Reproduction of Pea Aphids (*Acyrthosiphon pisum*) in Response to Autocorrelated Temperatures

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Abstract:

Both mean and variation in temperature have profound effects on ectotherms. Thermal Performance Curve is asymmetrical (left-skewed), and it is sensitive near hot extreme. Positive autocorrelation in temperatures may trigger negative effects on aphids due to elongated duration of hot extreme condition. However, most studies on pea aphids (Acyrthosiphon pisum) were based on mean temperatures. Will positive autocorrelation have additional effects on the performance of pea aphids? Does the sequence of extreme temperatures matter? In this research, given the same mean temperature of 27 °C and same variance range. I hypothesized that 1) Strong, positive autocorrelation in temperature will decrease the reproductive outputs of pea aphids compared with no autocorrelation in temperature. 2) Different sequences of extreme temperatures will have significant difference in terms of the results. Even aged aphids were put into incubator for 5 days under temperature treatments. Unfortunately, both hypothesis were rejected due to large p-values. Significance and possible explanation to this results were discussed.

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Introduction:

Ectotherms are organisms that highly depend on the external source of temperature to control their body heat (Angilletta et al., 2002). The body temperature of ectotherms may have profound effects on speed, age at maturity, and reproduction (Angilletta et al., 2002). Pea aphids, *Acyrthosiphon pisum*, are studied widely in the laboratory environment because of their rapid life cycles. Like other ectotherms, the mean temperature experienced by pea aphids would have direct effects on their life history (Lu & Kuo, 2008; Ahn et al., 2020; Mastoi et al., 2020). Nonetheless, variation in temperatures occurs frequently in the nature. Therefore, it is of importance to understand how such factors will affect the life history of pea aphids in order to understand such species better for future researches.

Like most ectotherms, the relationship between mean temperature and performance (development time, reproductive inputs, body size, etc.) of pea aphids is always non-linear. Huey and Stevensen et al. (1979) suggested that a typical thermal performance curve, which relates a metric of ectotherm life history to temperature, would skew to the left (Figure 1). Since the thermal response of ectotherms is asymmetrical, and the optimal temperature for their best performance is located at the right part of the curve (i.e. relatively high temperatures). Additionally, once the temperature reaches the optimal, there will be a sudden drop with aspect to

performance, which indicates that hot extreme temperatures may be more sensitive than cold extreme temperatures to performance (Martin & Huey, 2008).
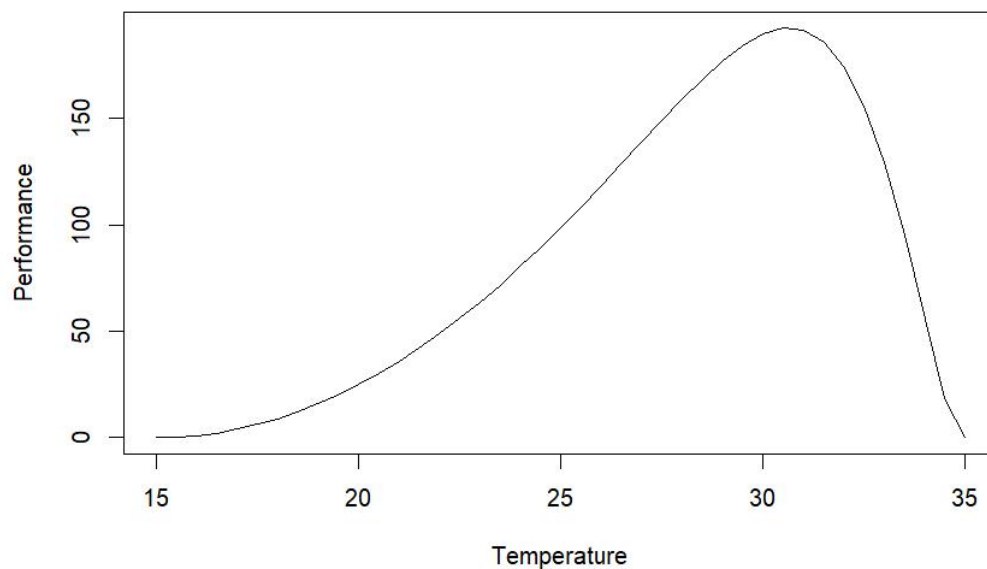

Figure 1. A normal form of thermal performance curve of ectotherms based on Huey & Stevensen (1979)'s suggestion. The equation was based on equation from Ratkovsky et al. (1983).

Such relationship has been confirmed by several studies. Lu & Kuo (2008) found that both the development time and longevity of pea aphids decreased when temperatures increased from 10°C to 35°C, and they indicated that the optimal range is from 20°C to 25°C. This applies to the results that the intrinsic rate of increase was the highest 25°C but decreased to negative at 30°C (Ahn et al., 2020). In addition, Mastoi et al. (2020) stated that the optimal temperature for the pea aphids was at 27°C, and the population growth of pea aphids increased from 27°C to 36°C then declined at 39°C.

Overall, all three papers proved that the relationship between temperature and the performance of pea aphids is non-linear.

However, other than mean temperature, several essential factors can also influence the performance of ectotherms. For instance, frequency distribution, especially the variance, might drive different estimations in biology (Ruel & Ayres, 1999). For another, a sine wave fluctuation would decrease the growth rate of duckweed, *lemna minor*, compared with that in constant temperature (Novich, 2012).

Autocorrelation, another important factor discussed frequently in the recent researches, describes how the deviations from the mean are correlated among each other in a temporal or spatial interval. A stronger, positive autocorrelation indicates that the deviations are more likely to be grouped together on the same side above or below the mean (i.e. the direction of deviations are the same). In contrast, a stronger, negative autocorrelation implies that the deviations are more likely to be followed by a deviation in a different direction (i.e. jump up and down the mean). Environmental factors such as temperature are usually positively autocorrelated with time (Steele, 1985). This means that extreme temperatures will be more likely to group together even after detrending the temperature series (i.e. not following the trend). Groupings of extreme temperatures may have additional effects on the performance since the duration of similar temperatures is long enough for them to respond. Thus, increasing

the duration of such unfavourable conditions may exaggerate the existing effects on ectotherms.

Lee (2015) found that positive autocorrelation around optimal temperatures would have a significantly negative impact on the nematode, *Caenorhabditis elegans*, in terms of offspring number, maturation time, and body length. Chaves et al. (2014) found increased mortality of mosquito, *Aedes aegypti*, in autocorrelated environments. Di Cecco & Gouhier (2018) proposed that the risk of extinction might have been elevated due to increased duration and magnitude of unfavourable conditions in temperature. However, these studies might overlook the fact that extreme temperatures can occur at either the beginning or the end in a strongly, positively autocorrelated temperature series. In this sense, the sequence of autocorrelated temperatures may have different effects on the ectotherms. For example, extreme high temperatures could cause a delay in outbreak (sudden increase in abundance) of mosquitoes (Chaves et al., 2014).

All in all, it seems like that autocorrelation in temperatures may be a great influencer in the performance of pea aphids. Therefore, in this study, I will focus on how positive autocorrelation will affect the reproduction of pea aphids. As described above, temperatures higher than optimal may have a greater negative impact on performance, and the increased duration of such condition may magnify such impact. Therefore, my

prediction is that, based on the same mean temperature at optimal (27°C), 1) positive autocorrelation will significantly decrease the reproductive outputs of pea aphids compared with the aphids experiencing no autocorrelation in temperature. 2) the sequence of extreme temperatures occurred will have significant difference on the reproductive outputs.

Methods:

I examined the thermal responses of pea aphid (*Acyrthosiphon pisum*). This species is an model study target because the development time of pea aphids is relatively short (5 to 20 days) (Lu & Kuo, 2008), and they can reproduce both sexually and asexually (Simon et al., 2002). Laboratory populations of aphids were maintained on peas (*Pisum sativum*) and fava (*Vicia faba*). 3-week old fresh plants were provided every week as habitat and food.

To measure the impact of temperature autocorrelation on aphid thermal responses, I used the reproductive output of adult aphids exposed to thermal regimes with differing autocorrelation regimes, but at the same mean temperature of 27°C. There were 3 treatments of autocorrelation regimes in the experiment: 1) no autocorrelation (alpha = 0) with mean temperature at 27°C (Appendix 1. Treatment 1 sample temperature profile), 2) strong, positive autocorrelation (alpha = 0.95) with mean

temperature at 27°C and warmer than average temperature aggregated in the first part of the sequence (Appendix 2. Treatment 2 sample temperature profile), 3) strong, positive autocorrelation (alpha = 0.95) with mean temperature at 27°C and colder than average temperature aggregated in the first part of the sequence (Appendix 3. Treatment 3 sample temperature profile). Ten replicated temperature sequences for each treatment were created using R (R Core Team, 2013) where the autocorrelated temperatures were generated using the equation (Appendix 4. Equation to generate alpha) by R (R Core Team, 2013). In this case, all control sequences sent to the incubators had same mean temperature, standard deviation, and autocorrelation coefficient.

For the experiment, fresh pea plants, 1 to 2 weeks old, were prepared as host plants for aphids since the reproductive output is related to the nutrition acquired from host plants (Bouvaine et al., 2012). In addition, in order to keep the even age of pea aphids used for experiment, one week prior to starting date, 3 adult aphids were transferred to new host plants and reproduced under the constant temperature at 20°C. After 24 hours, adult aphids were removed, and the nymphs would develop for a week at the same temperature of 20°C. After the nymphs developed into adults, each one of them was transferred to a new host plant. 3 plants with 3 aphids were counted as a replicate and incubated in Memmert incubators (IPP-500) for 5 days. At the end of the experimental period, the number of offspring was counted.

Temperature sequences were sent to the incubator using the manufacturer's software by Celsius (Version 10.1.6). Light in the incubator was provided by Enover Plug-in Digital Outlet Timer from 6 a.m. to 10 p.m. every day to provide 16 hours of light and 8 hours of darkness. The temperature records were collected and saved as excel file type. They were all save on the GitHub website and were retrieved directed by R. Then, one way ANOVA test ("anova" function in R) was used to determine if there were significant differences in the number of offspring in each of the 3 autocorrelation treatments. I expected the p-values, that were greater than 0.05, to support my hypothesis 1 & 2. Otherwise, they would fail to prove them if the p-values were smaller than 0.05.

Results:

For a better indication on the results, we combined the total number of nymphs on each replicate (3 plants), and it is defined as the reproductive outputs. I expected the reproductive outputs to be lower in the high autocorrelation (alpha = 0.95) treatment than the no autocorrelation (alpha = 0) treatment. In addition, the reproductive outputs in highly autocorrelated treatments with colder temperatures coming first would be higher compared with treatments with warmer temperatures coming first. However, there was no significant difference in reproductive outputs at 27°C between each of the three autocorrelation treatments (Figure 2.) (p=0.666, F=0.412, df=2).
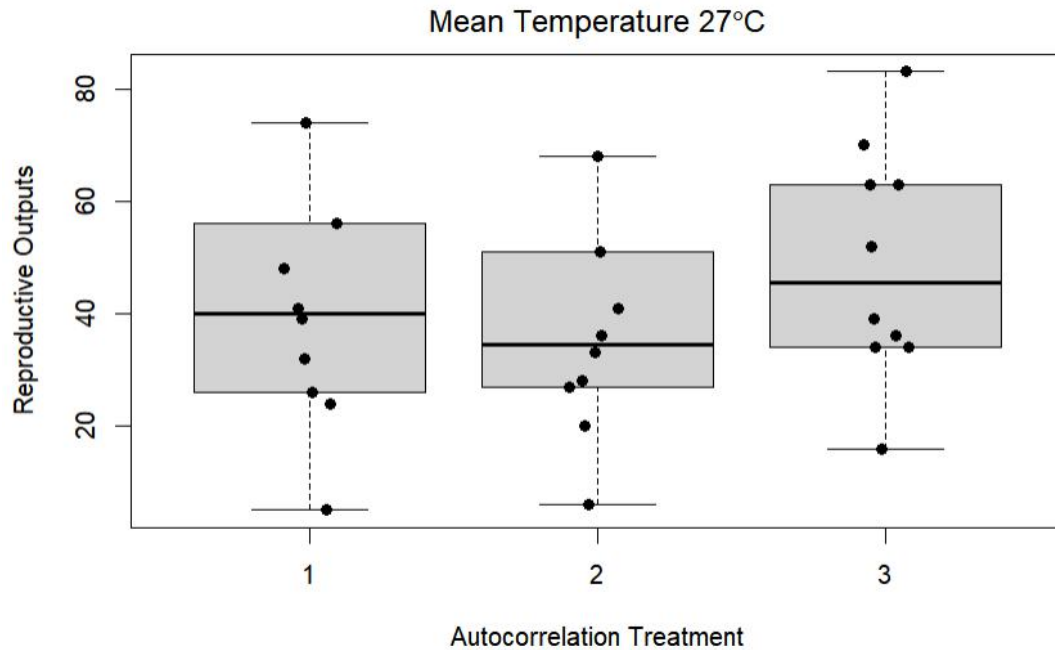
Figure 2. Total offspring for each replicate at 27°C in each autocorrelation treatment (1 = no autocorrelation, 2 = strong, positive autocorrelation (0.95) with warmer temperatures coming first, 3 = strong, positive autocorrelation (0.95) with colder temperatures coming first). Horizontal Line represents the mean reproductive outputs. Summed offspring is the total number of nymphs in each replicate (3 plants).

Discussion:

I conclude that strong, positive autocorrelation did not decrease the reproductive outputs of pea aphids at the mean temperature of 27 °C, compared with no autocorrelation. In addition, when warm extreme temperatures came first, high autocorrelation did not have significant effects on decreasing the reproductive outputs of pea aphids at the mean temperature of 27 °C, compared with treatments with warm extreme temperatures came in the second part of time series.

One possible reason that I did not find a significant difference in the mean number of offspring in each treatment could be due to observation errors. It was difficult to count the aphids' nymphs accurately at the end of each experiment, especially when the number was over 20 on each plant. In addition, we found that aphids like to hide beneath the leaves, which inevitably increased the difficulty of having a an accurate count. There was also a potential for damage and loss of aphids during the experimental setup and take down.

Another potential issue is the microclimate under leaves. Insects can shelter in the microclimate where temperature is distinct from air temperature (Vermunt et al., 2012). Like I observed in the laboratory before, pea aphids preferred to hide under leaves where both light and temperature could be lower than the temperature manipulated by the incubator. If this was the case, it would be less likely to have accurate effects of autocorrelated temperatures on the reproductive outputs since the duration of unfavorable conditions (above 31 °C) was not long enough as expected. Lu & Kuo (2008) found no reproduction of nymphs at the constant temperatures of 30 °C and 35 °C. Therefore, the temperatures pea aphids experienced in the incubators might not fall under such range so that results gained little difference between each treatment.

For future research, I would like to suggest increasing both the variance and mean of the temperature tested. This could make the hottest temperature high enough to reach the stage that pea aphids cannot reproduce in order to gain more obvious results. Additionally, the short experimental time, 5 days in my experiment, could bring potential fluctuation and uncertainty to the results. Usually, pea aphids need 5 to 10 days to develop into adults (Lu & Kuo, 2008). In this sense, it could be good to increase the time length to 10 days to make sure pea aphids can all be at adult stage.

In conclusion, I found non-significant relationship between autocorrelation in mean temperature at 27°C and the reproductive outputs of pea aphids. It is of importance but difficult to understand such relationship. This experiment could be considered for studies involving temperatures in the future.
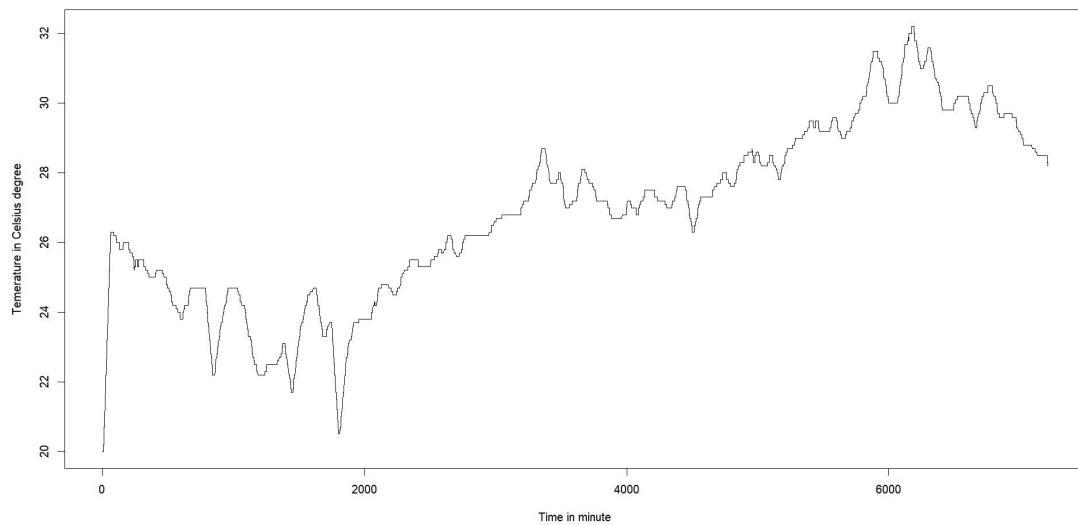
Appendix:

Appendix 1. Sample temperature profile of treatment 1: no autocorrelation (alpha = 0) with mean temperature at 27°C.
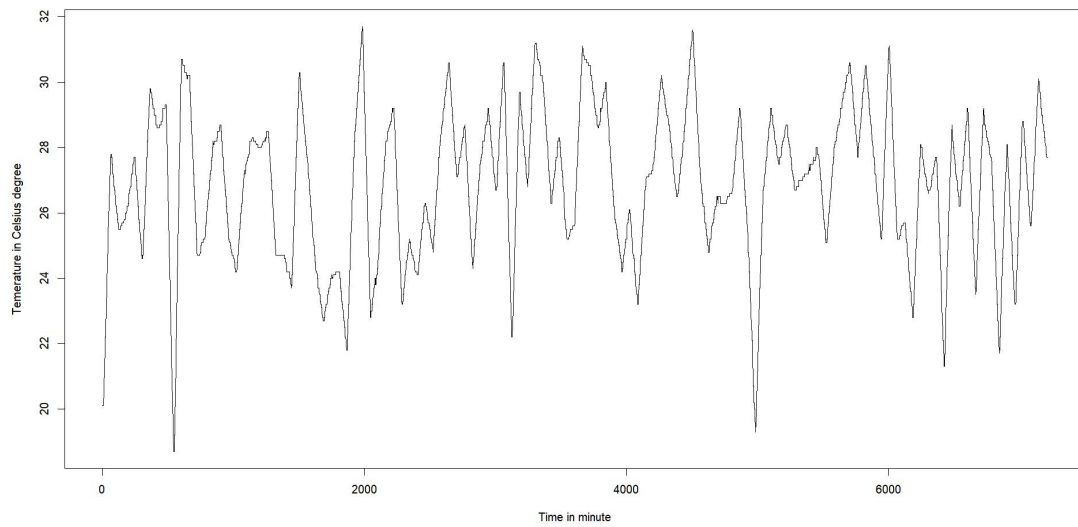
Appendix 2. Sample temperature profile of treatment 2: strong, positive autocorrelation (alpha = 0.95) with mean temperature at 27°C and warmer than average temperature aggregated in the first part of the sequence.
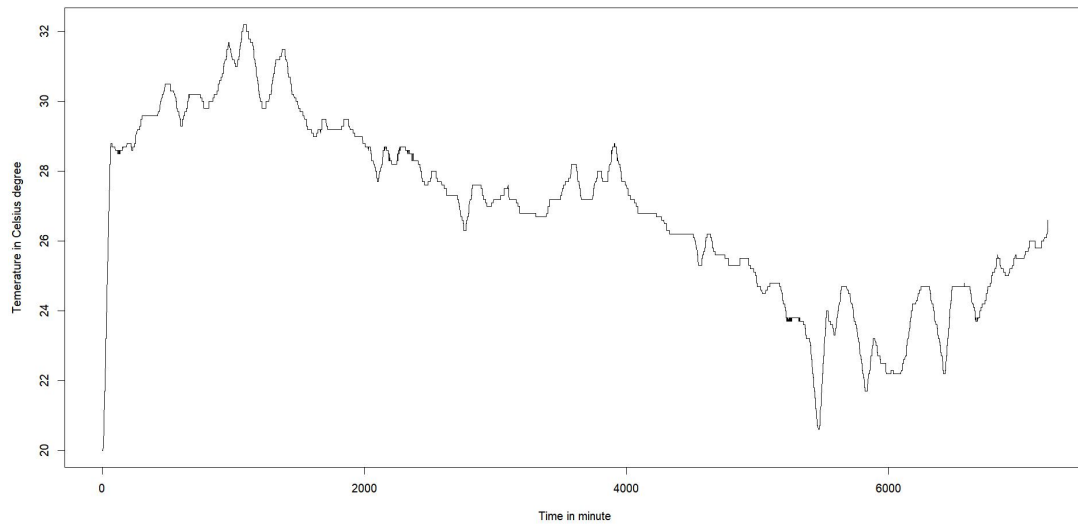
Appendix 3. Sample temperature profile of treatment 3: strong, positive autocorrelation (alpha = 0.95) with mean temperature at 27°C and colder than average temperature aggregated in the first part of the sequence.

Appendix 4. Equation to generate alpha (autocorrelation coefficient).

The code below was used to generate autocorrelation coefficient when we generated temperature profile for autocorrelation treatments. This is written by Dr. Kim Cuddington.

```
#generate sequences with desired degree of autocorrelation (using Ar(1) process) within given
# error tolerance
lbet=c(0,0.6,0.99)
tol2=0.01;
gs=vector('double',leg)
tseq=matrix(NaN, leg,length(lbet)+1)
acz=vector('double', length(lbet))
for (j in 1:length(lbet)) {#loop1
acoef=lbet[j];
ac=-99
while ((ac<(acoef-tol2))||(ac>(acoef+tol2))) {#loop2
```

```
gs[1]=rnorm(1,0,1);

crand=rnorm(leg, 0,1);

for (cnts in 2:leg) {#loop2

gs[cnts] =acoef*gs[cnts-1]+crand[cnts]*sqrt(sdev)*sqrt(1.-acoef^2);

} #loop3 through desired sequence length

ac=acf(gs,1)$acf[2]

ac

} #loop2 until generate sequence with desired autocorr within tolerance

z=mimicry(noise,gs)

acz[j]=acf(z,1)$acf[2]

tseq[,j]=round(z,1)

} #loop1 through autocorr values
```

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