

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

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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 (Lawson et al., 2013). 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 (Lawson et al., 2013). 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., 2006). 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., 2016).

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 only June mean temperature as the only significant contributor. 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 using cook’s distance; ggExtra to display additional graphics; GGally for scatter matrices; performance to create model checking plots; MASS to produce model checking boxcox plots; KableExtra and gt to produce model tables; 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; $W = 0.98003$, $p\text{-value} = 0.851$) and female forewing length was also normally distributed (95% CI; $W = 0.96938$, $p\text{-value} = 0.5223$). 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	57.7
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 a positive linear relationship between forewing length and mean June temperature across all *Hesperia comma* because juvenile hormones, ecdysteroids, and other signalling molecules are critical for proper growth and development in butterfly genes involved in growth and development (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, I used 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. Results show a significant, weak positive linearity between June Mean Temperature and Forewing Length. This model suggests that for every 1 OC increase, butterfly forewing length increases by 0.23 cm (95% CI 7.68 ~ 13.29; $p = 0.027$, figure 2b). Results may derive from stimulated growth hormones in response to heat. Comparable results are observed in other species, including Lycaenidae (*Celastrina argiolus*), Nymphalidae (*Polygonia c-aureum*), Papilionidae (*Atrophaneura alcinous*, *Papilio macilentus*, *Parnassius stubbendorffii*), and Pieridae (*Colias erate*, *Pieris canidia*, *Pieris dulcinea*, *Pieris rapae*) (Na et al., 2021), indicating a general trend in molecular biology. However, results are inconsistent with studies finding size increases at lower temperatures for *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. Plots shown in figure 2b.

Butterfly Forewing length against June Mean Temperature

<i>Predictors</i>	forewing length		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(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 2a: *Visual model summary for Hesperia comma Forewing length against June Mean Temperature across all samples.* Samples were placed through an OLS model to analyse body size variations with temperature, the only significant contributor. Predictors indicate the dependent variable (intercept = Forewing length) and independent variable (june mean temperature). CI indicated 95% confidence intervals. Observations indicate sample sizes. R² and R²-adjusted indicate the strength of regression, scaled between 0 ~ 1. Estimators indicate the mean estimate for each variable. *p* values indicate significance.

Butterfly Forewing Length against June Mean Temperature

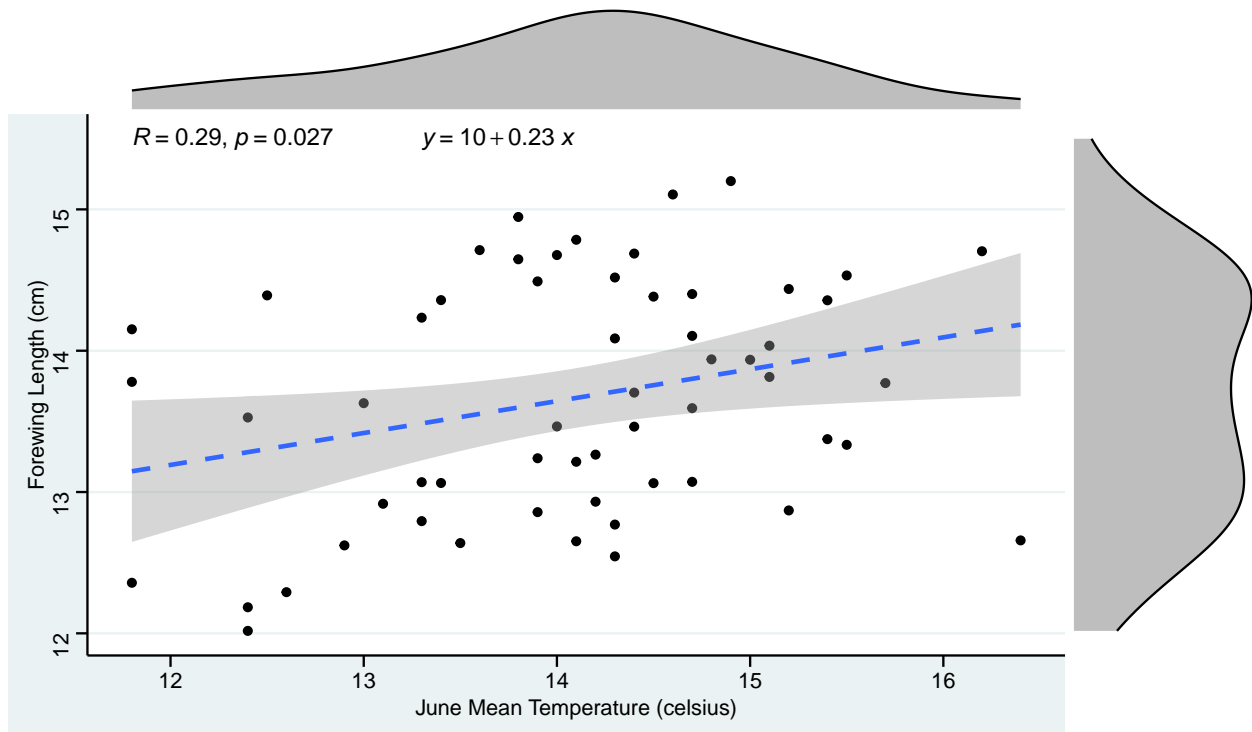


Figure 2b: *Hesperia comma* forewing length increases with June temperature. Slope was fitted as temperature was the only significant contributor (Adj. $R^2 = 0.068$). This model suggests that for every 1 OC temperature increase, butterfly forewing length increases by 0.23 cm (95% CI 7.68 ~ 13.29; $p = 0.027$). 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 density plot distributions for forewing length (right) and June Mean Temperature (top).

I hypothesised a positive correlation between Male 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 assessed the strength and significance of this correlation. The analysis showed a strong positive correlation, whereby a 1 OC temperature increase results in a 0.37 cm Forewing length increase (95% CI 0.2338876 ~ 0.5015223; $p < 0.001$; Figure 2; Adj. $R^2 = 0.544$). Similar results are observed in studies examining the effects of temperature on body size and mass of male and female butterflies in the genus *Colias* (Kingsolver, Diamond and Buckley, 2013). Results may derive from upregulated ecdysteroid or ecdysone genes in response to increased temperature. Trends shown in Figure 3b.

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

forewing length[-26]			
<i>Predictors</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(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 3a: **Visual model summary for Male Forewing length against June Mean Temperature.** Samples were placed through a reduced OLS model, whereby temperature was the only significant contributor. Predictors indicate the dependent variable (intercept = Forewing length) and independent variable (june mean temperature). CI indicated 95% confidence intervals. Observations indicate sample sizes. R2 and R2-adjusted indicate the strength of regression, scaled between 0 ~ 1. Estimators indicate the mean estimate for each variable. p values indicate significance. [-26] represents a removed outlier at row=26

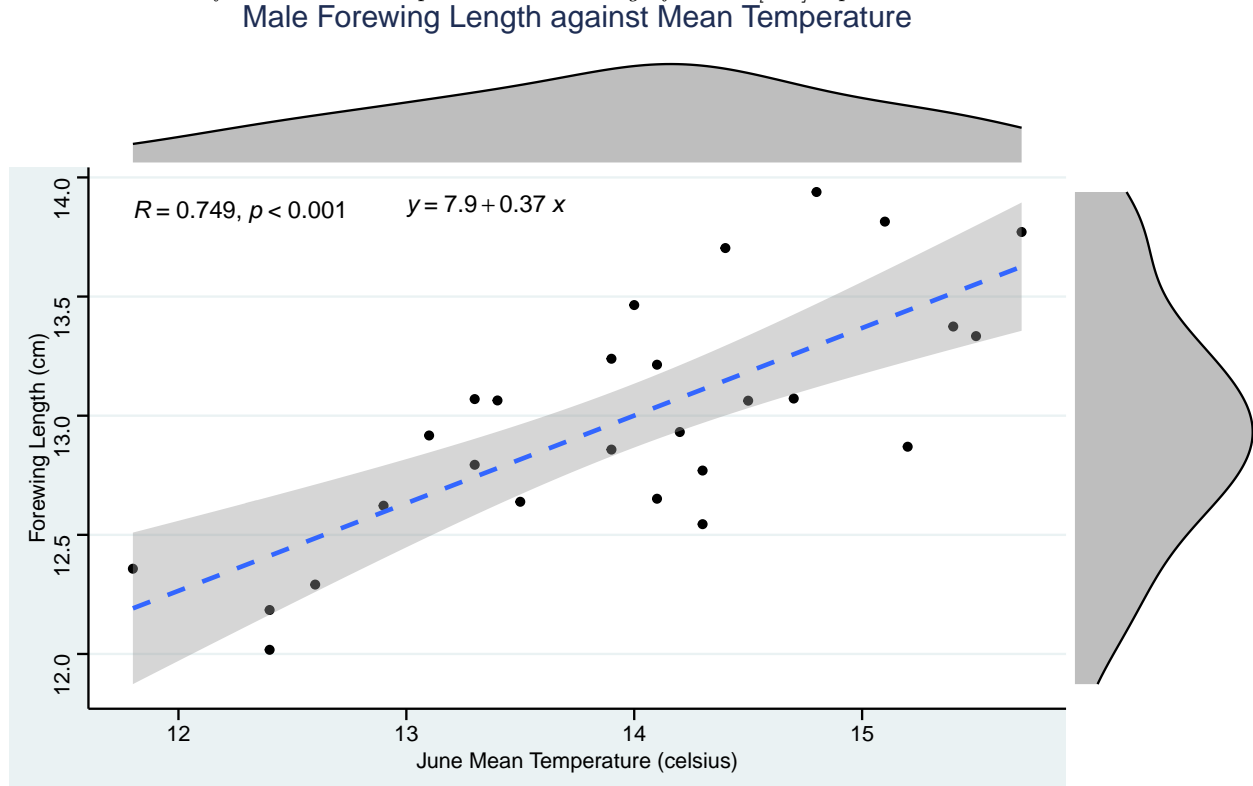


Figure 3b: **Male *Hesperia comma* forewing length increases with temperature.** The fitted slope is from the reduced linear model (the only significant factor being June Mean Temperature) excluding outliers (Adj. $R^2 = 0.5441$). This model suggests that for every 1-degree Celsius temperature increase, 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 regression line of best fit, shaded regions surrounding the line of best fit indicate regions of 95% confidence, and marginal curves indicate density plot distributions for Forewing length (right) and June Mean Temperature (top).

I also hypothesised a positive relationship between female forewing length and June Mean Temperature using a Pearson correlation coefficient test and OLS model. However, an insignificant relationship was observed between forewing length and June mean temperature in Female silver spotted skippers (95% CI; $p = 0.142$, Adj. $R^2 = 0.042$). These results are consistent with a study that found no relationship between environmental temperature and Forewing length (Johnson, 2022). This is also consistent with another study showing June temperatures can predict adult body size within only Male *Hesperia comma* (Fenberg et al., 2016). This shows that this is a trend in biology whereby Female butterflies do not behave similarly to male butterflies. Plots shown in Figure 4b.

Female Forewing Length against June Mean Temperature

<i>Predictors</i>	forewing length		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(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 4a: **Visual model summary for Female *Hesperia comma* Forewing length against June Mean Temperature.** Female *Hesperia comma* Samples were placed through an OLS model to compare their forewing length against their climate they lived in. Predictors indicate the dependent variable (intercept = Forewing length) and independent variable (june mean temperature). CI indicated 95% confidence intervals. Observations indicate sample sizes. R^2 and R^2 -adjusted indicate the strength of regression, scaled between 0 ~ 1. Estimators indicate the mean estimate for each variable. p values indicate regression insignificance.

Female Butterfly Forewing Length against June Mean Temperature

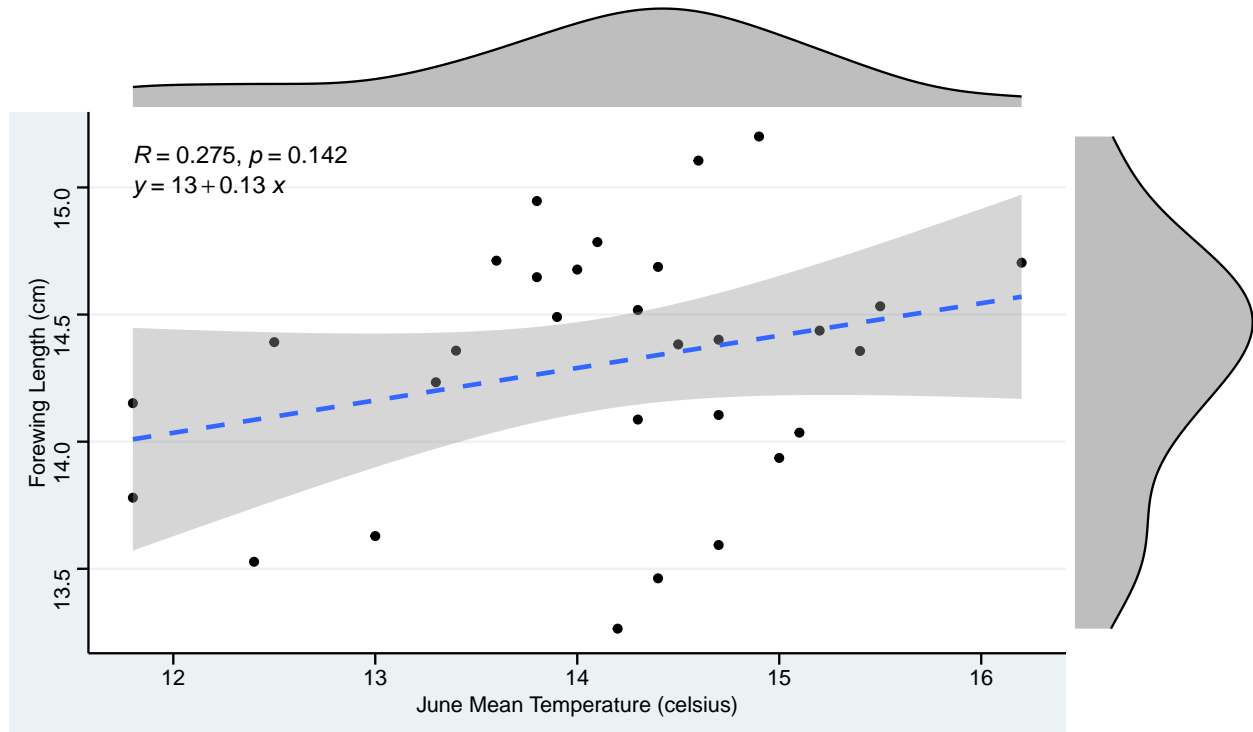


Figure 4b: **Non-significant positive linear relationship between Female *Hesperia comma* Forewing Length and June Mean Temperature.** Female silver spotted skipper specimens was collected from museums and measured against the corresponding June mean temperature that they lived in. Slopes were fitted from the reduced linear model as the temperature was the only significant contributing factor ($\text{Adj. } R^2 = 0.042$) excluding outliers. However, no significant linear relationship exists between Female forewing length and temperature (95% CI $-0.06937291 \sim 0.2545739$; $p = 0.244$). Circles indicate individual observations, the dashed line indicates the regression line of best fit, shaded regions surrounding the line of best fit indicate 95% confidence intervals 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 a mean forewing length of 14.30458 cm in females (95% CI 14.124618 ~ 14.48455), whereas Males had a mean forewing length of 12.97242 cm, offering a mean 1.332164 cm greater forewing length (95% CI $-1.591178 \sim -1.07315$; $t = 10.3$; $p < 0.05$). Results suggest that June temperature 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. Temperature effects may also result from differences in growth factor activity between the sexes, or the growth factor gene responding to temperature being present on the Y chromosome. Results shown in Figure 5. Results are consistent with a study that found Male *Hesperia comma* had significantly smaller forewing length than their counterpart Females (Thornton and Switzer, 2015). As such, the results of this study are valid.

Forewing Length Comparison between Males and Females

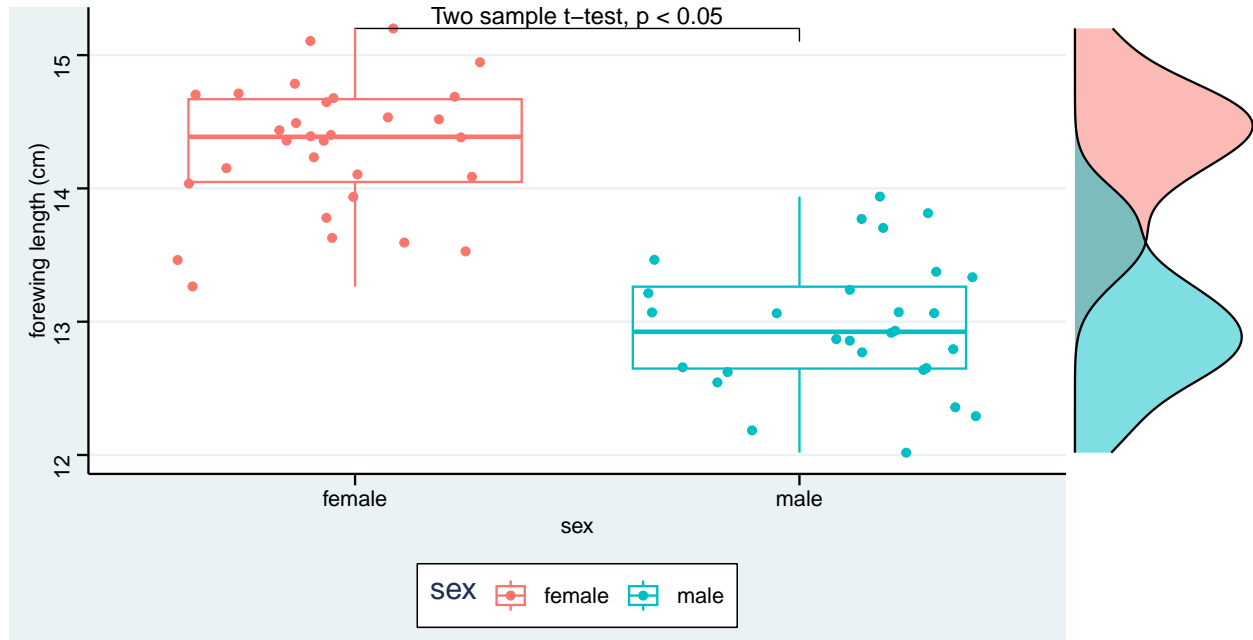


Figure 5: **forewing length comparison between Male and Female *Hesperia comma***. A two-sample *t*-test compared the mean forewing length between Male and Female butterflies. Females had a mean forewing length of 14.30458 cm (95% CI 14.124618 ~ 14.478455) whereas Males had a decreased forewing length of 12.97242 cm, indicating a 1.332164 cm difference (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 density plot distribution of forewing Length for Male and Female Butterflies (right).

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 only affect male *Hesperia comma* body sizes (Fenberg et al., 2016). This difference may result from upregulated growth factor genes, or the responsible growth factor genes existing on the Y chromosome. However, females may already exhibit excessive growth factors, causing little response to temperature changes. Results open opportunities to analyse genetic mechanisms in response to heat, and

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. This results in data loss and decreased model accuracy. The data measured males and females at arbitrary interval years. Consequently, female butterflies had more data than male butterflies, with male butterflies having twenty-eight samples and female butterflies having 30. This omission places bias towards female butterflies. As such, 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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