A latitudinal cline in a courtship song character of *Drosophila melanogaster*

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**ABSTRACT**

The courtship song of male *Drosophila melanogaster* is generated by wing vibration and contains an interpulse interval (IPI) which is species-specific and usually falls in the mean range of 30–40 ms. The IPI is extremely temperature-sensitive, so we wondered whether flies collected along the eastern coast of Australia between latitudes 16.9°S and 42.9°S might have adapted to the different thermal conditions and show differences in mean IPI. We observe a significant correlation between IPI and latitude in addition to the well-known association between latitude and body size (Bergmann’s Rule). However, somewhat surprisingly we could not detect a significant association between body size and IPI. We also examined flies collected from the North and South-facing slopes of ‘Evolution Canyon’ in Israel and observed differences in IPI that support the view that thermal adaptation can shape this important song character. We also examined the songs of flies from Kenya and observed no correlation between altitude of collection and IPI. In all three experiments, body size did not correlate with IPI. A global analysis of all three sets of populations on three continents revealed a strong association between IPI and latitude. We speculate that IPI is shaped by thermal and sexual selection whereas body size is also shaped by natural selection.

**Introduction**

It was a pleasant surprise to be asked by Jeff Dason to write a ms for a collection in honour of Marla Sokolowski’s 65th birthday. I remember first meeting her at a Behavior Genetics conference somewhere or other nearly 40 years ago and sitting with her and Jeff Hall in London at another meeting (maybe even the same one?) as Jeff explained to her how to map using balancers, deficiencies and compound autosomes. I’ve followed her career closely since the 1980s. Her work on the *foraging (for)* gene in *Drosophila* is a *cause celebre* and she has worked that story from the bottom up, from discovering the initial behavioural polymorphism, to its mapping and eventual molecular identification (Anreiter & Sokolowski, 2019). She has also covered almost every aspect of genetic analysis in her career, so from quantitative to population and evolutionary genetics, as well as neurogenetics, although most of her work would be classified as classic behavioural molecular genetics rather than neurogenetics. Indeed one of her papers on geographical variation in diapause in *Drosophila melanogaster* (Williams & Sokolowski, 1993) stimulated me (CPK) to work on this seasonal phenotype (Sandrelli et al., 2007; Tauber et al., 2007). With this in mind, and unashamedly using this early paper of Marla’s as an excuse, we would like to present some relevant, particularly as it concerns geographical variation in *D. melanogaster* courtship song, which is a character that has come under considerable molecular neurogenetic scrutiny in recent years (e.g. Clemens et al., 2015; Deutsch, Clemens, Thiberge, Guan, & Murthy, 2019; Wang et al., 2020).

When the *D. melanogaster* male courts the female, he extends his wing and vibrates it, generating a species-specific acoustic signal that has two major components, the pulses and the hums (Hall, 1994). More recently, Murthy and colleagues have detected two types of pulse song in *D. melanogaster* which is interesting as *Drosophila yakuba*, a member of the *melanogaster* subgroup of species also has two types of pulse song (Demetriades, Thackeray, & Kyriacou, 1999). The pulses in *D. melanogaster* have an interpulse interval (IPI) that can vary widely in *D. melanogaster* from 15 to 100 ms within a courtship but an average IPI for individual males will come in between 28 and 40 ms depending on the strain that is studied (Kyriacou & Hall, 1986). The sympatric sibling species *Drosophila simulans* has a mean IPI that on average is longer, between 40–80 ms, again, depending on the strain (Kyriacou & Hall, 1986). IPI is extremely temperature-sensitive and in *D. melanogaster* mean IPI can vary from 32 ms (at 35°C) to 56 ms (at 16°C) (Kyriacou & Hall, 1980). Other characteristics of IPI, such as the hotly debated ‘song cycle’ (Kyriacou, Green, Piffer, & Dowse, 2017; Kyriacou, Dowse, Zhang, & Green, 2020; Stern, 2014; Stern et al., 2017) which is not temperature-sensitive and is species-specific may serve species-
identification functions (Kyriacou & Hall, 1982; Kyriacou & Hall, 1986; Ritchie, Halsey, & Gleason, 1999).

The work that we will describe is represented by a study that takes a step back(wards) and asks a simple question. Given that IPI is so temperature-sensitive, could there exist a latitudinal cline in the IPI of *D. melanogaster* males? One would imagine that within each of the northern or the southern hemispheres, flies collected from the north or south of each hemisphere would be exposed to quite different thermal conditions throughout the seasons. For example on the eastern coast of Australia, flies from the north would have to adapt to temperatures that are generally hotter for much longer periods, both daily and seasonally, than those in the cooler south (James, Azevedo, & Partridge, 1995; James, Azevedo, & Partridge, 1997). What would happen to the IPIs of such flies, if after collection in their different thermal habitats, they were raised and recorded at the same temperature? Would the flies from the hotter north maintain a shorter IPI than those from the south? In addition, Bergmann’s rule would mean that flies under hotter conditions would be smaller than those from colder regions (Partridge & Coyne, 1997). Larger *D. melanogaster* males tend to be more successful in mating than smaller ones (Partridge & Farquhar, 1983; Partridge & Fowler, 1993) and one might also suspect that body size, or thorax length in particular, might also have an influence on IPIs which are generated from the motoneurons innervating the wing muscles (Shirangi, Wong, Truman, & Stern, 2016). Thus our study investigates how IPI length and thorax length covary with latitude from populations taken from a large latitudinal transect from eastern Australia and Tasmania, from ‘Evolution Canyon’ near Haifa in Israel and from Kenya, in the ancestral homelands of sub-Saharan Africa where *D. melanogaster* is believed to have evolved (Lachaise, Cariou, David, & Lemeunier, 1988; Mansourian et al., 2018).

**Methods and materials**

**Fly strains**

**Australia**

The strains of *D. melanogaster* were captured from a 2600 km transect from the eastern coast of Australia in February 1993. They were collected in duplicate from two different but neighbouring sites from 13 different latitudes within Queensland, New South Wales and Tasmania. Many isofemale lines from each site were established from the collections and these lines were maintained at 16.5 °C until they were kindly donated by Avis James and Linda Partridge in 1996 (James et al., 1995). All our experiments were performed between 1996–1997. Temperature data was taken from the weather station closest to the collecting site and obtained from the Australian Government Bureau of Meteorology website (http://www.bom.gov.au/climate/data/). A mean annual temperature was taken from all the years that temperature recordings were made, in most cases, from the early 1900s to 2020.

**Israel**

We also obtained replicate isofemale lines collected in Israel at ‘Evolution Canyon’ on Mount Carmel near Haifa in 1994 at 7 stations across the canyon, each station being approximately 30 m higher than the preceding one (32°43′N, 34°58′E) (Nevo, 1995). Station 1 lies at the highest altitude of 120 m ASL, station 4 at the valley floor and stations 4–7 on the N-facing slope with station 7 at the highest altitude of ~120 m, generating a V-shape. The highest stations 1 and 7 are only ~400 m apart. One year’s mean air temperature data from September 1996 exists for the top two stations on each slope (Pavlíček, Sharon, Kravchenko, Saaroni, & Nevo, 2003).

**Kenya**

These lines were collected from ‘shambas’ which are cultivated areas or mango gardens. Replicate collections from each sampling site were made during October and November 1995 by Stephan Esher (Escher, Eriksson, & Bachli, 1997). The locations sampled were Ngoruman Mango Garden (NGMI, NGCM, ~1000 m above sea level, 1.76°S, 36.05°E), Kericho Shamba (KESI, ~2000 m, 0.37°S, 35.29°E), Matuga Mango Farm (MA, ~200 m, 4.17° S, 39.58° E), ICIPE (Nairobi ~1800 m, 1.29°S, 36.82°E). All these replicate lines were isofemale in origin. Two lines from Kiambu Shamba (K & P, outside Nairobi ~1900 m, 1.17°S, 36.83°E) were generated from less than 5 females each.

All lines were maintained at 18 °C but for the experiments were raised at 25 °C in a 12 h:12 h light–dark cycle. From the Australian collections, two isofemale lines were selected at random from the different latitudes. Three pairs of virgin parental flies were placed in a food vial and allowed to mate for 48 h. Parents were then removed ensuring the progeny were raised at low density. Once hatched this procedure was repeated for one further generation. The first 4–6 males to emerge with intact wings from each line were used for song analysis and thorax measurement using an eyepiece graticule fitted to a stereomicroscope. The next 30 or so flies to emerge from the same vials were then also measured for thorax length (both males and females) in order to obtain a more accurate average measurement for each line. The same procedures were carried out for the two replicate lines at each station for the populations collected in Israel, and we selected a few flies from each available African isofemale line for analysis.

As mentioned earlier *D. melanogaster* IPI length is inversely influenced by temperature so all recordings were performed at a strictly controlled 25 ± 0.5 °C inside a modified Insectavox. Song was recorded for 2 min, amplified, and recorded on a Revox reel-to-reel tape recorder before digitisation using a Cambridge Electronic Design (CED) 1401 A/D converter (Ritchie, Yate, & Kyriacou, 1994). Mean IPIs were measured for each fly using the Spike 2 software package from CED and custom written programmes. While IPIs can be analysed automatically with the software, the error rate is relatively high, so manual inspection of all IPIs was necessary. Most flies produced between 200–400 individual IPIs.

Spatial autocorrelation was studied using statistic Moran’s I (Sokal & Oden, 1978) representing the similarity between
populations as a function of their separation. The computations for spatial autocorrelation were carried out using the SAAP program developed by David Wartenberg (Version 4.3, October 1989) and the correlations by Statistica (Statsoft). IPI data was further analysed using the Royaltey–Astrachan–Sokal (RAS) non-parametric test of departure from random geographical variation (Royaltey, Astrachan, & Sokal, 2010). Spearman’s ρ was used rather than Pearson-product moment R to correlate pairs of variables as it makes no underlying assumptions about the normality of the data.

Results

Australia

Mean male and female thorax length at each location was calculated (Figure 1(A) and see Supplementary Table 1) and mean IPI for the males (Figure 1(B) and Supplementary Table 2). The mean annual temperature closest to each collection site is shown in Figure 1(C). One-way ANOVA gave highly significant effects for male ($F_{39, 907} = 27.5, p < 5 \times 10^{-7}$) and female thorax ($F_{39, 963} = 43.8, p < 5 \times 10^{-5}$) and for male IPI ($F_{38, 138} = 7.5, p = 5 \times 10^{-5}$). We used Spearman’s rank-order correlation to correlate latitude with each variable. Table 1, part A, reveals that male and female mean thorax length generates a highly significant relationship with latitude ($p = 0.014, p < 0.0001$, respectively). Latitude and mean IPI generate a marginally significant correlation, ($p = 0.05$) whereas male thorax length and IPI, perhaps surprisingly, show no significant relationship. We also analysed independently the data from those individual male flies that had both their thorax length and IPI measured (Table 1, part B). Spearman rank-order correlations were highly significant for latitude and male thorax ($p = 0.005$) and latitude and IPI ($p = 0.001$) with again, no significant relationship between IPI and thorax length.

Spatial autocorrelation reflects the dependence of the value of a particular variable at one location with the value of the same variable at neighbouring locations. Consequently, IPI, male and female thorax length were summarised using the spatial autocorrelation statistic Moran’s I representing the similarity between populations as a function of their separation (Sokal & Oden, 1978). The data were divided into 6 distance classes with each IPI class containing 114–143 comparisons while the thorax classes (male and female) contained 120–140 comparison pairs. Moran’s I tends to $+1$ when IPI or thorax values show a similar trend (i.e. depart from the mean in a uniform direction) and tend towards $-1$ when the values are dissimilar and is expected to equal $-1/(n-1)$ under a random model. A cline is present if Moran’s I decreases from significantly positive to significantly negative with increasing distance between populations (Sokal & Oden, 1978). A steady decline of Moran’s I value from positive to negative was observed for both male and female thorax length, with a similar but less steep relationship between distance classes and IPI. Figure 2

Table 1. Australian populations.

| A. Variables (Means) |   |   |   |
|----------------------|---|---|---|
| Latitude and male thorax | 40 | 0.39 | 0.014 |
| Latitude and female thorax | 40 | 0.44 | <0.0001 |
| Latitude and IPI | 39 | 0.31 | 0.051 |
| Male thorax and IPI | 39 | 0.2 | 0.212 |
| B. Variables (Individual) |   |   |   |
| Latitude and male thorax | 175 | 0.21 | 0.0049 |
| Latitude and IPI | 177 | 0.29 | 0.0001 |
| Male thorax and IPI | 175 | 0.02 | 0.83 |

Spearman’s rank order correlations $p$ for latitude, IPI and thorax size from (A) group means and (B) individual fly measurements.

Figure 1. Mean IPI and thorax length (as graticule units) in Australian populations collected from the East coast. Bars indicate ± SEMs. (A) Male (blue) and female (red) thorax lengths. (B) IPI of males. (C) Annual mean temperature from weather station closest to collection sites (see Methods).

Figure 2. Correlograms for IPI (green), male (blue) and female (red) thorax size. Gradient of the lines demonstrates the overall trend from positive to negative for all three parameters, indicating the likelihood of spatial patterning.
Table 2. Australian populations.

| Distance Classes (km) | Minimum | Maximum | Pairs | Moran’s I | p-Values |
|-----------------------|---------|---------|-------|-----------|----------|
| A. IPI – Bonferroni approximation 0.078 | 0       | 347.9   | 133   | 0.055     | 0.139    |
| 347.9                 | 765.2   | 128     |       | 0.048     | 0.167    |
| 765.2                 | 1065.8  | 114     |       | 0.055     | 0.154    |
| 1065.8                | 1388.2  | 122     |       | -0.03     | 0.484    |
| 1388.2                | 1778.3  | 120     |       | -0.11     | 0.152    |
| 1778.3                | 2989.3  | 124     |       | -0.181    | 0.013    |

B. Male thorax length – Bonferroni approximation <0.0005
| Minimum | Maximum | Pairs | Moran’s I | p-Values |
|---------|---------|-------|-----------|----------|
| 0       | 347.9   | 140   | 0.42      | >0.0001  |
| 347.9    | 765.2   | 120   | 0.62      | >0.0001  |
| 765.2    | 1065.8  | 130   | 0.119     | 0.032    |
| 1065.8   | 1388.2  | 134   | 0.049     | 0.168    |
| 1388.2   | 1778.3  | 128   | -0.404    | >0.0001  |
| 1778.3   | 2989.3  | 128   | -0.966    | >0.0001  |

C. Female thorax length – Bonferroni approximation <0.0005
| Minimum | Maximum | Pairs | Moran’s I | p-Values |
|---------|---------|-------|-----------|----------|
| 0       | 347.9   | 140   | 0.475     | >0.0001  |
| 347.9    | 765.2   | 120   | 0.714     | >0.0001  |
| 765.2    | 1065.8  | 130   | 0.02      | 0.279    |
| 1065.8   | 1388.2  | 134   | -0.083    | 0.228    |
| 1388.2   | 1778.3  | 128   | -0.454    | >0.0001  |
| 1778.3   | 2989.3  | 128   | -0.823    | >0.0001  |

Results of spatial autocorrelation analyses.

shows the spatial autocorrelogram reflecting these relationships. Significance testing was performed using a stringent Bonferroni procedure (Oden, 2010). For IPI the overall Bonferroni p-value was 0.078, so narrowly missing significance but the most extreme distance class comparison was significant (p = 0.013, Table 2, part A) and the general trend for Moran’s IPI is from positive to negative as the distance increases (Figure 3) suggesting spatial patterning. The Bonferroni value was highly significant for both male and female thoraces (p < 0.0005) with most of the individual distance class comparisons significant for Moran’s I for male and female thoraces (Table 2, parts B and C).

We then examined the IPI data using the RAS non-parametric test of departure from random geographical variation (Royaltey et al., 2010). The 20 localities were connected by a Delauney network which is a triangulation system connecting neighbouring localities (Brassel & Reif, 2010). The mean IPI values were ranked with respect to localities and rank differences between the mean IPIs of adjacent localities were calculated. These are called edge lengths and an expected edge length corrected for continuity was also calculated. The mean deviation from the expected mean edge length by the observed mean edge length was examined as follows:

\[ T = \frac{\text{Observed mean edge length}-\text{expected mean edge length} \pm \text{Correction for continuity}}{\text{Standard deviation of mean edge length}} \]

(1)

Brassel & Reif, 2010. A t-value of 2.21 was obtained (<0.05 > 0.01) meaning that IPI variation with respect to latitude is not random.

**Israel**

We examined the mean IPIs of flies collected from Evolution Canyon in Israel. The African south-facing slope is warmer and drier than the European, north-facing slope which has dense maquis forest. A prediction based on our results above might be that flies facing south would have shorter IPIs than flies facing north assuming that flies do not simply mate randomly with each other across the various localities in the canyon. 4–5 males from two isofemale lines from each station were recorded and we examined whether there was any correlation between altitude and IPI and slope and IPI. Figure 3 illustrates the results from the 7 stations for IPI and thorax length. In both, there is a general V shape that is reminiscent of the spatial arrangement of the stations within the canyon with the apex at the valley floor (station 4). One-way ANOVA revealed significant effects for IPI (F₁₃, 3₇ = 3.44; p = 0.0016, Figure 3(A)), but a marginal effect for thorax size (F₅, ₂₈ = 2.43; p = 0.05, Figure 3(B)). We also correlated mean male thorax length with altitude, altitude and IPI and thorax and IPI for the 7 stations but none were significant (Supplementary Table 3). When we examined correlations taken from measurements of individual flies rather than means we found no significant correlation between altitude and IPI but a marginal effect with altitude and thorax size (Spearman’s r = 0.35, p = 0.04). A technical problem precluded us from measuring IPI and thorax length on the same flies.

One-way ANOVA revealed a significant effect of IPI comparing flies from the N-facing, S-facing, and valley floor...
It was not possible to generate a global correlation between IPI and thorax size at the level of individual flies because of the technical problems encountered with the Israeli lines which resulted in IPI and thorax measurements made on different flies.

\[ F_{2,59} = 5.7; \ p = 0.005 \]. Post-hoc testing using the conservative Newman–Keuls procedure revealed significant differences between flies on the N-facing slope (mean IPI = 34.29 ms) compared with those from the S-facing slope (mean IPI, 33.04 ms, \( p < 0.05 \)) or the valley floor (mean IPI, 32.4 ms, \( p < 0.05 \)). ANOVA also gave a significant effect of thorax length (\( F_{2,32} = 4.37, \ p = 0.02 \)) with post hoc testing revealing a significant difference between flies located on the S-facing slope (mean = 0.913 mm) compared to flies on the valley floor (0.893 mm) and N-facing slope (0.87 mm).

**Kenya**

One way ANOVA revealed no significant differences in mean IPI (\( F_{5, 64} = 1.83, \ p = 0.12 \)) nor mean thorax length (\( F_{5, 84} = 1.89, \ p = 0.11 \)) among the lines (Supplementary Table 4). Spearman’s \( \rho \) showed no significant correlations between altitude and mean male thorax size (\( \rho = -0.09, \ p = 0.87 \) df = 4), altitude and mean IPI (\( \rho = -0.6, \ p = 0.2 \)) nor male thorax and mean IPI (\( \rho = 0.12, \ p = 0.83 \)) among the six different locations. When we examined individual flies which had both song and thoracic parameters measured we obtained a marginally negative relationship between IPI and altitude (\( \rho = -0.23, \ p = 0.05 \)) but not for thorax size and altitude (\( \rho = 0.16, \ p = 0.15 \)) nor IPI and thorax size (\( \rho = 0 \)).

**Global analysis**

We finally considered the relationship among IPI, thorax length, latitude and altitude using all our data from three continents using Spearman’s \( \rho \). Table 3 reveals the various combinations of variables calculated using the population means. Latitude was calculated as a deviation from the Equator regardless of whether this was northern or southern hemisphere. A strongly significant positive \( \rho \) was observed between latitude and IPI (\( \rho = 0.003 \)). There was also a significant positive correlation between latitude and thorax (\( \rho = 0.025 \)) and the altitude-thorax correlation just missed significant (\( \rho = 0.06 \)). If we use the individual data rather than the means for each location the same pattern reoccurred with a highly significant correlation between latitude and IPI (\( \rho = 5 \times 10^{-5} \)), latitude and thorax length (\( \rho = 0.002 \)) and an inverse relationship between altitude and IPI (\( \rho = 0.001 \)), but not altitude and thorax (\( \rho = 0.17 \)).

**Discussion**

There is considerable literature on the effects of body size in Drosophilids. Larger body size appears to be an advantage in many but not all studies (Partridge & Coyne, 1997; Pavković-Lučić & Kekić, 2013). We were therefore interested in correlating body size and latitude with one of the major features of the courtship song, the IPI. In Australia, we have observed a significant relationship between IPI and latitude from populations collected on the eastern coast as well as the expected thorax length/latitude relationship, but we did not observe a body-size/IPI relationship. In Israel, somewhat remarkably given the very small distances between collecting stations, we observed that higher mean IPIs are observed in flies collected on the N-facing compared to the S-facing slope. Body size measurements differed between the slopes and the valley floor. African flies showed a significant inverse association between IPI and altitude when both individual and mean measurements were used as variables and this relationship was also carried forward to the global analysis.

Flies from the northern or southern slopes of the canyon also show differences in the Thr-Gly length repeat of the clock gene *period* (Zamorzaeva, Rashkovskety, Nevo, & Korol, 2005) which are consistent with the latitudinal clines found in repeat length in Europe and Australia and the relationship of the repeat to circadian temperature compensation (Costa, Peixoto, Barbuji, & Kyriacou, 1992; Peixoto et al., 1998; Sawyer et al., 1997; Sawyer et al., 2006). Consequently, the differences observed in IPI and in Thr-Gly repeat lengths from different sides of the canyon are consistent with an underlying thermal basis for these observations. Overall, our results reveal a relationship between IPI and latitude on both local and global scales. The thorax length-latitude correlation is expected for poikilotherms via Bergmann’s Rule (Partridge & Coyne, 1997; Stillwell, 2010). Interestingly, we also observed an inverse relationship between IPI and altitude, but only on a global scale using individual measurements (Table 3). The correlation was negative revealing that higher altitude flies sing with shorter IPIs, the opposite of what we would expect given that higher altitudes are generally colder. Inspection of Supplementary Table 4 reveals that all of the higher altitude Kenyan flies sang with generally shorter IPIs than those from Israel and Australia. Consequently, this will generate the inverse correlation with latitude as the African flies were the only ones collected at higher altitudes. We could speculate that the consistently warmer seasonal temperatures in Kenya compared to the more variable thermal environments of Israel and Australia would favour shorter IPIs in general, and that would provide a convenient explanation for the inverse relationship we observe between IPI and altitude.

The two results that most interest us are those between IPI and latitude, and, in sharp contrast, the independence of our measure of body size with IPI. Smaller flies from lower latitudes that are generally warmer might be expected to have a shorter IPI length. Furthermore, flies from warmer climates have smaller, narrower wings and the mechanics associated with smaller wings may favour faster beating and

| Variables – thorax | \( n \) (mean) | \( \rho \) | \( p \) | \( n \) (individual) | \( \rho \) | \( p \) |
|-------------------|--------------|-------|------|-------------------|-------|------|
| Latitude – thorax | 53 | 0.31 | 0.025 | 291 | 0.18 | 0.002 |
| Latitude – IPI    | 52 | 0.41 | 0.003 | 298 | 0.41 | 5 \times 10^{-7} |
| Altitude – thorax | 53 | 0.26 | 0.062 | 291 | 0.08 | 0.166 |
| Altitude – IPI    | 52 | 0.12 | 0.933 | 298 | -0.19 | 0.001 |
| Thorax – IPI      | 52 | 0.23 | 0.101 |  |   |   |

*It was not possible to generate a global correlation between IPI and thorax size at the level of individual flies because of the technical problems encountered with the Israeli lines which resulted in IPI and thorax measurements made on different flies.

Table 3. Global analysis of Australian, Israeli and African populations.
shorter IPIs (Pezzoli, Guerra, Giorgi, Garoia, & Cavicchi, 1997). Consequently, smaller wings would be expected to correlate positively with thorax length, so the absence of the body size-IPI relationship is unexpected. Many mating signals and fitness traits show a positive correlation with body size (Partridge & Farquhar, 1983; Partridge & Fowler, 1993) but our finding suggests that species signalling is not related to body size in the case of Drosophila IPI. These results resonate with a study that found little correlation between male body mass or wing size with wingbeat frequency or mating success (Barnes, Sullivan, & Villella, 1998) and another that observed that body size was affected by temperature but there was no correlation with mating success (Pavković-Lučić & Kekić, 2013). These results however are in contrast to several older studies that did observe a positive relationship between body size and various measures of mating success ((Ewing, 1964; Markow & Ricker, 1992; Partridge & Farquhar, 1983; Partridge, Hoffman, & Jones, 1987; Partridge & Coyne, 1997). Irrespective of whether male body size does or does not correlate with mating success, our results would suggest that IPI is not a critical character in any such association.

The response of IPI to temperature is not linear in that an increase of temperature from 25 to 35°C shortens mean IPI by about 3 ms (from 35 to 32 ms) whereas reducing temperature from 25°C to 16°C increases mean IPI from 35 ms to 56 ms (Kyriacou & Hall, 1980). The mean annual temperatures for the east coast of Australia are 25°C in the extreme north to 12.4°C in the extreme south (Figure 1(C)). Flies have a reproductive arrest, often referred to as ‘diapause’ but it is predominantly temperature-driven and is reflected in the loss of eggs in female ovaries at temperatures below 12°C (Anduaga, Nagy, Costa, & Kyriacou, 2018; Zonato, Collins, Pegoraro, Tauber, & Kyriacou et al., 2017). Consequently, there will be some reproductive and metabolic quiescence in southern Australia, but it is unlikely to occur in central or northern regions of the continent where flies will be active throughout the year. The maximum IPI differences observed were 28.5 ms from an isofemale line from Cairns in the extreme north compared to 37.6 and 36.4 ms in lines from Melbourne and Tasmania respectively in the extreme south (Supplementary Table 2). This maximum ~9 ms IPI difference will represent the effects on IPI of the annual differences in mean temperature of ~10°C between the two locations (24.95°C versus 14.5°C, Figure 1(C)) as well as the stochastic element of genetic drift. Nevertheless, in the laboratory, a change in temperature of this magnitude in that thermal range would generate a mean IPI difference greater than 20 ms (Kyriacou & Hall, 1980). Consequently, this suggests that over time there has been some local genetic adaptation of IPI to a temperature which is reflected in the linear relationship of mean IPI and latitude (Figure 1(B)).

At 25°C D. melanogaster females prefer simulated temporally patterned conspecific D. melanogaster 34 ms IPIs compared to patterned heterospecific D. simulans 48 ms IPIs (Kyriacou & Hall, 1982; Kyriacou & Hall, 1986; Ritchie et al., 1999). As this represents a 14 ms difference in mean IPI it might be expected that the 10 ms IPI difference discussed above between some of the northern and southern lines might also generate some local female IPI preferences possibly leading to a runaway process with genetic variation favouring and generating longer or shorter IPIs that become established at different latitudes. It would thus have been interesting to test the IPI preferences of females from the relevant isofemale lines collected at the extreme latitudes.

Raising higher and lower latitude flies at the same temperature in the laboratory appears to have maintained these differences in male IPI because we presume that some of the genetic variations for longer or shorter IPIs has been fixed. The same can be stated for the fly lines collected in Haifa. Here the mean annual differences in air temperature between the upper northern and southern facing slopes of the canyon are ~1°C (see Figure 3(B)). The maximum differences observed in mean IPI are from 35.6 ms at the top of the northern slope to 31.8 and 31.3 ms at the top of the southern slope and the bottom of the valley respectively (Figure 3(A)). This difference in mean IPI is rather more than we might expect from a laboratory experiment in which we would compare IPIs from 22–23°C which is the relevant mean annual temperature range for the two slopes. However, the maximum mean monthly ground temperature difference between the top of the two slopes was observed to be in excess of 10°C during October 1996 (Pavlíček et al., 2003) reflecting the difficulty in attempting to correlate IPI with measures of external thermal conditions. It may be that if we had raised and recorded the flies from Australia and Israel at lower or higher temperatures, say 18 versus 30°C rather than the usual 25°C, these IPI differences among lines may even be accentuated although it is difficult to predict what the IPI reaction norms might be.

To conclude, we have argued rather speculatively that the IPI differences we see among males from different latitudes may be a consequence of sexual selection. In contrast, the relationship between body size and latitude will not only reflect a component of sexual selection (Partridge & Farquhar, 1983; Partridge & Fowler, 1993; Pavković-Lučić & Kekić, 2013) but will have additional components based on natural selection within the framework of Bergmann’s Rule. The lack of relationship between IPI and body size further underlines the separation between this component of courtship and natural selection.

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