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A novel parasitoid for an invasive mealybug: temperature-dependent biology of *Anagyrus aberiae* (Hymenoptera: Encyrtidae) and implications for the biological control of *Delottococcus aberiae* (Hemiptera: Pseudococcidae)

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Anagyrus aberiae (Guerrieri) (Hymenoptera: Encyrtidae), an imported parasitoid from South Africa, was released in Spain for the classical biological control of the invasive citrus mealybug *Delottococcus aberiae* (De Lotto) (Hemiptera: Pseudococcidae). However, limited information is available about the parasitoid's biological traits. Temperature is a key factor influencing insect performance and population dynamics. This study examines the effect of 7 constant temperatures on the development and survival of *A. aberiae* and determines its developmental thresholds and thermal constants using linear and nonlinear regression models. The parasitoid successfully developed and survived at temperatures between 18 and 33 °C but failed to develop at 36 °C. Development time and adult longevity decreased with increasing temperature, from 58.15 d at 18 °C to 19.27 d at 33 °C. Female progeny production peaked at 24 °C, whereas offspring production was lowest and male-biased at 18 and 33 °C. The estimated lower and upper developmental thresholds were 11.4 and 36 °C, respectively, with an optimal temperature of 32.1 °C. The thermal constant was calculated as 250 degree-days (DD). *A. aberiae* can survive, develop, and reproduce within the typical temperature range found in Mediterranean orchards, suggesting strong adaptive potential in regions beyond its native range. These results should be considered when optimizing mass-rearing programmes and assessing the field adaptation and establishment of *A. aberiae* as a biological control agent of *D. aberiae* across different citrus-growing regions.

Keywords: classical biological control, developmental thresholds, regression models, pest management

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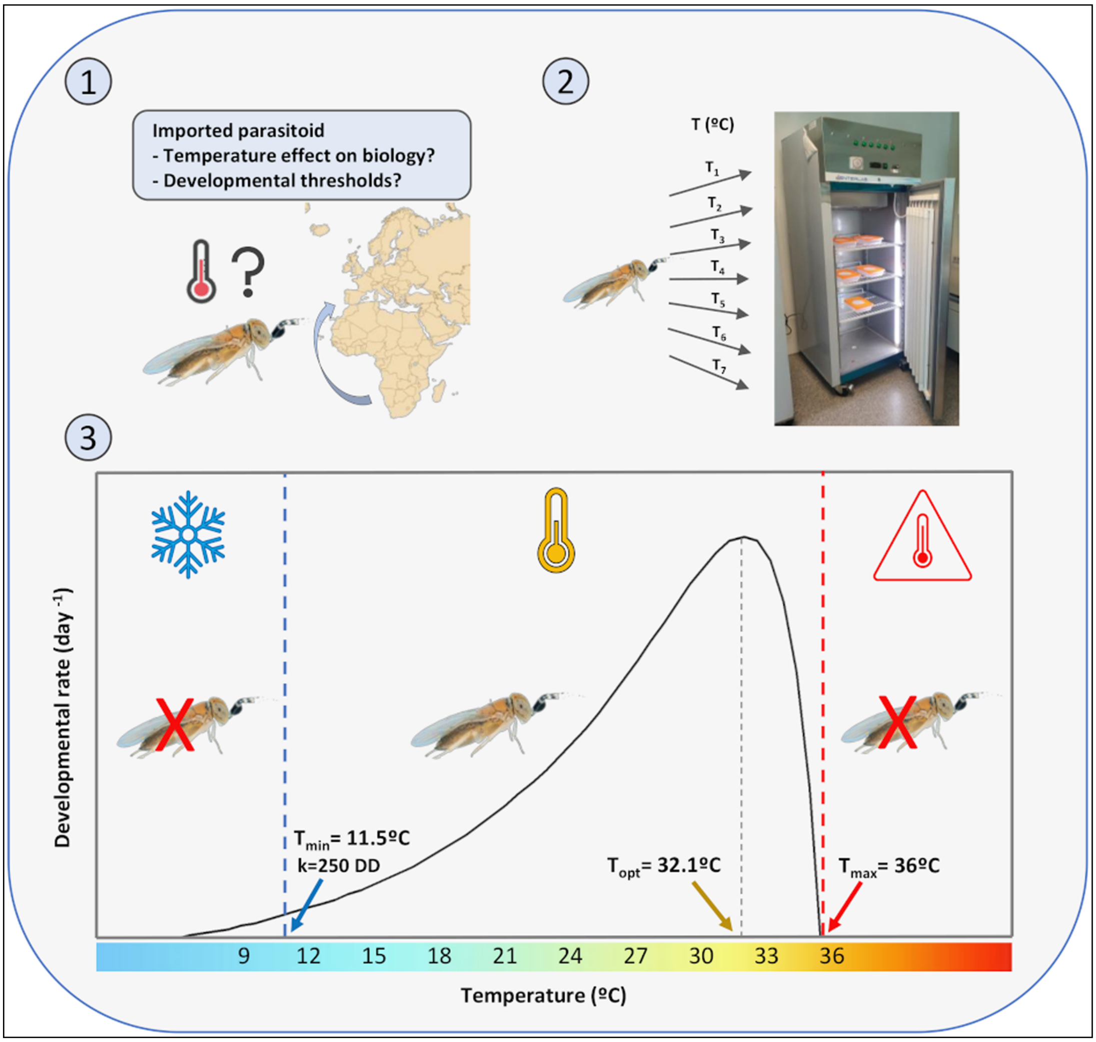
Graphical abstract



Introduction

The South African mealybug, *Delottococcus aberiae* De Lotto (Hemiptera: Pseudococcidae), is an invasive pest first detected causing damage in citrus orchards in the region of "Les Valls" (Valencia, eastern Spain) in 2009 (Beltrà et al. 2012, 2015, García-Marí 2012). Since then, populations of this insect have spread rapidly, causing severe damage by deforming and reducing the size of citrus fruits, therefore increasing production losses (Pérez-Rodríguez et al. 2017, Martínez-Blay et al. 2018a, 2018b). The use of insect parasitoids in biological control programmes has been successful in controlling pseudococcidae populations worldwide (Moore 1988, Neuenschwander and Herren 1988, Colmenarez et al. 2018). However, the absence of effective natural enemies to control populations of *D. aberiae* in Spain (Soto et al. 2016, Tena et al. 2017) has led to the use of broad-spectrum insecticides (Pérez-Rodríguez et al. 2017), which interfere with the complex of natural enemies of other citrus pests (Franco et al. 2009, Tena and García-Marí 2011). Hence, introducing the natural enemy of the mealybug from the pest's region of origin, the province of Limpopo, South Africa (Beltrà et al. 2015), may help to control *D. aberiae* populations. A survey in its native area during the period from 2017 to 2018 resulted in the identification of several species of parasitoids belonging to the genus *Anagyrus* and *Allotropa*. Among these, the most widely found was a parasitoid, later described as *Anagyrus aberiae* Guerrieri sp. Nov. (Guerrieri and Cascone 2018), which accounted for 75% of the emerged pupae examined (Soto et al. 2020). As a result, *A. aberiae* was imported to Spain in 2019 and the release of the parasitoid in citrus orchards affected by *D. aberiae* was authorized in 2020, following several laboratory studies that indicated minimal ecological risk (Soto et al. 2020, 2021).

Many parasitoids belonging to the Encyrtidae family (Hymenoptera), such as *A. aberiae*, are well known for their high host-specificity among mealybugs (Moore 1988), suggesting that an introduced host-specific parasitoid to control an exotic mealybug is unlikely to affect native, nontarget mealybug species (Charles 2011). The study of the parasitoid's life history on the target pest species is one of the first and most important steps to evaluate its potential as a biological control agent. Life cycle studies are essential to understand the host-parasitoid interactions under different environmental factors, especially temperature. Effects of temperature on developmental rate, adult longevity, fecundity, oviposition behavior and sex ratio of hymenopterous parasitoids have been widely documented (Löhr et al. 1989, Mani and Krishnamoorthy 1992, Sagarra et al. 2000a, 2000b, Ferreira De Almeida et al. 2002, Arai and Mishiro 2004, Daane et al. 2004, 2005, Chong and Oetting 2006, Pandey and Johnson 2006, Milosavljević et al. 2019, 2020, Wang et al. 2019, Power et al. 2020, Tougeron et al. 2021). A thorough understanding of a parasitoid's temperature requirements is crucial to design an adequate parasitoid mass-rearing in the laboratory and to predict seasonal occurrence of populations under field conditions (Pandey and Johnson 2006, McCalla et al. 2019, Milosavljević et al. 2019, Power et al. 2020, Yi et al. 2020). The relationship between temperature and developmental rate (1/Development time in days) of an insect can be evaluated by various linear and nonlinear regression models (Uvarov 1931, Davidson 1944, Wagner et al. 1984, Rebaudo and Rabhi 2018). Models provide estimates on temperature thresholds as well as the thermal constant. Linear models have proven useful in determining the degree-days (DDs) needed for an insect to complete development, as well as its lower temperature threshold (Uvarov 1931, Campbell et al. 1974). On the other hand, nonlinear models give good estimates for the optimal temperature and upper temperature threshold above which the insect is unable to develop (Régnière et al. 2012, Shi et al. 2017, Rebaudo and Rabhi 2018).

The aim of this study is to assess the impact of temperature on the development, longevity, progeny production, and sex ratio of *A. aberiae* under controlled conditions. This parasitoid was recently described and is still largely unexplored in terms of its performance and biological characteristics.

To this end, experiments were conducted to obtain the time needed for the parasitoid to complete development from egg to adult, and to study the longevity and offspring at the adult stage of the parasitoid at each temperature. The data were subsequently used to develop linear and nonlinear thermal developmental models and to estimate the thermal constant and threshold temperatures (maximum, minimum, and optimal temperatures for insect development).

These findings will help optimize mass rearing and assess the effectiveness of *A. aberiae* as a biological control agent against this mealybug in the Mediterranean Basin.

Materials and Methods

Insect and Plant Materials

A laboratory colony of *D. aberiae* was established in an insectary at the Universitat Politècnica de València (UPV) with field-collected material from citrus orchards located in the region of Les Valls (Valencia, Spain). Mealybugs were reared on *Cucurbita maxima*, which was cleaned in a 2% bleach solution to prevent mold growth. Mealybug infested pumpkins were placed in containers which consisted of sleeve cages (47.5 × 47.5 × 47.5 cm) containing between 3 and 5 pumpkins each. For colony maintenance, between 3 and 4 new pumpkins were infested every 2 wk to renew the old, dried ones. The mealybug colony was maintained in darkness in a climate-controlled chamber at a temperature of 24 °C and 70% ± 10 RH.

The parasitoid *A. aberiae* was reared on the *D. aberiae* colony described above, using individuals introduced from South Africa in 2020. The mealybug-infested pumpkins were replaced every 3 wk to provide the parasitoid with a continuous supply of mealybugs. Drops of diluted honey were provided through the cage walls to feed the parasitoid 3 times a week. The parasitoid colony was maintained in an environmental chamber at 24 °C, 70% ± 10 RH and a photoperiod of 14:10 L:D. For experiments, mummified mealybugs were observed daily, and newly emerged parasitoids were collected, sexed, and individualized in plastic tubes with a drop of honey and covered with cotton until used.

Effect of Temperature on Development

The development time from *A. aberiae* parasitoid egg to adult stage was assessed at 7 constant temperatures: 18, 21, 24, 27, 30, 33, and 36 °C. For each treatment, bean pods were inoculated with 50 preovipositional adult mealybugs and placed in a 1.6L plastic container covered with a fine mesh to allow ventilation. After inoculation, mealybugs were allowed to settle and begin feeding for 3 h before exposure to the parasitoids. The container was then transferred uncovered into a parasitoid rearing cage with at least 50 pairs of *A. aberiae*, where the parasitoids were able to forage and oviposit in the mealybugs for 24 h. After this time, the plastic containers were removed from the parasitoid cages and transferred to each of the 7 experimental temperatures. Three plastic containers were assigned to each of the 7 temperature regimens to provide sufficient development data for a minimum combined total of 80 emerged adult male and female of *A. aberiae*. Mealybugs were examined daily, and mummified mealybugs were individualized in plastic tubes and checked daily for parasitoid emergence. The emerged parasitoids were counted and sexed. The time between egg deposition and adult emergence was recorded for each temperature. The experiments were conducted in environmental chambers with a relative humidity of 70% ± 10% and a photoperiod of 14L:10D.

Effect of Temperature on Female Longevity, Progeny, and Sex Ratio

Female adult longevity, progeny production and the sex ratio of the offspring were determined at 6 temperatures: 18, 21, 24, 27, 30, and 33 °C. Based on results from the developmental study, the 36 °C treatment was excluded from the longevity and progeny production assessment. A couple of newly emerged female and male parasitoids from the temperature-dependent development experiment were transferred into a 150 mm Petri dish containing a bean pod infested with 30 mealybugs. Every 5 d, 20 mealybugs were added to the Petri dishes to provide the parasitoids with new mealybugs. Petri dishes were covered with a lid to allow ventilation and to feed the parasitoids with drops of diluted honey. A minimum of 20 replicates were prepared for each temperature regimen. Newly emerged male parasitoids replaced those which died before the females. When mummification of the mealybugs began, individuals were isolated and incubated in plastic tubes sealed with a cotton at the designated temperature regimen. All the experiments were conducted in environmental chambers with a relative humidity of 70% ± 10% and a photoperiod of 14L:10D. The emerged parasitoids were counted and sexed. Adult longevity and the number and sex ratio of progeny were determined for each female parasitoid in her lifetime.

Thermal Requirements and Mathematical Models

The performance of 1 linear and 9 nonlinear regression models was evaluated to predict the relationship between temperature and the development rate of *A. aberiae*. Model selection was guided by previous studies reporting their effectiveness in modeling temperature-dependent development in encyrtid parasitoids (Daane et al. 2004, Chong and Oetting 2006, Rebaudo and Rabhi 2018, Milosavljević et al. 2019, Power et al. 2020). The development time data of *A. aberiae* reared at 7 temperatures were used to build the models. The models were evaluated based on the goodness of fit to the observed development rates for each temperature tested and the thermal threshold estimations.

A linear approximation equation is suitable to extrapolate the relationship between temperature and developmental rate within a moderate range of temperatures, such as 18 to 30 °C (Davidson 1944). Hence, a linear regression model was used to calculate the lower developmental threshold (ie, the temperature below which the insect is unable to develop), and the thermal constant (ie, the number of DD above T_{min} needed to complete a developmental stage). The linear model is defined by the equation $Dr = a + bT$, where a and b are constants and T is the temperature. In the linear model, the lower development threshold and the thermal constant were calculated as $T_{min} = -a/b$ and $k = 1/b$, respectively (Campbell et al. 1974). The adjusted coefficient of determination (R^2_{adj}) was used as a measure of goodness of fit to the linear model, with high values of R^2_{adj} indicating a good adjustment to the data. The number of *A. aberiae* generations per year was estimated by dividing the total annual DDs accumulated at a given location by the insect's thermal constant derived from the linear model.

Nonlinear models are commonly used to estimate the upper developmental threshold (T_{max}), the optimal temperature for development (T_{opt}), and, in some cases, the lower developmental threshold (T_{min}). Several of these models were evaluated, based on their accuracy in describing the development rates observed for *A. aberiae*. The models evaluated include Briere 1 and 2 (Briere et al. 1999), Lactin 1 and 2 (Lactin et al. 1995), Logan 6 (Logan et al. 1976), Wang (Wang et al. 1982, Shi and Ge 2010), Performance-2 (Shi et al. 2011), Ratkowski (Ratkowsky et al. 1983), and Lobry–Rosso–Flandrois (LRF) (Rosso et al. 1993). The performance of each nonlinear model was assessed based on the goodness of fit to observed data (ie, estimations of T_{min}, T_{max}, and T_{opt}), the residual sum of squares (RSS) and the Akaike information criterion (AIC), to compare models with different numbers of parameters and thus, different degrees of freedom (Akaike 1974). Models with lower values of RSS and AIC are considered the best fit. Based on these criteria, 3 nonlinear models were selected, and their parameters presented (Table 1). The coefficient of determination (R^2) along with its adjusted value (R^2_{adj}), although widely used in the literature to assess the validity of a nonlinear regression, fails to accurately describe the fit of nonlinear models to developmental data (Spiess and Neumeyer 2010) and was therefore excluded as an indicator of goodness of fit for nonlinear models.

Statistics

To assess the effect of temperature on the parasitoid development rate, each temperature regimen (18, 21, 24, 27, 30, 33, and 36 °C) and parasitoid sex was considered separately. We used regression analysis to describe the relationship between temperature and developmental rate (egg to adult emergence), and to calculate temperature thresholds and the thermal constant. Variables were tested for normality using the Kolmogorov–Smirnov test before subjecting them to the analysis. If required, percentage data were subjected to an angular transformation, arcsine of the square root of the proportion, before analysis to satisfy model assumptions regarding homogeneity of variances and to approximate a normal distribution (Kasuya 2004). Experimental data were tested using an analysis of variance (ANOVA) with treatment means separated using Fisher's LSD test at a significance threshold of 0.05 (STATGRAPHICS Centurion 2018). When data could not be adjusted to a normal distribution, a Kruskal–Wallis 1-way ANOVA test followed by a pairwise Wilcoxon rank-sum test with Bonferroni's correction ($P < 0.05$) (STATGRAPHICS Centurion 2018) was used.

Results

Effect of Temperature on Parasitoid Development, Longevity, Progeny Production, and Sex Ratio

Temperature significantly affected the development of *A. aberiae*. There were no significant differences in the development times (egg to adult emergence) of male and female parasitoids at any temperature treatment (Wilcoxon rank-sum test, $P > 0.05$; Supplementary Table S1); therefore, *A. aberiae* male and female development times are shown combined (Table 2). Mean development times were significantly different among all temperature treatments ($H = 567.29$; df = 5; $P < 0.0001$). *A. aberiae* was able to complete development from egg to adult at temperatures between 18 and 33 °C. However, at the highest temperature tested, 36 °C, no emergence was observed. Development times for *A. aberiae* ranged from 40 d at 18 °C to 12.91 d at 33 °C (Table 2). At those temperatures where development was successful, the mean development time decreased with increasing temperature until reaching 36 °C.

Mean longevity of female adult *A. aberiae* was also affected significantly by temperature ($F = 22.52$; df = 5, 136; $P < 0.00001$) (Table 3). The longevity of the female adult parasitoid decreased linearly with rising temperatures from 18 to 33 °C. Parasitoids kept at a lower temperature lived significantly longer than those held at higher temperatures. The maximum longevity of 18.15 d was observed at 18 °C, which was 3 times longer than those kept at 33 °C. Temperature significantly affected the number ($F = 18.19$; df = 5, 131; $P < 0.00001$) and sex ratio ($H = 40.05$; df = 5; $P < 0.00001$) of progeny produced by a female parasitoid (Table 3). *A. aberiae* oviposited at all temperature regimens at which development was observed. The number of offspring increased with rising temperature up to 24 °C and then fell above 27 °C. Oviposition at the temperature extremes was significantly lower compared to intermediate temperatures. The number of progeny produced ranged from 2.46 to 14.7 eggs laid per female, with the highest offspring observed at 24 °C and the lowest at 33 °C. Production of male progeny was highest at temperature extremes, peaking at 33 °C with a male-biased sex ratio of 0.95. In contrast, the highest proportion of female progeny was observed at 21 and 24 °C, with a male sex ratio of 0.59.

Thermal Requirements and Mathematical Models

Since there were no significant differences in development times of male and female *A. aberiae* at any temperature treatment (Table 2), developmental rates were pooled before subjecting them to regression analysis.

Table 1. Parameter estimates of 1 linear and 3 nonlinear mathematical models describing the relationship between the development rate and temperature for *A. aberiae*

Model	Model equation	Parameter	Estimation	Reference
Linear	$D_r = a + bT$	a	−0.0458	Campbell et al. (1974)
		b	0.0039	
		k (DD)	250.42	
		$T_{\min}$	11.46	
		R^2_{adj}	0.9815	
Logan-6	$D_r = \psi\ (e^{\rho T} \text{-} e^{(\rho Tmax - (Tmax - T)/\Delta T)})$	Ψ	0.005562	Logan et al. (1976)
		ρ	0.09058	
		ΔT	2.0927	
		$T_{\max}$	36.002	
		T_{opt}[a]	32.0121	
		RSS	0.000044	
		AIC	−75.22	
Lactin-2	$D_r = \lambda + e^{\rho T -} e^{(\rho Tu \text{-} (Tu - T)/\Delta T)}$	λ	−1.0442	Lactin et al. (1995)
		ρ	0.003762	
		ΔT	1.3538	
		T_u	39.3065	
		$T_{\min}$[a]	11.5	
		$T_{\max}$[a]	36.001	
		T_{opt}[a]	32.1	
		RSS	0.00001714	
		AIC	−82.44	
Wang	$D_r = \dfrac{m\left[1 - e^{\left(-\frac{T - T\min}{c}\right)}\right]\left[1 - e^{\left(-\frac{T\max - T}{c}\right)}\right]}{1 + e^{(-rm(T - T\text{opt}))}}$	m	0.1126	Wang et al. (1982)
		r_m	0.1411	Shi and Ge (2010)
		C	1.195	
		$T_{\min}$[a]	15.7084	
		T_{opt}[a]	32.3343	
		$T_{\max}$	36.0002	
		RSS	0.000014	
		AIC	−79.99	

In all models, T is the rearing temperature (°C), and Dr is the developmental rate at temperature T (d^{-1}). $T_{\min}$ and $T_{\max}$ are the lower and upper temperature thresholds, respectively, at which the development rates equal zero. T_{opt} is the optimal temperature for insect growth. Parameters a and b in the linear model are constants to be fitted; k is the thermal constant defining the DD needed for insect development. In Logan 6 model, ψ represents the developmental rate at some base temperature above the developmental threshold. In Lactin 2 model, λ forces the curve to intercept the abscissa at a value below zero, thus allowing for the estimation of the lower developmental threshold and T_u represents the upper lethal temperature, which is higher than the conceptual upper threshold. In Logan 6 and Lactin 2 models, ρ is a constant defining the biochemical reaction rate as the ambient temperature increases to optimal temperature (T_{opt}) and ΔT is the temperature range between the optimal and upper temperature. In the Wang model, m is the value of the upper asymptote of the curve; r_m is the exponential increase rate, and c is the boundary width at the upper and lower temperatures.

[a] Parameters that were mathematically measured by Excel with the model equation.

Table 2. Effect of temperature on parasitoid development time

		Development time (d)		
Temperature (°C)	***n***	**Male**	**Female**	**Pooled mean (d)**
18	93	40.28 ± 0.40	39.85 ± 0.21	40.00 ± 0.19a
21	86	26.26 ± 0.49	25.63 ± 0.24	25.77 ± 0.22b
24	117	20.31 ± 0.21	20.15 ± 0.15	20.19 ± 0.12c
27	102	15.44 ± 0.13	15.11 ± 0.14	15.26 ± 0.10d
30	129	13.59 ± 0.14	14.10 ± 0.14	13.98 ± 0.12e
33	89	12.67 ± 0.13	13.02 ± 0.08	12.91 ± 0.07f
36	102	–	–	–
Kruskal–Wallis *H* value		0.2873		567.29
***P*-value**		0.5919		<0.00001

Development times are not significantly different between sexes (corrected P-value = 1; Kruskal–Wallis, $P > 0.05$). Pooled means followed by the same lowercase letter are not significantly different among temperatures (Kruskal–Wallis, $p < 0.05$). Detailed post hoc pairwise comparisons (Wilcoxon test with Bonferroni correction) are provided in Supplementary Table S1.

Table 3. Effect of temperature on female parasitoid longevity, progeny and sex ratio

Temperature (°C)	*n*	Female longevity (d)	Number of progeny/female Male	 Female	 Total	Sex ratio (proportion of males)
18	20	18.15 ± 1.46a	5.85 ± 0.73	1.55 ± 0.46	7.4 ± 0.81c	0.79 ± 0.06ab
21	20	16.70 ± 0.98a	5.3 ± 0.68	3.75 ± 0.59	9.05 ± 1.04bc	0.59 ± 0.06c
24	20	9.70 ± 0.77b	8.70 ± 0.98	6.00 ± 0.92	14.7 ± 1.36a	0.59 ± 0.05bc
27	27	8.3 ± 0.70b	7.79 ± 0.79	3.79 ± 0.73	11.58 ± 1.17b	0.67 ± 0.05bc
30	30	7.83 ± 0.47b	7.65 ± 0.69	1.80 ± 0.39	9.46 ± 0.76bc	0.81 ± 0.04ab
33	25	6.36 ± 0.56c	2.33 ± 0.46	0.13 ± 0.09	2.46 ± 0.49d	0.95 ± 0.02a
ANOVA *F*-value		22.52			18.19	
Kruskal–Wallis *H* value						40.05
P-value		<0.00001			<0.00001	<0.00001

Mean longevity, progeny and sex ratio proportions followed by the same lowercase letter are not significantly different among temperatures (ANOVA or Kruskal–Wallis, $P < 0.05$). Detailed post hoc pairwise comparisons (Fisher's LSD and Wilcoxon test with Bonferroni correction) are provided in Supplementary Table S2.

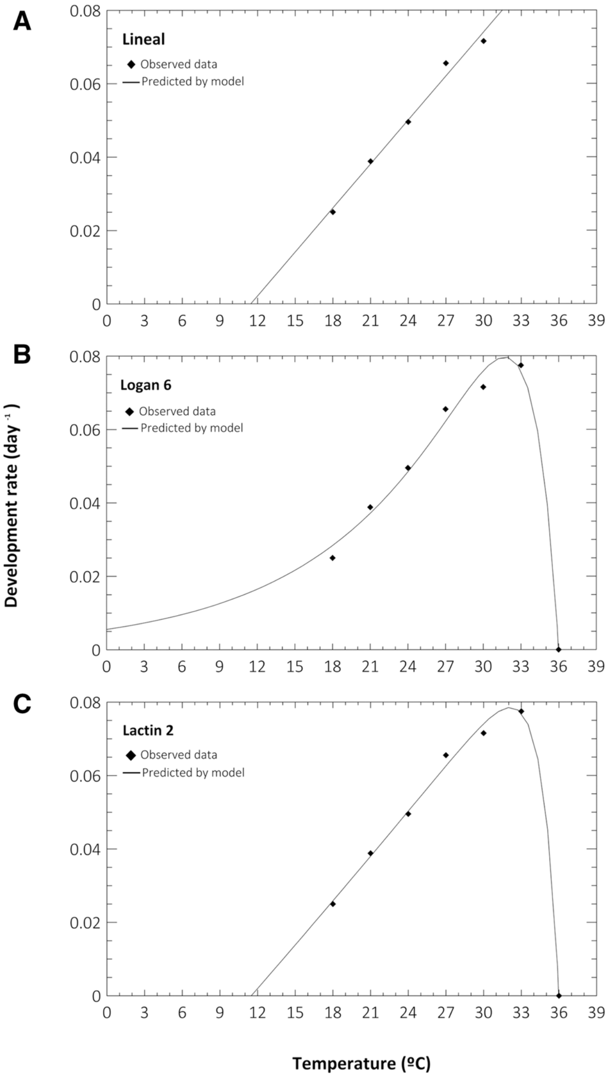


Fig. 1 Development rate fitted into 1 linear (A) and 2 nonlinear (B, C) development regression models.

Using development data from temperatures ranging from 18 to 30 °C, the linear model provided a good description for the relationship between temperature and development rate of *A. aberiae* with an adjusted R^2 of 0.98. The development rate of *A. aberiae* at temperatures between 18 and 30 °C was a positive linear function of temperature (Fig. 1A) ($y = 0.00399323x - 0.0457572$; $F = 213.41$; $df = 1, 4$; $P < 0.001$). The lower temperature threshold (T_{min}) was estimated at 11.46 °C and the thermal constant (k) was 250.42 DD.

The linear model fitted the linear part of the curve from 18 to 30 °C and predicted a lower temperature threshold (T_{min}) but failed to estimate the optimal (T_{opt}) and upper temperature threshold (T_{max}); therefore, we estimated these parameters using nonlinear models with data from all temperatures tested (18 to 36 °C). Of the 9 nonlinear models evaluated, 3 models were selected based on their goodness of fit to the observed data (Table 1). Wang's model gave an accurate estimate on the upper threshold and optimal developmental temperature (36 and 32.3 °C, respectively; Table 1) but failed to represent the linear part of the curve at lower and intermediate temperatures. Both the Logan 6 and Lactin 2 models provided an excellent fit to the whole dataset, as both had low RSSs values ($RSS < 0.00005$). The AIC value was lower for the Lactin 2 model than for the Logan 6 one (−82.44 and −74.22, respectively), indicating a better fit. In both models, development is steadily linear until nearing 33 °C, after which the curve shows an abrupt decline when the zero development is reached at 36 °C (Fig. 1B and C). Both Logan 6 and Lactin 2 models predicted a T_{max} of 36 °C. The Logan 6 model estimated a T_{opt} of 32.01 °C, while Lactin 2 estimation was 32.1 °C. The Lactin 2 model was able to estimate the lower development threshold at 11.5 °C. Differences between the mean development times predicted by the models and the actual mean development times observed varied from 0 to ± 1.72 d in all cases except for the Logan 6 model at the lowest temperature (18 °C), where the difference was −4.76 d (Table 4).

Discussion

This study is the first to evaluate the development and behavior of the newly introduced parasitoid *A. aberiae* under different temperature regimens. The development and survival of insects are strongly linked to environmental temperature, and physiological processes tend to cease above and below thermal thresholds. The relationship between these biological aspects and constant temperature has been observed in many hymenopteran

Table 4. Observed mean development times ± SE (d) compared to times predicted by the linear, Logan 6 and Lactin 2 models for *A. aberiae*.

Temperature (°C)	Observed development time (d)	Predicted development time (d)			Predicted-observed		
		Linear	Logan 6	Lactin 2	Linear	Logan 6	Lactin 2
18	40.00 ± 0.19	38.28	35.24	38.67	−1.72	−4.76	−1.34
21	25.77 ± 0.22	26.25	26.91	26.31	0.48	1.15	0.54
24	20.19 ± 0.12	19.97	20.65	19.89	−0.22	0.46	−0.29
27	15.26 ± 0.10	16.11	16.07	15.98	0.85	0.81	0.71
30	13.98 ± 0.12	13.51	13.16	13.49	−0.47	−0.82	−0.48
33	12.91 ± 0.07	–	13.16	12.99	–	0.02	−0.16

parasitoids including encyrtids (Avidov et al. 1967, Daane et al. 2004, Chong and Oetting 2006, Milosavljević et al. 2019). Organisms are unable to develop below a critical minimum temperature. As the temperature rises, their development rates increase peaking at an optimal temperature. Beyond this point, development declines and eventually ceases when the lethal maximum temperature is reached. The experimental temperatures used in our study were designated based on a range of predominant conditions in citrus-producing regions of Valencia (Spain). These temperatures are of biological importance, and they represent realistic exposure conditions for the parasitoid. Mediterranean climatic data indicate that temperatures in eastern Spain generally remain within the thermal tolerance range observed for *A. aberiae*, allowing for its survival throughout the year. During the coldest months (December and January), mean temperatures are around 12 °C, while in the hottest months (July and August), they average 27 °C. Although extreme temperatures that could disrupt parasitoid development (ie, 36 °C) may occasionally occur, such short-term events are not expected to significantly affect development, as daily mean temperatures remain within *A. aberiae*'s tolerable range. Furthermore, under field conditions, insects are exposed to fluctuating temperatures, allowing them to acclimate to conditions beyond their tolerable thermal limits, which may enhance survival under extreme temperatures (Romoser 1981).

Our results show that the development time of *A. aberiae* decreased with increasing temperature from 18 to 33 °C, but ceased at or above 36 °C indicating a positive correlation up to a critical threshold. These data are consistent with those reported by other authors regarding the *Encyrtidae* family. Avidov et al. (1967) and Daane et al. (2004) reported the successful development of *Anagyrus pseudococci* at 20 to 32 °C and 14 to 34 °C, respectively. Likewise, Mani and Krishnamoorthy (1992) observed the development of *Anagyrus dactylopii* at a range of 20 to 35 °C, while Power et al. (2020) reported that *Ooencyrtus mirus* completed development at 18 to 34 °C.

The linear and nonlinear models used in this study were chosen based on their ability to estimate biologically relevant parameters with limited previous information, and because they closely fitted the data experimentally obtained. Of the 9 nonlinear models tested, Briere 1 and 2, Lactin 1, Ratkowski, Performance 2, and LRF failed to fit the dataset and were excluded from further analysis. The Wang model fitted the data of this study well, as indicated by the low value of RSS (<0.00002). However, Wang's adjustment at lower temperatures was poor, indicating that this model could not accurately describe the temperature-dependent development of *A. aberiae*. Both the Logan 6 and Lactin 2 models met all the criteria and predicted feasible temperature threshold parameters and therefore were the most suitable for analysis. The relationship between temperature and developmental rate is widely studied for estimating the thermal thresholds (Davidson 1944, Campbell et al. 1974, Wagner et al. 1984). Finding the lower thermal threshold of insects is fundamental to predict their behavior under various environmental conditions, such as their moment of appearance and population peaks at different field locations (Skendžić et al. 2021). The estimates of the lower developmental threshold and thermal constant for *A. aberiae* (11.46 °C and 250.52 DD) are similar to those reported for other encyrtids, such as *A. pseudococci* (11.6 °C and 223.5 DD; Daane et al. 2004), and *Anagyrus* sp. nov. *sinope* (11.1 °C and 222.2 DD; Chong and Oetting 2006). Similarly, the estimated of upper thermal threshold (T_{max}) for *A. aberiae*'s at 36 °C is consistent with values observed in other parasitoids of the *Encyrtidae* family (Daane et al. 2004, Chong and Oetting 2006, Milosavljević et al. 2019). Both Logan and Lactin models gave comparable predictions for T_{max}, which aligned with the temperature experimentally observed in the laboratory. Regarding the optimal development temperature (T_{opt}) for *A. aberiae*, both models produced similar estimates, predicting values close to 32 °C. Additionally, mean development times predicted by the linear and Lactin 2 models between 18 and 33 °C closely matched the experimentally observed values (Table 4), indicating a good fit of these models.

The development of endoparasitoids, including *A. aberiae*, is strongly linked to the development of their hosts (Hentz et al. 1998). Hence, knowledge of developmental threshold temperatures and the thermal constant of both insects is also crucial to predict their performance within a specific environment. In this study, we estimated that *A. aberiae* completes between 9 and 10 generations per year in eastern Spain, based on local meteorological data. Preliminary results in our laboratory show that *D. aberiae* mealybug has a longer development time than the parasitoid and completes between 4 and 5 generations per year (unpublished data). A larger number of generations of a parasitoid with respect to the host might be potentially beneficial for its role as a biological control agent (Daane et al. 2004, Pandey and Johnson 2006). Therefore, *A. aberiae* could complete 2 generations for every one of the mealybugs, which allows for a more rapid population growth of the parasitoid, provided there is an available mealybug population.

In addition to development time, temperature had a significant effect on the duration of the adult lifespan and fecundity of *A. aberiae*. We observed a maximum fecundity peak of *A. aberiae* at intermediate temperatures, in contrast to the lower values exhibited at temperature extremes of 18 and 33 °C. Lifetime fecundity of parasitoids often shows a dome-shaped relationship with temperature, where fecundity rises from a low temperature, peaks at a medium temperature, and then falls again at a high temperature. In accordance with our results, *A. aberiae* followed this pattern. The sex ratio of the progeny varied significantly with temperature regimes, showing a higher proportion of females emerging at intermediate temperatures (24 °C), in contrast to a male-biased sex ratio at extreme temperatures (18 and 33 °C). Previous studies on other parasitoids have also shown reduced fecundity and a larger proportion of male offspring under extreme temperatures (Chong and Oetting 2006, Zhang et al. 2019, Yi et al. 2020).

This study provides the first insight into the biology of the recently introduced parasitoid *A. aberiae*. Temperature is a critical factor for parasitoid development and its performance as a biological control agent. Developmental thresholds, as well as the thermal constant, are useful tools to evaluate the suitability of a parasitoid introduced to a new ecosystem. The total developmental and survival period of *A. aberiae* ranged from 58.15 and 19.27 d at temperatures from 18 to 33 °C, respectively. This result suggests that higher temperatures shorten the immature development time and adult longevity of *A. aberiae* while lower temperatures have the opposite effect. Consequently, rearing the parasitoid at high temperatures would accelerate the development and emergence of *A. aberiae* adults. However, the maximum peak of fecundity at intermediate temperatures combined with the highest proportion of female progeny suggests that moderate temperatures are more suitable for *A. aberiae* population growth. Our study shows that *A. aberiae* can develop and survive within the tested temperature range of 18 and 33 °C suggesting that the parasitoid would indeed be suitable for the temperature range common in Spanish citrus fields. These aspects are of vital importance and must be taken into consideration when mass-rearing *A. aberiae* in the laboratory and releasing the parasitoid in the field.

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Author Contributions

Elena Romero (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal]), Marco Benito (Data curation [equal], Investigation [equal], Methodology [equal]), and Antonia Soto (Conceptualization [equal], Data curation [equal], Funding acquisition [equal], Methodology [equal], Supervision [equal], Validation [equal], Writing—review & editing [equal])

Supplementary Material

Supplementary material is available at *Journal of Economic Entomology* online.

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Conflicts of Interest

The authors declare no conflict of interest.

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