instars survived an average of 3.5 ± 0.2 d and fifth instars

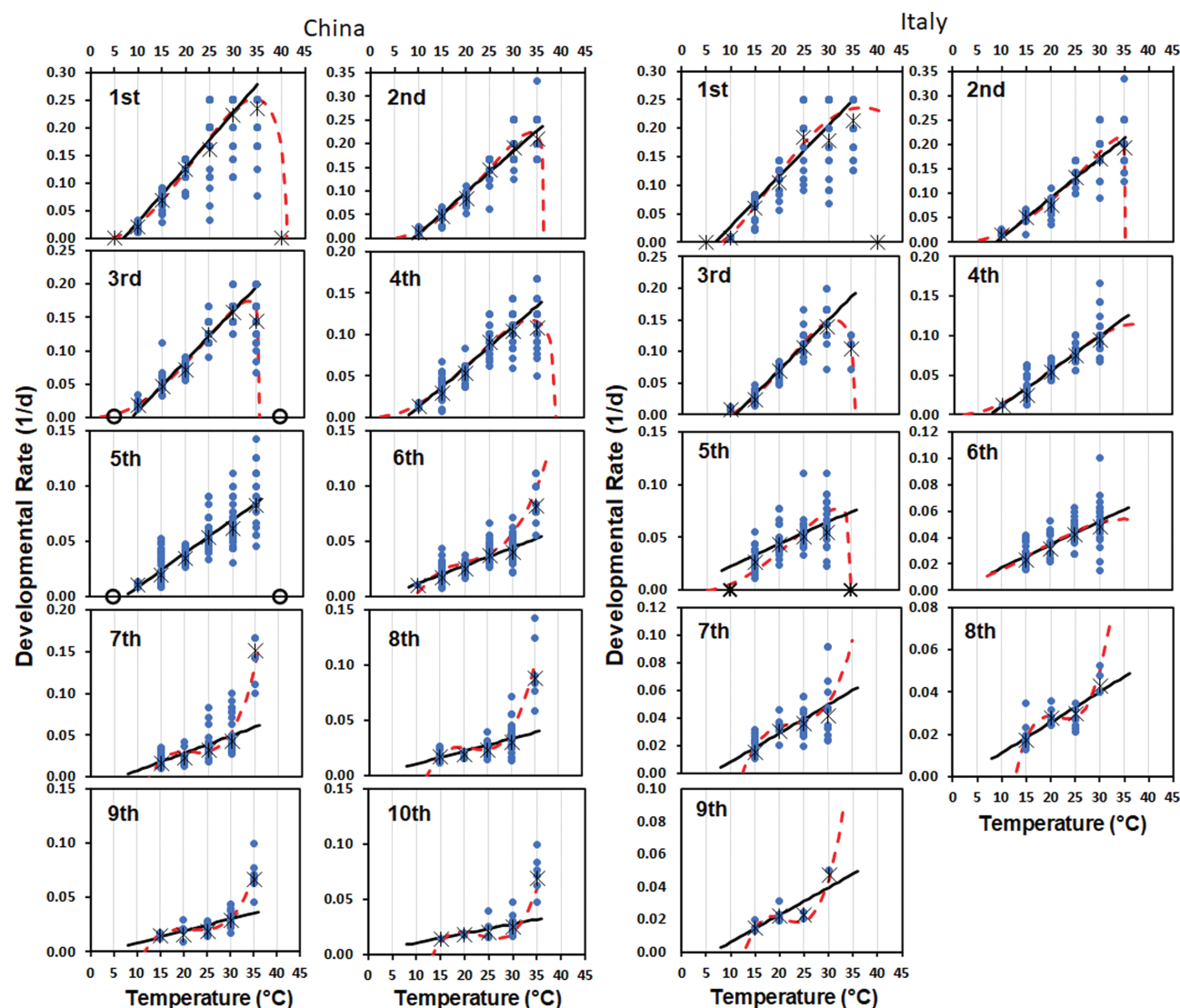


Fig. 2. Relationships between temperature and mean developmental rate (1/d) for each larval instar of *A. chinensis* from two populations. The solid line represents the estimated linear relationship, the dashed line is the estimated nonlinear relationship (Briere model or third-degree polynomial), the stars are the mean observed values, and the observed points are shown as dots. When larvae were reared to a specific instar (third or fifth) and then moved to a temperature to determine if they could complete an instar, the values are shown as open circles.

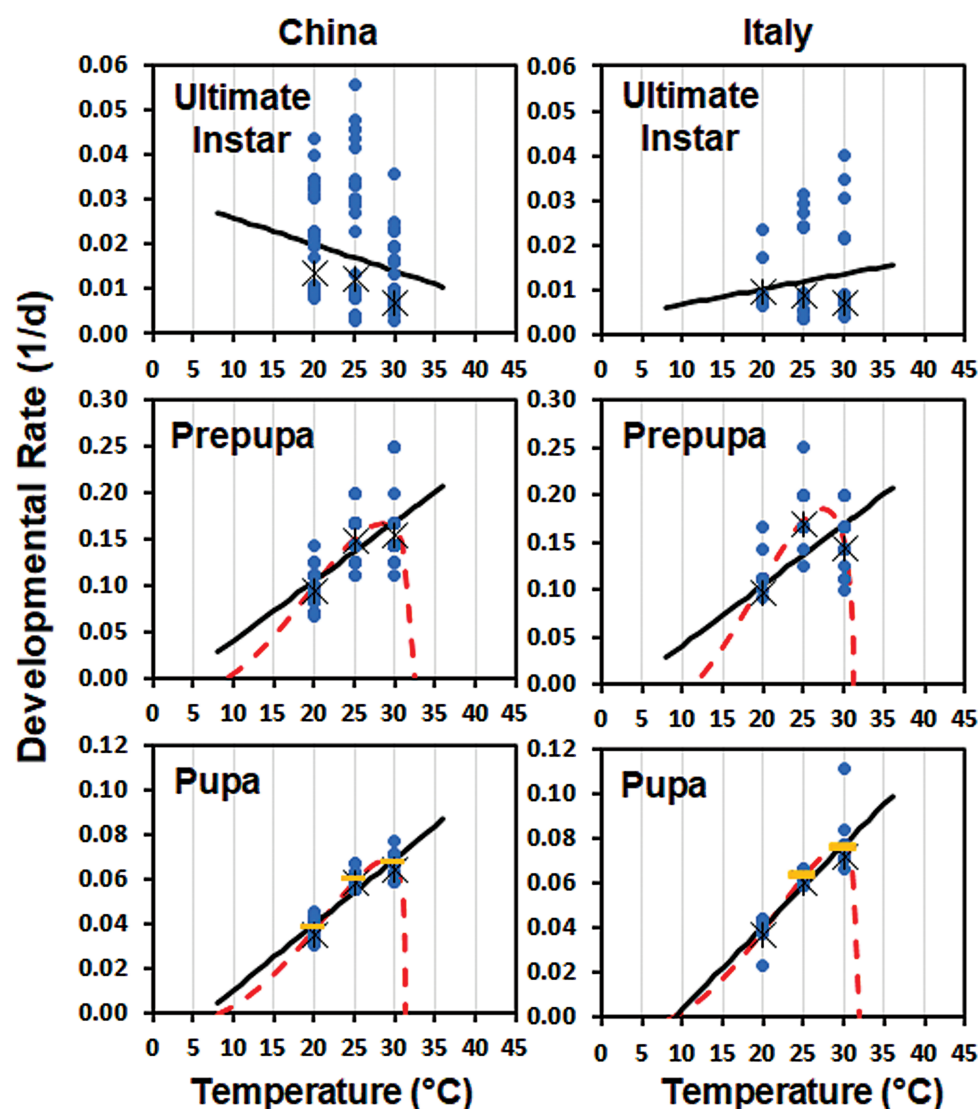


Fig. 3. Relationships between temperature and ultimate instar, prepupal and pupal development rate (1/d) for *A. chinensis* from two populations. The line represents the estimated linear relationship using both populations and observed points are shown. The solid line represents the estimated linear relationship, the dashed line is the estimated nonlinear relationship (Briere model), the stars are the mean observed values, and the observed points are shown as dots. In the pupal graphs, the stars are the female means and the dashes are the male means.

survived 3.1 ± 0.1 d. At both temperatures there was some evidence of feeding by a few of the third instars but none of the fifth instars.

The number of days spent in each instar/stage generally decreased as temperature increased from 10 to 30°C (Fig. 2; Table 1). Developmental time for the immature stages differed significantly between temperatures or the interaction between population and temperature for all but the ultimate instar (Table 1). Mortality for the Italian population was lowest at 30°C and increased at temperatures both above and below that (Fig. 1). Mortality for the Chinese strain was lowest at 20 and 25°C and increased at temperatures above and below that. Chinese larvae survived longer and were able to complete more instars at 35°C than the Italian larvae. However, no Chinese larvae were able to complete development at 35°C, even with a chill period at 10°C.

The relationships between temperature and developmental rate (fitted to linear or nonlinear models as shown in Figs. 2 and 3) and the estimated upper (T_{max}) and lower (T_{min}) thresholds for each immature stage are given in Table 2. The T_{min} for the first six instars and the pupa was <10°C, while for the higher instars it was closer to 12°C. Larvae in the fifth instar and higher

responded less to temperature changes than did those in the lower instars, as evidenced by the slopes of the linear portion of the developmental rate curves (Fig. 2). The ultimate instar for both populations was the longest instar and was insensitive to temperature (Fig. 3; Table 1).

The instar at which larvae received chill(s) and the number of instars they went through before pupation for the 10–30°C range are given in Table 3. In addition to the extended time spent in the ultimate instars for all temperatures, several larvae held at 15°C spent more than 80 d in other instars: 8 Italian larvae in a seventh instar or higher and 15 Chinese larvae in the fourth to ninth instars (the majority in the fifth). Extended times in an instar were only seen at the seventh instar for the 20–35°C range (6 Italian and 18 Chinese larvae in total) and only when they received a chill at that instar and then molted after chill before they pupated. Although Table 1 only provides data up to the thirteenth instar, at 15, 20, and 30°C six larvae went through 14–15 instars before they died and another three pupated in the 14th instar (Table 3).

When the T_{max} thresholds could be estimated they were between 31 and 41°C for all larval instars and the prepupal and pupal stages,

Table 2. Parameter values (mean $\pm$ SE) for the best linear or nonlinear models used to describe the relationships between temperature ($^{\circ}\text{C}$) and time spent (d) in each immature stage of *Anopheles chinenensis*

Source	Instar/ stage	Model	$T_{\max}$	$T_{\min}$	a	b	c or m	d	F-statistics	Adj. R^2	Temp. Range ($^{\circ}\text{C}$)	Opt. Temp. ($^{\circ}\text{C}$)
China	1st	Briere	41.1 $\pm$ 7.9	4.50 $\pm$ 3.53	0.000114 $\pm$ 0.000135		2.36 $\pm$ 2.26		$F = 856.9$; df 4, 220; $P < 0.0001$	0.733	10–35	34.3
China	2nd	Briere	36.4 $\pm$ 1.7	5.49 $\pm$ 2.23	0.000198 $\pm$ 0.000037		5.96 $\pm$ 3.87		$F = 2041.7$; df 4, 205; $P < 0.0001$	0.877	10–35	33.8
China	3rd	Briere	35.5 $\pm$ 0.8	2.04 $\pm$ 3.27	0.000148 $\pm$ 0.000015		6.52 $\pm$ 3.76		$F = 1819.3$; df 4, 201; $P < 0.0001$	0.838	10–35	33
China	4th	Briere	39.1 $\pm$ 4.9	2.04 $\pm$ 4.53	0.000063 $\pm$ 0.000041		3.01 $\pm$ 2.47		$F = 1192.5$; df 4, 189; $P < 0.0001$	0.771	10–35	33.6
China	5th	Linear		7.11 $\pm$ 1.27	-0.0217 $\pm$ 0.0039	0.00305 $\pm$ 0.000156			$F = 379.7$; df 1, 180; $P < 0.0001$	0.678	10–35	
China	6th	Poly.		9.48	-0.1168 $\pm$ 0.0321	0.0196 $\pm$ 0.00433	-0.00091 $\pm$ 0.000187	0.000015 $\pm$ 0.0000026	$F = 161.8$; df 3, 162; $P < 0.0001$	0.745	10–35	
China	7th	Poly.		12.51	-0.4023 $\pm$ 0.1249	0.0608 $\pm$ 0.0165	-0.00284 $\pm$ 0.000695	0.000044 $\pm$ 0.0000094	$F = 73.8$; df 3, 85; $P < 0.0001$	0.7128	15–35	
China	8th	Poly.		12.21	-0.3284 $\pm$ 0.1178	0.0501 $\pm$ 0.0154	-0.00234 $\pm$ 0.000637	0.000036 $\pm$ 0.0000085	$F = 54.4$; df 3, 59; $P < 0.0001$	0.721	15–35	
China	9th	Poly.		11.49	-0.1973 $\pm$ 0.1020	0.0307 $\pm$ 0.0130	-0.00143 $\pm$ 0.000528	0.000022 $\pm$ 0.0000069	$F = 48.9$; df 3, 37; $P < 0.0001$	0.782	15–35	
China	10th	Poly.		13.37	-0.3671 $\pm$ 0.2045	0.0528 $\pm$ 0.0251	-0.00235 $\pm$ 0.000986	0.000034 $\pm$ 0.000013	$F = 24.5$; df 3, 21; $P < 0.0001$	0.761	15–35	
China	Ult.	Linear		53.7 $\pm$ 0.15	0.0317 $\pm$ 0.0073	-0.00059 $\pm$ 0.000294			$F = 4$; df 1, 103; $P < 0.0483$	0.028	20–30	28.4
China	PP	Briere	32.6 $\pm$ 0.7	9.24 $\pm$ 2.01	0.000182 $\pm$ 0.000032		2.74		$F = 62.5$; df 2, 102; $P < 0.0001$	0.542	20–30	
China	Pupa	Briere	31.3 $\pm$ 0.2	8.10 $\pm$ 0.80	0.000095 $\pm$ 0.0000056		4.8		$F = 622.5$; df 2, 100; $P < 0.0001$	0.9257	20–30	29
Italy	1st	Briere	66.5 $\pm$ 93.2	8.03 $\pm$ 3.77	0.00000044 $\pm$ 0.0000099		0.55 $\pm$ 1.47		$F = 331$; df 4, 125; $P < 0.0001$	0.622	10–35	36.8
Italy	2nd	Linear		8.88 $\pm$ 1.47	-0.0722 $\pm$ 0.0095	0.0038711 $\pm$ 0.0003			$F = 480.4$; df 1, 122; $P < 0.0001$	0.796	10–35	
Italy	3rd	Briere	35.8 $\pm$ 0.6	9.92 $\pm$ 1.17	0.000133 $\pm$ 0.000002		2.92 $\pm$ 0.84		$F = 825$; df 4, 100; $P < 0.0001$	0.873	10–35	31.4
Italy	4th	Linear		7.38 $\pm$ 1.16	-0.0364 $\pm$ 0.0043	0.00488 $\pm$ 0.000186			$F = 690.2$; df 1, 162; $P < 0.0001$	0.809	10–30	
Italy	5th	Briere	35.0 $\pm$ 0.0	6.06 $\pm$ 5.23	0.000074 $\pm$ 0.000013		4.95 $\pm$ 4.25		$F = 166.1$; df 4, 90; $P < 0.0001$	0.367	10–35	
Italy	6th	Poly.		0	0.00346 $\pm$ 0.1859	0.000413 $\pm$ 0.0263	0.000099 $\pm$ 0.0012	0.00000199 $\pm$ 0.0000018	$F = 21.19$; df 3, 77; $P < 0.0001$	0.431	15–30	
Italy	7th	Poly.		12.47	-0.3123 $\pm$ 0.2735	0.0451 $\pm$ 0.0389	-0.00197 $\pm$ 0.000177	0.000029 $\pm$ 0.000026	$F = 13.3$; df 3, 25; $P < 0.0001$	0.721	15–30	
Italy	8th	Poly.		12.65	-0.4094 $\pm$ 0.1210	0.0615 $\pm$ 0.0174	-0.00286 $\pm$ 0.000803	0.000044 $\pm$ 0.000012	$F = 25.1$; df 3, 59; $P < 0.0001$	0.721	15–30	
Italy	9th	Poly.		12.86	-0.4267 $\pm$ 0.1496	0.0652 $\pm$ 0.0217	-0.00312 $\pm$ 0.00101	0.000049 $\pm$ 0.000015	$F = 17$; df 3, 7; $P < 0.0014$	0.828	15–30	
Italy	Ult.	Linear		-9.97 $\pm$ 29.2	0.0337 $\pm$ 0.00714	-0.000338 $\pm$ 0.00028			$F = 1.5$; df 1, 54; $P < 0.2323$	0.008	20–30	
Italy	PP	Briere	31.1 $\pm$ 0.3	11.52 $\pm$ 1.67	0.000259 $\pm$ 0.000042		2.64		$F = 26.9$; df 2, 52; $P < 0.0001$	0.49	20–30	27.4
Italy	Pupa	Briere	32.0 $\pm$ 0.9	8.83 $\pm$ 3.47	0.000103		4.72 $\pm$ 2.40		$F = 136.3$; df 2, 48; $P < 0.0001$	0.844	20–30	29.4

$T_{\min}$ = lower temperature threshold and $T_{\max}$ = upper temperature threshold. The temperature range used did not always include all the data points since some were not part of the linear portion of the response line based on preliminary plots. Ult. = ultimate, PP = prepupa, Poly. = third degree polynomial.

except for the Italian first-instar larvae. The Chinese third and fifth instar larvae that were moved to 40°C seem to confirm this, as even though larvae survived for a time they did not molt.

Individual Weights and Head Capsule Sizes

First instars from each population did not vary in weight between temperatures at hatch, with the exception that Italian individuals were significantly heavier than Chinese individuals in the 15°C treatment (Supp Table 1 [online only]). Temperature (all instars except 9 and 12) and the interaction between temperature and population (first, fourth, and sixth instars; pupae and adults) had significant effects on the weights of individuals (Supp Table 1 [online only]). At the fourth instar Italian larvae at 10, 15, and 35°C weighed less than those at 25 or 30°C and Chinese larvae at 20–30°C weighed more than those at the other temperatures (Fig. 4). By the fifth instar the Italian larvae held at 20°C also weighed more than those held at 10 or 35°C. At higher instars, the weights of the Chinese larvae held at 10, 15, and 35°C continued to be significantly lighter than those at the other temperatures, but the larvae from the Italian population held at 15 and 20°C caught up in weight to those held at 25°C by the 10th instar. Difference in weight gain began to be evident between the populations by the fourth instar, with Chinese larvae weighing more than Italian larvae at most temperatures (Fig. 4). The maximum weight attained by an Italian larva was 3.3 g, while the maximum for the Chinese larvae was 5.4 g, both at 25°C.

Head capsule width increased with successive instars but began to plateau around the seventh instar (Fig. 4; Supp Table S2 [online only]). The widths of head capsules from larvae within a population held at 20–30°C were very similar over all instars, but Italian larvae held at 10 and 15°C and Chinese larvae at 10 and 35°C were smaller than those of larvae at other temperatures from the fourth instar on. Although the head capsule widths of larvae from both populations were the same at hatch, the head capsule width of Chinese larvae increased in width faster than those of larvae from the Italian population.

Degree Days Required for Each Instar and Stage

The data fit the Gompertz function with an adjusted $R^2 > 0.94$ for all instars (Table 4). The number of degree days for 50% of the population to molt to the next instar increased with increasing instar for both populations up through the sixth instar (Fig. 5). The degree days to complete the seventh and higher instars were more variable between individuals from the Chinese population than those from the Italian population. Also, the difference in the predicted DD accumulations at which 50 and 90% of the population complete an instar are estimated to be <160 DD in the lower instars (1–5) and increase to >290 DD for Chinese and >220 DD for Italian larvae for instars 7–10 (Table 4).

The proportion of individuals completing the prepupal and pupal stages versus predicted degree days above the T_{min} fit the Gompertz curve well with R^2 values >0.97 (Fig. 6; Table 4). The Italian population individuals completed both the prepupal and pupal stages in fewer DD than did the individuals from the Chinese population. This was most likely due to the Chinese individuals weighing more than the Italian individuals at pupation (Supp Table 1 [online only]).

Discussion

The two *A. chinensis* populations, each from a different subspecies, differed in a few key responses to temperature. The Chinese

population both survived and developed better at 10 and 35°C than did the Italian population (origin Japan), although neither completed development at those temperatures. The T_{min} for the Chinese larvae was 3–7°C lower than that of the Italian larvae for the first four instars, which further supports the finding that the Chinese larvae developed better than Italian larvae at lower temperatures. The Chinese larvae also weighed significantly more than the Italian larvae from the fourth instar on across all temperatures, despite weighing the same at hatch (except those held at 15°C). However, in general larval weights were lower and head capsule sizes smaller at temperatures outside the 15–30°C temperature range than within. Larvae of both populations in the fifth and higher instars responded less to temperature and had higher T_{min} values than did larvae at lower instars. Larvae from both populations in the ultimate instar were insensitive to temperature, while in general the other larval instars, the prepupa, and the pupa developed faster with increasing temperature 10–30°C (35°C for some instars). At 5 and 40°C no development occurred and the T_{max} was between 31 and 40°C (when it could be estimated). The T_{max} values are consistent with the average monthly maximum climate normals for July and August in the locations the two populations came from; in Guangzhou, China the maximums are 34°C, and in Milan, Italy the maximums are 30°C (timeanddate.com/weather). These results provide a complete set of data that can be used in modeling the phenology of this invasive species while taking population differences into account.

Previous estimates of *A. chinensis* time spent in each of the first seven instars were generally shorter for a population from Japan on citrus twigs at three constant temperatures (20, 25, and 30°C; Adachi 1994) than those found in this study for the same temperatures on artificial diet. The T_{min} estimated for the first five instars of the Italian population (6.1–9.9°C) was lower than those estimated in the study for the Japanese population (9.9–13.3°C). In a study that evaluated a Chinese *A. chinensis* population held at 25°C on an artificial diet containing host material, the days in the pupal stage were longer for the female and comparable for the male to those in this study at 25°C (Cai et al. 2016). The Chinese larvae on diet containing host material had peak weights of 2.1 g while those in this study peaked at about 3.0 g at 25°C. A major difference between both of the previous studies and the current one was that some larvae from both Japan (20°C) and China (25 and 28°C) were able to complete development without a chill period, while none of the larvae from either the Chinese or Italian population in this study pupated without a chill period. These differences are most likely attributable to the diet the larvae fed on, since twigs and the diet containing host material were both drier and would contain different qualities and quantities of nutrients than the artificial diet used in this study. It is also possible that there could be phenotypes in the original populations of the insects that do not require chill and they are not present in the laboratory colonies used in these studies.

There were several interesting differences in responses to temperature between the *A. chinensis* populations evaluated in this study and *A. glabripennis* populations that have been previously evaluated (Keena and Moore 2010). First, *A. chinensis* larvae completed instars faster than *A. glabripennis* larvae at 15°C (instars 2–7), 20°C (instars 4–10), and 30°C (instars 2–10), but had similar developmental rates at 25°C. Second, the T_{min} for the Chinese *A. chinensis* population, but not the Italian population, was lower than that of the *A. glabripennis* for instars 1–6 and the pupa. Third, *A. glabripennis* larvae survived better at 5°C than did *A. chinensis* larvae and were able to complete development when moved to 25°C, even though they both had similar survival at 10°C. Fourth, *A. chinensis* larval survival (75–77%) was higher at 30°C than was

Table 3. Number of *Anoplophora chinensis* larvae that pupated at each instar, with one or two chill periods (84 d at 10°C) at the instar indicated, for four constant temperatures. Larvae at 10 and 15°C had to be moved to higher temperatures to complete development before they were chilled

Instar			Population										Grand total
			Italy					China					
			Temperature (°C)										
First chill	Second chill	Pupation	10	15	20	25	30	10	15	20	25	30	
6		6			9		2						11
7		7			5	5	8	1	2	27	19	10	77
		8					2				1	1	4
	7	7				4	1				2		7
	9	9					1				1		2
	10	10			1	1				1			3
	12	12										1	1
8		8	1	1		4	4	5	2	9	2	1	29
	8	8				1							1
	11	11							1				1
9		9		1	1	4	1	2	3	2	4	5	23
		10		1									1
	9	9				1					1		2
10		10		2			1		2		4	4	13
	10	10							2		1	2	5
11		11		1						1	1	3	6
	11	11		2									2
	14	14							1				1
12		12			1								1
	14	14							1				1
13		13							1				1
	13	13									1		1
14		14			1							2	3
		Totals	1	8	18	20	20	8	15	40	37	29	196

Half of the larvae held at 20–35°C were chilled at the 7th instar and the other half were only chilled if/when they spent 80 d in an instar without a molt. The top shaded rows for each instar show the individuals that pupated without molting after they were removed from chill.

A. glabripennis larval survival (23–32%) and *A. chinensis* larvae also survived longer and were able to complete more molts at 35°C. Finally, *A. chinensis* larvae on average weighed more than *A. glabripennis* larvae from the seventh instar on and attained higher maximum weights; the largest *A. glabripennis* larva was 3.5 g (25°C) while the largest Chinese *A. chinensis* larva weighed 5.4 g (30°C). The ability of *A. glabripennis* larvae to survive the colder temperatures better than *A. chinensis* larvae fits with earlier findings that *A. glabripennis* is freeze tolerant and has a higher respiratory rate than *A. chinensis* so can maintain its body temperature better in the cold (Feng et al. 2016, Javal et al. 2018). However, the lower respiratory rate of *A. chinensis* is inconsistent with the faster development and greater sizes attained when compared to *A. glabripennis*. The greater heat tolerance of *A. chinensis* is consistent with its native range extending further south than that of *A. glabripennis*.

The cues for pupation in *A. chinensis* are not well defined. The time spent in the ultimate instar did not differ significantly with temperature but was more variable at 30°C than at 20 or 25°C and lasted more than 70 d. Most or all of the time in that ultimate instar the larva was not feeding. The larvae that pupated did so in an average of 55 and 39 d after a chill, for the Italian and Chinese populations respectively, although in previous studies some larvae pupated without a chill (Adachi 1994, Cai et al. 2016), so temperature alone does not seem to be the cue but there could be upper or lower temperature gates that prevent larvae from proceeding to pupation at the wrong time. The lowest pupal weight was 0.83 g and 96% of the larvae weighed more than 1.0 g as pupae (7% exceeded

3 g, maximum of 3.88 g). This may indicate that there is a threshold weight that larvae must attain before they can pupate as is the case for *A. glabripennis*, although the threshold weight is lower in that species (0.5 g; Keena and Moore 2010). It is possible that diet moisture content may play a role in cuing pupation in this species for the following reasons. First, although temperature changes in the lower bole and roots that *A. chinensis* infests may be small, sap flow changes drastically with season in the broadleaf trees this species infests, with little sap flow when foliage is removed. Second, larvae were able to pupate without a chill on cut host material and drier artificial diets than used in this study. The chill period that larvae received in this study was accompanied with no diet change for 84 d. The diet would dry substantially during that time and could vary between individuals based on when it was last changed before chill. Third, time to pupation was decreased without chill when its congenitor *A. glabripennis* was reared in drier diets (Whitney and Keena 2010). In *A. glabripennis* this response to decreasing moisture in the larval environment may cue larvae to accelerate development to escape declining host wood quality, but in the case of *A. chinensis* it may be an important cue to proceed to pupation under natural conditions. Further work will be needed to determine exactly what cues *A. chinensis* uses for proceeding to pupation when conditions are appropriate for adults to emerge and lay eggs.

As mentioned previously the thermal environment that the larva is exposed to in the wood can be very different from ambient temperature and will vary with location in the tree along with many other factors. For example, the deeper in the wood the longer

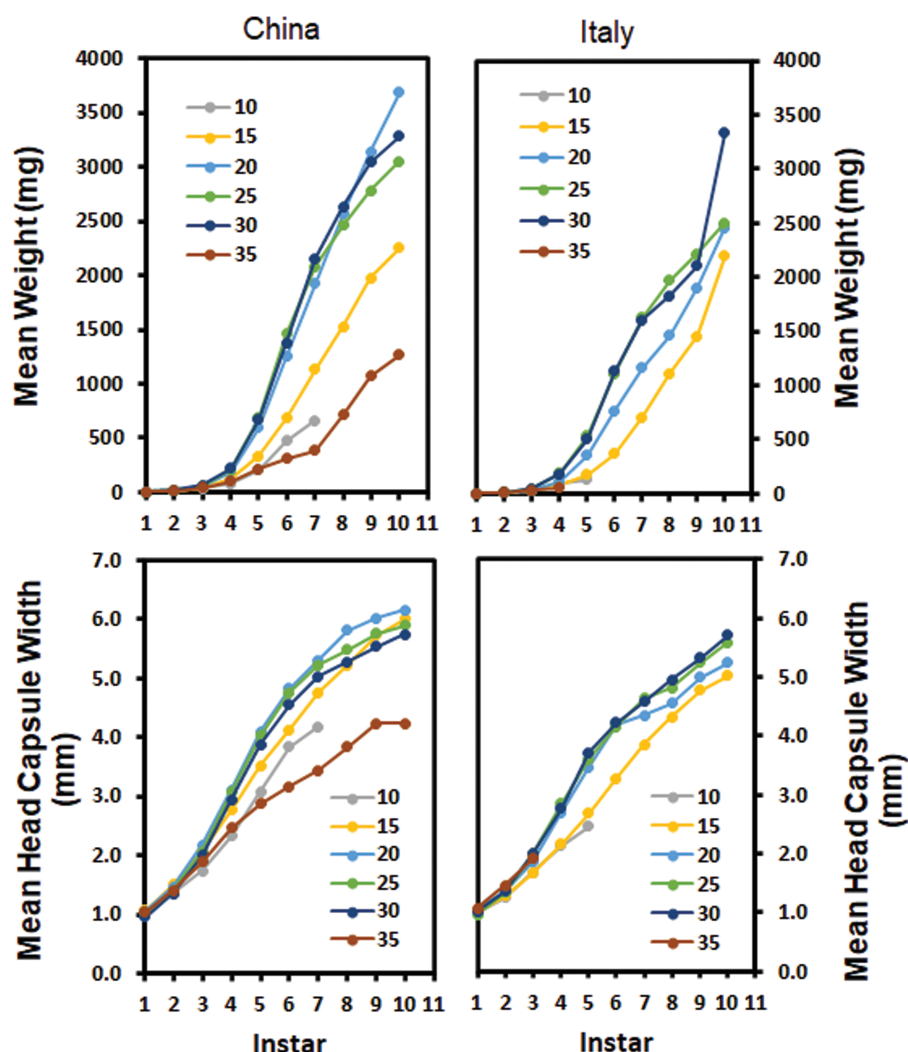


Fig. 4. Increasing weights (mg) and head capsule sizes (mm) of *A. chinensis* larvae with each successive instar for two populations and six constant temperatures.

external temperature changes will take to affect the temperatures the larvae experience, and daily minimum phloem temperatures average 2°C warmer than ambient temperatures (Derby and Gates 1966, Bolstad et al. 1997). These thermal differences may also help explain the differences in responses to temperature between the early instars that feed in the phloem region and the later instars that feed deeper in the wood. Studies to determine the microhabitat temperatures actually experienced in the tree bole and roots have not been done so the temperature deviation from ambient needed for use in modeling the phenology of this species still needs to be determined. Also, caution should be taken in extrapolating laboratory-derived thresholds and degree-day estimates for use in the field since fluctuating temperatures and larval diets can have impacts on phenology.

Given the temperature effects found in this study, the difference from ambient temperature in the thermal microclimate in the wood, and the native range of this insect, *A. chinensis* larvae should be able to develop and pupate in most of the continental United States where suitable hosts are found. The citrus growing regions of California and Florida are most likely at risk due to the ability of at least the larvae of the Chinese population to handle warmer conditions if there is sufficient time when the temperatures drop below 10°C for larvae that require a chill. The larger maximum larval and pupation weights of *A. chinensis* compared to *A. glabripennis* suggest that this species may cause greater damage to the tree bole and root system,

seriously weakening trees and predisposing them to blow over in high winds. It is also harder to identify trees infested *A. chinensis* than *A. glabripennis* because of the location in the tree they inhabit and the fact that prior to pupation they spend a longer time not feeding, further reducing signs of infestation. These data combined with those for adults and eggs (Keena et al. 2021) can be used to develop a phenology model for this insect that will be able to predict its range in uninfested regions and the timing of each stage in for management purposes.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

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Table 4. Parameter values predicted using a Gompertz function (Brown and Mayer 1988) for the relationship between proportion of *Anoplophora chinensis* progressing to the next instar or stage and number of accumulated degree-days above the lower threshold for each immature stage

Source	Instar/stage	a	b	Predicted accumulated degree days (95% CI)		Adj R ²	Base °C	F-statistics
				10%	50%			
China	First	0.0269 ± 0.00119	2.831 ± 0.150	74.2 (71.8–76.5)	118.9 (118.5–119.2)	188.9 (186.3–191.8)	4.5	F = 7896.8; df = 2, 47; P < 0.0001
China	Second	0.0337 ± 0.00109	4.557 ± 0.164	110.5 (109.1–111.7)	146.1 (146.0–146.2)	202.0 (200.4–203.7)	5.49	F = 13069.8; df = 2, 40; P < 0.0001
China	Third	0.0207 ± 0.00055	3.982 ± 0.118	152.1 (150.4–153.7)	210.1 (210.0–210.2)	301.1 (298.8–303.5)	2.04	F = 16776.7; df = 2, 44; P < 0.0001
China	Fourth	0.0164 ± 0.00037	4.325 ± 0.107	212.9 (211.2–214.6)	286.1 (286.1–286.1)	401.0 (398.5–403.6)	2.04	F = 20949.9; df = 2, 62; P < 0.0001
China	Fifth	0.0129 ± 0.00025	3.942 ± 0.082	240.9 (239.3–242.6)	334.0 (333.8–334.2)	480.0 (477.1–483.1)	7.11	F = 17838.3; df = 2, 87; P < 0.0001
China	Sixth	0.0112 ± 0.00020	4.028 ± 0.076	285.2 (283.5–286.9)	392.4 (392.2–392.6)	560.6 (557.4–563.9)	9.48	F = 20976.2; df = 2, 88; P < 0.0001
China	Seventh	0.0064 ± 0.00013	1.433 ± 0.034	92.9 (89.4–96.3)	279.4 (279.0–279.7)	571.9 (565.7–578.3)	12.51	F = 11229.2; df = 2, 71; P < 0.0001
China	Eighth	0.0052 ± 0.00019	1.779 ± 0.077	182.8 (174.3–190.8)	415.0 (414.9–415.1)	779.4 (766.7–793.1)	12.21	F = 3819.0; df = 2, 50; P < 0.0001
China	Ninth	0.0054 ± 0.00018	2.384 ± 0.091	289.2 (281.8–296.1)	513.2 (512.7–513.6)	864.6 (852.7–877.4)	11.49	F = 5099.6; df = 2, 32; P < 0.0001
China	Tenth	0.0050 ± 0.00021	1.978 ± 0.097	228.7 (218.5–238.1)	468.8 (468.4–469.2)	845.6 (829.9–862.7)	13.37	F = 2827.4; df = 2, 24; P < 0.0001
China	Prepupa	0.0510 ± 0.00242	5.292 ± 0.265	87.4 (86.3–88.4)	110.9 (110.8–111.0)	147.9 (146.1–149.8)	9.24	F = 3242.2; df = 2, 17; P < 0.0001
China	Pupa	0.0407 ± 0.00248	12.064 ± 0.749	275.9 (274.2–277.4)	305.4 (305.2–305.6)	351.7 (348.8–354.9)	8.1	F = 2124.0; df = 2, 19; P < 0.0001
Italy	First	0.0304 ± 0.00203	2.703 ± 0.206	61.4 (58.6–64.0)	100.9 (100.9–101.0)	162.9 (159.1–167.3)	8.03	F = 2951.1; df = 2, 42; P < 0.0001
Italy	Second	0.0473 ± 0.00138	5.178 ± 0.161	91.8 (91.1–92.5)	117.2 (117.1–117.3)	157.1 (155.9–158.3)	8.88	F = 10136.0; df = 2, 39; P < 0.0001
Italy	Third	0.0311 ± 0.00109	4.133 ± 0.157	106.1 (104.7–107.4)	144.7 (144.7–144.7)	205.3 (203.2–207.5)	9.92	F = 8325.6; df = 2, 47; P < 0.0001
Italy	Fourth	0.0217 ± 0.00065	4.673 ± 0.147	176.9 (175.4–178.3)	232.2 (232.1–232.4)	319.0 (316.3–321.9)	7.38	F = 8477.9; df = 2, 42; P < 0.0001
Italy	Fifth	0.0123 ± 0.00034	3.798 ± 0.111	241.0 (238.5–243.3)	338.5 (338.3–338.8)	491.7 (487.4–496.3)	6.06	F = 10490.6; df = 2, 51; P < 0.0001
Italy	Sixth	0.0071 ± 0.00010	3.786 ± 0.054	414.6 (412.6–416.5)	583.2 (582.9–583.5)	847.8 (844.0–851.7)	0	F = 31815.7; df = 2, 41; P < 0.0001
Italy	Seventh	0.0084 ± 0.00030	1.590 ± 0.064	90.1 (85.5–94.4)	233.2 (232.6–233.8)	457.7 (449.5–466.5)	12.47	F = 5133.0; df = 2, 43; P < 0.0001
Italy	Eighth	0.0077 ± 0.00034	1.485 ± 0.077	84.3 (77.6–90.3)	239.8 (239.3–240.2)	483.8 (473.2–495.4)	12.65	F = 2857.0; df = 2, 25; P < 0.0001
Italy	Ninth	0.0075 ± 0.00085	1.690 ± 0.235	114.2 (93.3–130.8)	274.2 (273.7–274.6)	525.4 (500.4–556.8)	12.86	F = 427.1; df = 2, 9; P < 0.0001
Italy	Prepupa	0.0434 ± 0.00305	3.416 ± 0.265	59.5 (57.4–61.3)	87.2 (87.1–87.2)	130.6 (127.7–133.9)	11.52	F = 1566.8; df = 2, 16; P < 0.0001
Italy	Pupa	0.0526 ± 0.00430	13.970 ± 1.162	249.7 (247.9–251.3)	272.6 (272.4–272.8)	308.4 (305.5–311.8)	8.83	F = 1245.1; df = 2, 14; P < 0.0001

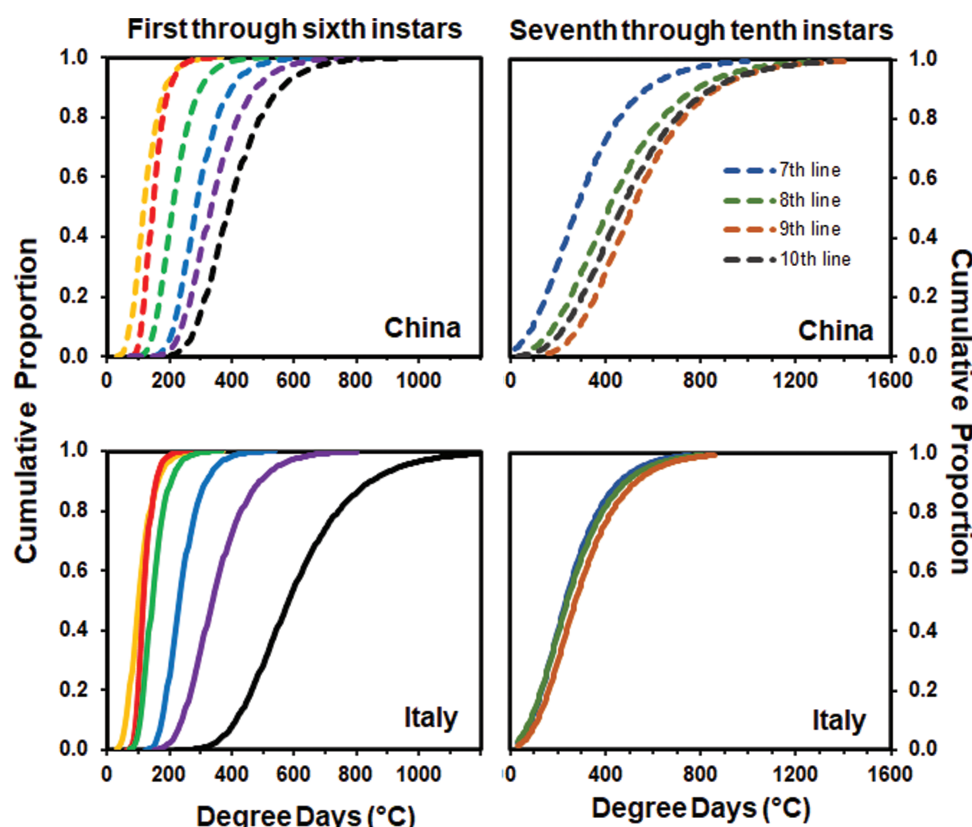


Fig. 5. Fitted models for cumulative proportion of *A. chinensis* larvae from each source population molting from one instar to the next over accumulated degree-days based on estimated T_L for each instar by population combination. First through sixth instar curves occur in sequence from left to right on each graph, while the seventh to tenth instars either overlap (Italy) or occur out of sequence (China).

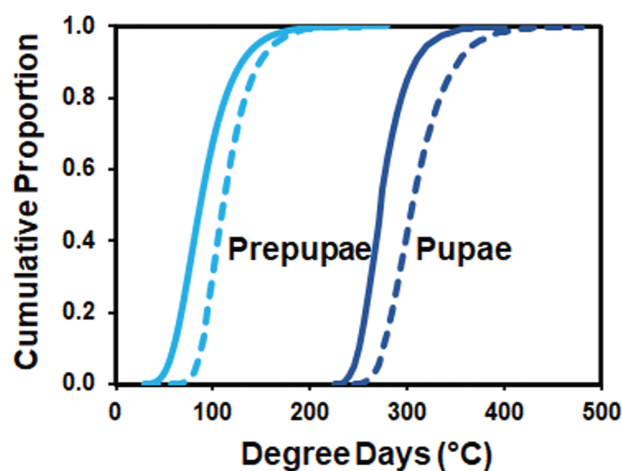


Fig. 6. Fitted models for cumulative proportion of *A. chinensis* prepupae and pupae completing the stage over accumulated degree-days based on estimated T_L for each stage by population combination. The curves for the Italian population are solid lines and the dashed lines are for the Chinese population.

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