“Can global warming spread your wings? Temperature has a surprising effect on butterfly wing length”.

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2023-05-02

Introduction

The Silver-spotted Skipper (*Hesperia comma*) is a butterfly species found in the United Kingdom. *Hesperia comma* resides in southern England, considered a rare species in the UK (Butterfly Conservation, n.d.). The species occurs in discrete colonies on open, sunny, short chalk grassland, where their larval foodplant, Sheep’s Fescue (Festuca ovina), grows next to patches of bare ground (UK Butterflies, n.d.). However, in recent years, the Silver-spotted Skipper has extended its range to previously unoccupied sites and has been observed egg-laying in short grassland despite the absence of bare ground, as a response to a warmer climate and microclimate in the areas where it breeds

The butterfly’s declining status resulted from the widespread reduction of sparse, short-turfed calcareous grassland containing the species’ sole larval host plant, sheep’s fescue grass Festuca ovina L. (Thomas et al., 1986). By 1982, rabbits had recovered, but the *Hesperia comma* population had not recolonized former sites. • Between 1982 and 1991, the number of populated habitat patches increased by 30% in the South and North Downs, primarily within East Sussex. (Thomas and Jones, 1993).

Studies have reported that *Hesperia comma* changes habitats in association with climate change – finding that the optimum percentage of bare ground within the habitat used for egg-laying shifted from 41% to 21% due to climate change (DAVIES et al.). The study also found that females adjust microhabitat usage in response to temperature variations. Climate warming has increased the availability of thermally suitable habitat for *H. comma* at the cool, northern edge of the species distribution, potentially increasing the rate of population increase and the number of habitat patches in the landscape currently available for colonies. In addition, another study found that temperature variation during different life stages of a butterfly species can affect changes in body size and phenology. Specifically, June temperatures affect adult body size, while July temperatures affect the timing of adult emergence. The study also suggests that August temperatures, which act on the adult stage, correlate to range change. (Fenberg et al.).

Analysis

This study follows up upon those findings, investigating how environmental changes, particularly temperature in June, affected the species size as a direct result of climate change. I used an ordinary least squares linear model to analyse forewing length, with June mean temperature as the numerical predictor. The best-fitting model explaining forewing length included sex as the interaction term. R version 4.2.3 was used . Additionally, This study uses R packages: tidyverse, for data importing, data tidying, exploratory data analysis, and data wrangling; ggpubr for visual model summaries; ISLR to spot anomalies; ggExtra to display additional graphics; KableExtra to produce model tables and ggthemes for ggplot themes; colorblindr to test for colour blindness; and sjplot to create model summary tables; webshot2 to produce a visual model summary table images; knitr and rmarkdown to produce a Rmarkdown document. I obtained the data by collecting museum samples of the Silver Spotted Skipper and comparing them against the measured weather data of each year.

To analyse the data, I used Cook’s distance transformations to remove outliers from the linear model from the male butterfly dataset. I also used box Cox plots and model-checking plots to assess each model fit. I used the Pearson correlation coefficient test to assess the strength and significance of each linear model.

I checked for normality within the datasets as sample sizes were 28 observations for males and 30 observations for Females. Using a Shapiro test, I found that forewing length was normally distributed in males (95% CI; W = 0.98003, p-value = 0.851) and female forewing length was also normally distributed (95% CI; W = 0.96938, p-value = 0.5223). The table below shows the dataset

year	forewing_length	sex	june_mean_temperature	june_mean_rain
1880	14.64700	female	13.8	87.9
1892	14.35800	female	13.4	83.1
1892	13.06400	male	13.4	83.1
1896	14.70400	female	16.2	54.7
1898	14.71200	female	13.6	44.9
1899	13.77100	male	15.7	37.7
1903	13.62875	female	13.0	55.3
1904	14.23375	female	13.3	14.1
1904	12.79400	male	13.3	14.1
1905	14.40100	female	14.7	69.9
1905	13.07200	male	14.7	69.9
1906	14.51800	female	14.3	74.2
1906	12.76975	male	14.3	74.2
1907	12.01750	male	12.4	55.6
1908	14.08700	female	14.3	37.7
1908	12.54475	male	14.3	37.7
1909	14.15150	female	11.8	81.8
1911	14.38275	female	14.5	72.1
1914	13.06300	male	14.5	577.0
1916	13.77950	female	11.8	60.9
1916	12.35775	male	11.8	60.9
1917	14.43675	female	15.2	47.8
1917	12.86975	male	15.2	47.8
1920	13.70350	male	14.4	56.2
1923	14.39150	female	12.5	16.5
1924	14.49050	female	13.9	48.8
1924	13.23900	male	13.9	48.8
1925	13.93600	female	15.0	5.8
1927	12.29125	male	12.6	96.6
1928	12.62225	male	12.9	70.4
1929	13.06975	male	13.3	30.3
1932	12.65150	male	14.1	15.4
1936	14.10500	female	14.7	89.2
1937	14.78500	female	14.1	32.0
1937	13.21400	male	14.1	32.0
1938	13.46250	female	14.4	31.7
1939	13.26450	female	14.2	52.1
1939	12.93150	male	14.2	52.1
1941	14.03550	female	15.1	22.7
1941	13.81450	male	15.1	22.7
1944	12.63875	male	13.5	47.1
1945	15.10550	female	14.6	57.6
1946	12.91700	male	13.1	70.8
1947	14.53250	female	15.5	46.5
1947	13.33400	male	15.5	46.5
1951	14.67700	female	14.0	30.8
1951	13.46425	male	14.0	30.8
1961	14.68750	female	14.4	28.9

1963	15.20025	female	14.9	62.0
1964	14.94650	female	13.8	77.8
1965	13.59350	female	14.7	51.0
1966	14.35675	female	15.4	73.2
1966	13.37425	male	15.4	73.2
1969	12.85775	male	13.9	39.4
1970	12.65775	male	16.4	27.1
1971	13.52750	female	12.4	71.5
1971	12.18450	male	12.4	71.5
1973	13.93875	male	14.8	76.1

*Figure 1: **Hesperia Comma dataset**. Data was obtained by analysing museum specimens in accordance with the climate they lived in.*

Results:

I hypothesised that forewing length would increase with mean June temperature because juvenile hormones, ecdysteroids, and other signalling molecules are critical for proper growth and development in insects, including butterfly genes that are involved in growth and development within butterflies (Jindra, Palli and Riddiford, 2013), as well as genes responsible for the insulin/insulin-like growth factor (IGF) signalling pathway, which are also involved in growth (Shingleton et al., 2007). To evaluate this hypothesis, I investigated how forewing length increases with temperature using an ordinary least squares model (OLS) with June mean temperature as a factorial predictor and forewing length as an ordinal scale. From this, I used a Pearson Correlation Coefficient test to assess the significance and strength of the OLS model. Across all butterflies, I found a significant, but weak positive correlation between June Mean Temperature and Forewing Length. This model suggests that for every 1 OC temperature increase, butterfly forewing length increases by 0.23 cm (95% CI 7.68 ~ 13.29; $p = 0.027$, figure 1). Results may derive from stimulated growth hormones in response to heat. Comparable results are observed in other species in Korea, including Lycaenidae (Celastrina argiolus), Nymphalidae (Polygonia c-aureum), Papilionidae (Atrophaneura alcinous, Papilio macilentus, Parnassius stubbendorfii), and Pieridae (Colias erate, Pieris canidia, Pieris dulcinea, Pieris rapae) (Na et al., 2021), showing that this is a general trend in molecular biology. However, results are inconsistent with studies finding size increases at lower temperatures for the copper butterfly Lycaena tityrus (Karl and Fischer, 2007). This suggests that although a trend, growth in response to temperature is not a universal law across all ectotherms. Results are shown in figure 2.

Butterfly Forewing length against June Mean Temperature

	forewing length		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	10.48	7.68 – 13.29	**<0.001**
june mean temperature	0.23	0.03 – 0.42	**0.027**
Observations	58		
R^2 / R^2 adjusted	0.085 / 0.068		

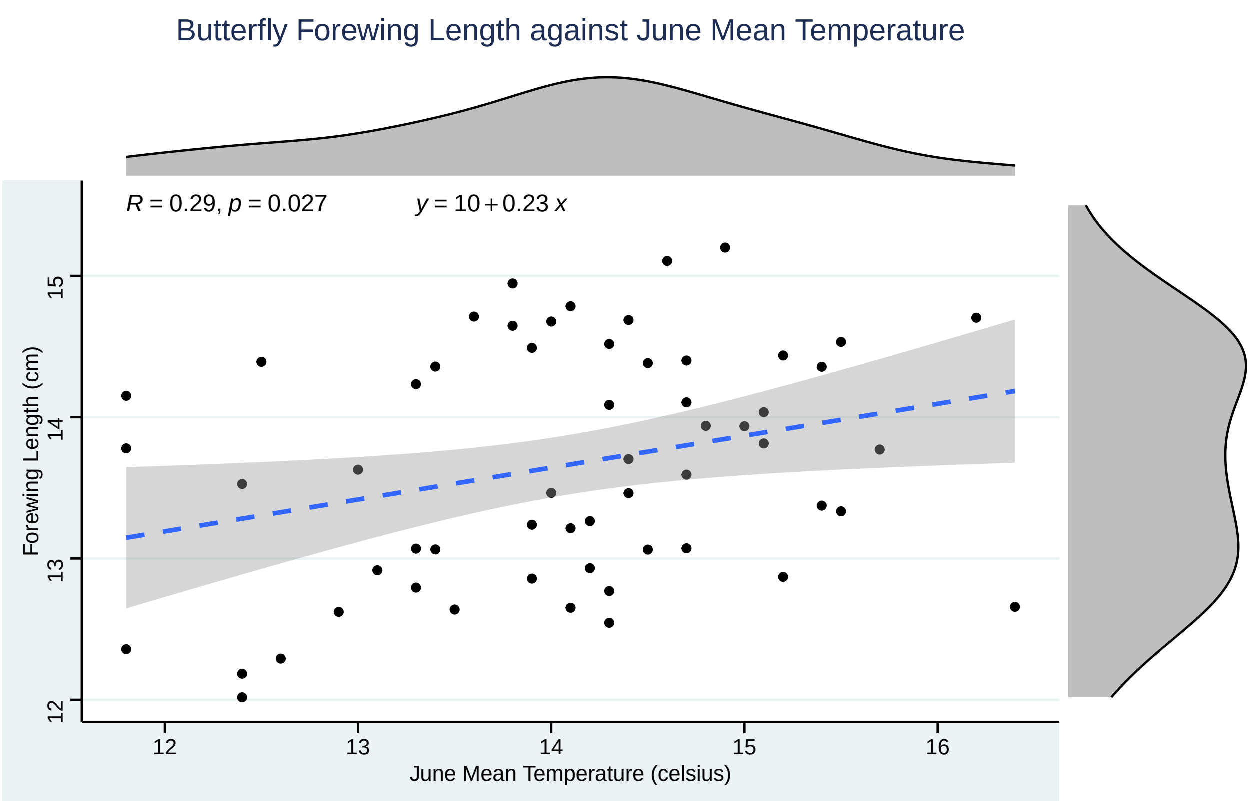

Figure 2: ***Butterfly forewing length increases with temperature****. Slope was fitted as temperature was the only significant contributor (Adj. R2 = 0.068). This model suggests that for every 1 OC temperature increase, butterfly forewing length increases by 0.23 cm (95% CI 7.68 ~ 13.29; p = 0.027). Circles indicate*

individual observations, the dashed line represents the line of best fit, shaded regions surrounding the line of best fit indicate 95% confidence intervals. Marginal curves indicate distributions across each variables.

Within male butterflies, I hypothesised a positive correlation between Forewing Length and June Mean Temperature. An OLS model was used to assess if correlation differences exist between Male and Female Silver-spotted skippers. A Pearson correlation coefficient test was then used to assess the strength and significance of the correlation between Forewing length and June mean temperature. The analysis showed a 0.37 cm Forewing length increase with every 1 OC increase (95% CI 0.2338876–0.5015223; $p < 0.001$, Figure 2). Similar results are observed in studies examining the effects of temperature on body size and mass of male and female butterflies in the genius Colias (Kingsolver, Diamond and Buckley, 2013). Results may derive from upregulated ecdysteroid or ecdysone genes in response to increased temperature. Trends are shown in Figure 3

Male Forewing length against June Mean Temperature. [-26] represents a removed outlier at the 26th datapoint

	forewing length[-26]		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	7.85	5.98 – 9.72	**<0.001**
june_mean_temperature[-26]	0.37	0.23 – 0.50	**<0.001**
Observations	27		
R^2 / R^2 adjusted	0.562 / 0.544		

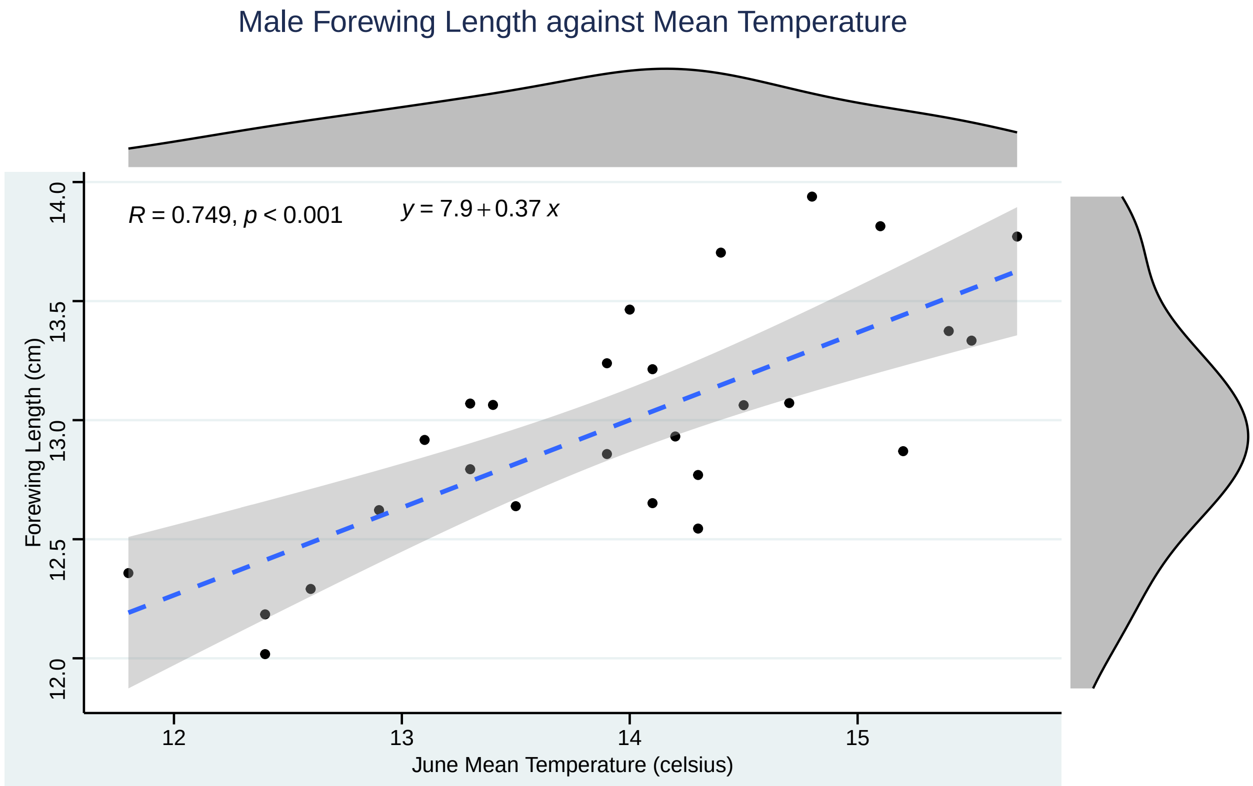

Figure 3: ***Male forewing length increases with temperature****. The fitted slope is from the reduced linear model (the only significant factor being June Mean Temperature) excluding outliers (Adj. R2 = 0.5441). This model suggests that for every 1-degree Celsius temperature increase, male forewing length increases by 0.37 cm (95% CI 0.2338876 ~ 0.5015223; p < 0.001). Circles indicate individual observations, the dashed line represents the line of best fit, shaded regions indicate regions of 95% confidence, and marginal curves indicate distributions for Forewing length and June Mean Temperature.*

I also hypothesised a positive relationship between female forewing length and June Mean Temperature. However, no statistically significant relationship was found between forewing length and June mean temperature in Female silver spotted skippers using a Pearson correlation coefficient test with an OLS model (95% CI; p = 0.142). These results are consistent with a study that found no relationship between environmental temperature and Forewing length (Johnson, 2022). This shows that this is a general trend in biology whereby female butterflies do not behave similarly to male butterflies. Trends are shown in Figure 4.

Female Forewing Length against June Mean Temperature

	forewing length		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	12.51	10.06 – 14.95	<**0.001**
june mean temperature	0.13	-0.05 – 0.30	0.142
Observations	30		
R^2 / R^2 adjusted	0.076 / 0.042		

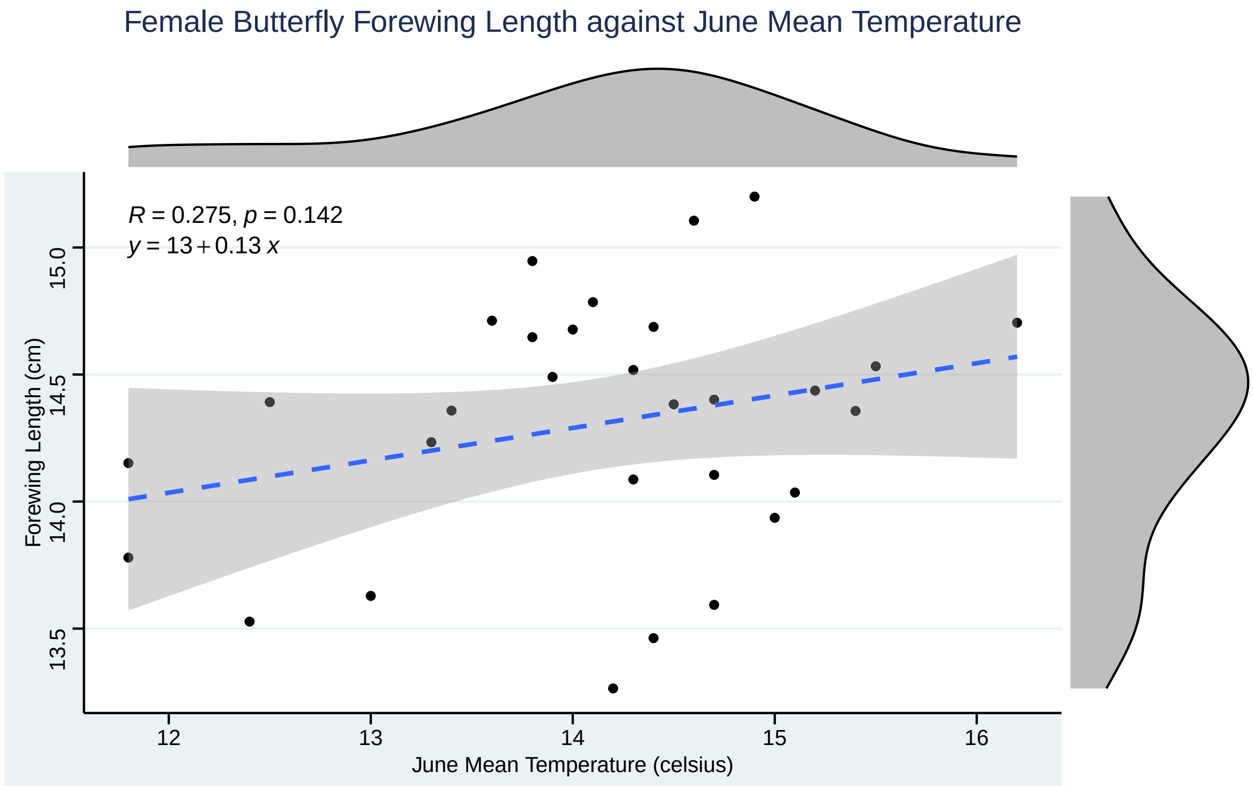

Figure 4: ***Non-significant positive relationship between Female Forewing Length and June Mean Temperature.*** *Female silver spotted skipper specimens was collected from museums and measured against the corresponding June mean temperature that they lived in. Slopes were fitted from the reduced linear model as the temperature was the only significant contributing factor (Adj. R2 = 0.07881) excluding outliers. However, no significant linear relationship exists between Female forewing length and temperature (95% CI -0.06937291 ~ 0.2545739; p = 0.244). Circles indicate individual observations, the dashed line indicates the line of best fit, and marginal curves display distributions for forewing length and temperature, respectively.*

Upon comparing the forewing length between Males and Females using a two-sample t-test, I found that Females, possessed a mean forewing length of 14.30458 cm (95% CI 14.124618 ~ 14.48455), whereas males had a mean forewing length of 12.97242 cm (95% CI -1.591178 ~ -1.07315), suggesting that females, on average, had a 1.332164 cm greater forewing length than males (95% CI; $t = 10.3$; $p < 0.05$). These results suggest that temperature in June induces the growth factors within the Hesperia comma. However, the same genes and signalling pathways responding to June temperature remain unclear. Further studies may reveal the mechanisms between light and growth factor genes. These results may be due to increased growth factor gene activity. Effects may also result from differences in growth factor activity between Males and Females, or the growth factor gene responding to temperature being present on the Y chromosome. Despite these findings, sample sizes were relatively low, and risks were unrepresentative towards the population. Results are shown in Figure 5.

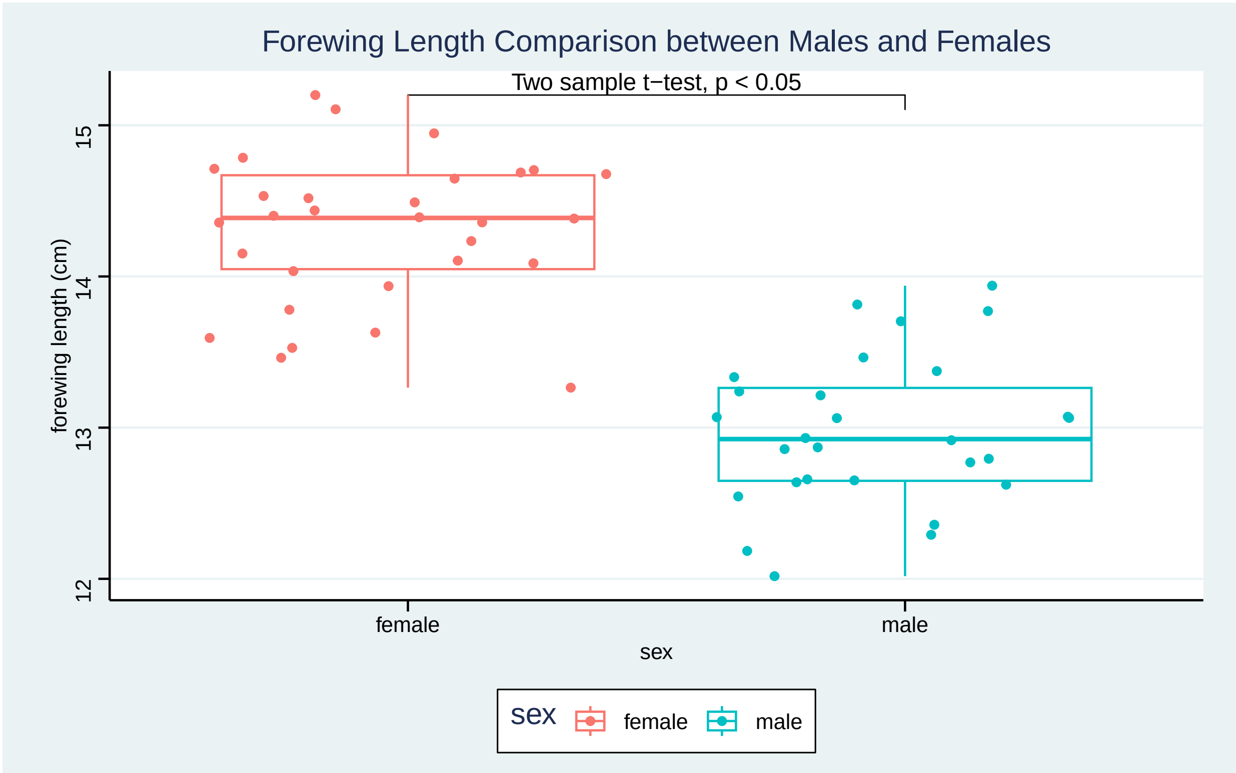

*Figure 5: **forewing length comparison between Male and Female Butterflies**. A two-sample t-test compared the mean forewing length between Male and Female butterflies. Females had a mean forewing length of 14.30458 cm (95% CI 14.124618 ~ 14.478455) whereas Males had a decreased forewing length of 12.97242 cm (95% CI -1.591178 ~ 1.07315; t = -10.303; p < 0.05). Circular points represent individual observations, blue colours represent male butterflies, red points represent female butterflies, and side curves represent the distribution of forewing Length for Male and Female Butterflies.*

Conclusion

Results show a weak but significant correlation between forewing length and June Mean Temperature across all butterflies. Results show a strong positive correlation between forewing length and June mean temperature in male butterflies. However, female butterflies do not share this - lacking a significant positive correlation. Results are consistent with studies suggesting that June temperatures affect butterfly body sizes (Fenberg et al.). This difference may result from upregulated growth factor genes, or the responsible growth factor genes being present on the Y chromosome. However, it is also possible that females already exhibited excessive growth factors, causing little response to temperature changes.

However, the data only measured mean temperatures and rainfall in June, failing to consider outliers or individual measurements annually. This reduces the available information, removing any understanding of the skewness within the data. Additionally, mean values are suspect to outliers... This results in data loss and decreased model accuracy. The data did not measure males and females for adjacent years, measuring at arbitrary intervals. Consequently, female butterflies had more data than male butterflies. This omission decreases model accuracy, placing bias towards female butterflies. Sample sizes were low, with male butterflies having twenty-eight samples and female butterflies having 30 samples. As such, the trends observed may be unrepresentative of the total population. The model assumes the relationship is linear, entailing the assumption of normality, linearity, and no multi-collinearity.

Despite this, further data and additional studies may reveal a substantial relationship between forewing length and June mean temperature in Female Butterflies.

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