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Abstract

Wings are primarily used in flight but also play a role in mating behaviour in many insects. *Drosophila* species exhibit a variety of pigmentation patterns on their wings. In some sexually dimorphic Drosophilids, a pigmented spot pattern is found at the top-right edge of the male wings. Our understanding of wing spot thermal plasticity in sexually dimorphic species is limited with wing spots being primarily associated with sexual selection. Here, we investigated the wing pigmentation response of two species with wing spots: *D. biarmipes* and *D. suzukii* species to thermal variation. We exposed freshly hatched larvae of both the species to three different growth temperatures and checked for wing pigmentation in adult males. Our results indicate wing pigmentation is a plastic trait in the species studied and that wing pigmentation is negatively correlated with higher temperature. In both species, wings were darker at lower temperature compared to higher temperature. Further, *D. suzukii* exhibits darker wing pigmentation compared to *D. biarmipes*. Variation in wing pigmentation in both *D. suzukii* and *D. biarmipes* could reflect habitat level differences; indicating a strong G*E interaction. Raman spectral analysis indicated a shift in chemical profiles of pigmented vs. non-pigmented areas of the wing. The wing spot was found enriched with carbon-carbon double-bond compared to the non-pigmented wing area. We report that C=C formation in spotted area is thermally controlled and conserved in two members of the suzukii subgroup i.e. *D. biarmipes* and *D. suzukii*. Our study indicated a conserved mechanism of the spot formation in two *Drosophila* species coming from contrasting distribution ranges.

Keywords Wing spot, Thermal plasticity, Drosophila, Pigmentation, Carbon

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Background

Body pigmentation diversity is well documented in the animal world [5, 36, 45, 68]. Selection of diverse body pigmentation patterns is likely to be strong due to several adaptive functions associated with pigmentation. Pigmentation is a labile trait which enables survival and fitness in variable environments. For instance an increase in predators also correlates with increased pigmentation in some fishes, which is likely an antipredatory strategy [26]. Pigmented body patterns in lizards enhance foraging in lizards compared to cryptic patterns [27]. However, pigmentation might also limit adaptation, for example pigmented plumage could limit birds from inhabiting niches exposed to high temperatures [18] as darker colours tend to absorb more heat, further disrupting thermoregulation. In insects, pigmentation plasticity is known to be adaptive through diverse functions ranging from evading predators; Biston betularia [8], aposematism in butterflies [17, 55], to thermoregulation in Harlequin bugs [60] and also mate choice in damselflies [24]. Body pigmentation, especially pigmentation of the abdominal segments in Drosophila melanogaster [21, 42, 48, 67, 71] has been speculated to be adaptive and studied in greater depths [43, 66]. However, pigmentation patterns

are often complex and challenging to decipher owing to their link to multiple physiological traits including those involved in fitness [31, 37–39].

An interesting case is the complexity of wing pigmentation patterns observed in several insects. In the Drosophilid lineage wing spots vary in their form and location on wings across species [35]. Striking wing spot patterns have been noted for both Hawaiian (e.g. in genera Idiomyia and Scaptomyza) (reviewed in [35]; and non-Hawaiian Drosophilds belonging to Zygothirca [25], Chymomyza and Drosophila (sub-groups- elegans, takahashii, suzukii and rhopaloa) [34] genera. In some species, wing spots are common in both sexes (monomorphic) while in some species only males have wing spots [61]. Although diversity in wing patterns is known, less is known about variation in wing pigmentation. Candidate genes associated with body pigmentation are known, however those related to wing spots need more attention. The cis-regulatory element (CRE) at the yellow locus in D. biarmipes plays a role in the expression of the *yellow* (*y*) gene in the wing spot [23]. The activity of the CRE is affected if the *distal-less* (Dll) gene is down regulated which is one of the key activators involved in spot formation [1]. At the proximate level, temperature has often been found to be a key player influencing

varying pigmentation patterns in Drosophila species [22]. Diversity in thermal regimes could also influence diverse norms for wing pigmentation. Here, we attempt to understand the role of temperature in regulating wing pigmentation of two wing-spotted Drosophila species, Drosophila biarmipes and Drosophila suzukii collected from the Indian subcontinent (Fig. 1). D. suzukii is found in the colder regions, typically spread at higher latitudes and elevations with a wider global distribution [46] while D. biarmipes is restricted to warmer regions and largely confined to the tropical South-east Asia. Wing pigmentation pattern in both D. biarmipes and D. suzukii occurs in the form of a spot only in males at the anterodistal region of the wing (Fig. 2). Recent studies [16, 70] on wing spot size in some Drosophila species indicate wing spots and wing size are sensitive to thermal changes to a certain extent and this sensitivity is lost above a threshold. Yet, if pigmentation of the wing or wing spot also responds to thermal variation has not been studied in detail. We also attempt to look into the chemistry of the wing spot using biophysical approaches. This work integrates biogeographic ecology, sexual dimorphism, thermal plasticity, and chemical properties of wing spot. Our findings reveal the intricate and complex behaviour of wing spot under different temperature conditions and its ecological relevance.

Material & methods

Stocks and maintenance

D. biarmipes and D. suzukii adults were collected from Karbatiya Farm, Gujarat (23.7925° N, 72.6408° E) and Kanatal, Uttarakhand (30.4137° N, 78.3458° E) respectively (Fig. 1). Both species were collected from remotely located fruit orchards (resident natural populations).



Fig. 1 Species distribution map is based on Markow & O'Grady [44], Ørsted and Ørsted [47], and Nair and Peterson [46]. Drosophila biarmipes is endemic to Southeast Asia [44] while Drosophila suzukii has a broader distribution. D. suzukii is found in Europe, North America, Southeast Asia and some parts of South America [46]. In India, D. suzukii is localized in the Himalayan regions [47]. The map highlights the specific collection sites for the two species. D. biarmipes adults were collected from Karbatiya Farm, Gujarat (23.7925° N, 72.6408° E), represented by a black dot on the map while D. suzukii adults were collected from Kanatal, Uttarakhand (30.4137° N, 78.3458° E), indicated by a blue dot on the map



Fig. 2 Species identification. The upper panel shows the males of *D. biarmipes*; A Dorsal view B Lateral view C Male wing with spot. The lower panel shows *D. suzukii*; D Dorsal view E Lateral view F Male wing with spot. The visual comparisons between the dorsal and wing views of *D. biarmipes* and *D. suzukii* males assist in accurately distinguishing between the two species based on morphological characteristics

All field-collected females were placed in separate vials (in isolation) to set up isofemale genetic lines. Upon arrival to the laboratory successful cultures of fifteen *D. biarmipes* isofemale lines, and seventeen *D. suzukii* isofemale lines were established. All the cultures were maintained at a density of 30–40 eggs per vial on cornmeal yeast-agar medium at 23°C temperature conditions.

Thermal plasticity experiment

All fifteen isofemale lines of *D. biarmipes* and seventeen isofemale lines of D. suzukii were used for the thermal plasticity experiment. All the lines were maintained at 23°C. Eggs were collected at a lower density (30–40 eggs per vial). Three sets of eggs were collected and each set was separately placed in BOD incubators set at 18°C, 23°C and 28°C temperatures. Seven days after adult eclosion, 10 males were randomly chosen from each isofemale line and preserved in alcohol (isofemale line-wise). Before imaging, both the right and the left wings were carefully dissected from preserved samples. Wings were placed next to each other on a clean grease free glass slide. Distorted wings during dissections were not used for slide mounting. Each slide was pre-marked for the left and right sides. Three to five wings were placed per slide. A drop of 70% ethanol was added to the area and covered with a glass cover-slip. Wings mounted in this manner were later imaged through a stereo-zoom microscope (Leica S9i, Germany) with an in-built camera. All images were captured under uniform light conditions and magnification (3X). A single wing was imaged at a time. The images were processed using ImageJ software.



Fig. 3 A representative image of a *D. biarmipes* male wing showing two different regions (spotted: S and unspotted: US) used for the Raman spectral analysis. The Raman spectral analysis was done for the both the spotted and the unspotted wing regions. From both the regions two squares were cut and placed under Raman PLC for further analysis

All the images were first binarised to 8 bit and then the entire wing area was selected using the polygon function in ImageJ. We extracted the entire wing surface area in gray scale (which represents a proxy for wing pigmentation) for the statistical analyses.

Raman spectroscopy of wing areas

Raman spectroscopy is widely used in analyses of carboniferous materials [6, 14, 15]. Raman spectra reveal the chemical composition and crystallite size of the analyzed sample [33]. We used Raman Spectroscopy to characterize the chemical properties of wing spot pigmentation. For the spectral analysis, the wings of both species were cut as two squares of 0.5 mm each (i.e. one from the spotted area of the wing and the other from the non-spotted area) using a sharp razor blade under a stereomicroscope (Leica S9i, Germany) (Fig. 3). The cut area was placed on a glass slide covered with adhesive tape. The analysis of the different areas was also made under RAMAN PLC with a confocal microscope (WiTec Germany, Alpha 300R). The lens used was 50x and a 532 nm laser was used for the analyses of the samples. The analyzed area was imaged and the peaks present in that area were analyzed using the Origin software.

Statistical analyses

We checked for variation in wing size and mean wing pigmentation across all isofemale lines for all the three temperatures in D. biarmipes and D. suzukii. We first tested if wing size and mean wing pigmentation were independently correlated across left and right wings in both species. We used the overall wing pigmentation as a proxy to understand the degree of pigmentation in the wing spot. Pigmentation was measured on a grayscale index where lower grayscale values are inversely correlated with the degree of pigmentation. Thus, higher grayscale values indicate less pigmentation compared to lower grayscale values. We expect the habitats of species D. biarmipes and D. suzukii to have an influence on their pigmentation patterns, here pigmentation of wings. Hence, we expect wing pigmentation to be affected by both species and temperature. We performed linear mixed effect modelling using the lme4 package [2] in R software [57] on the R Studio interface version 2023.6.0.421 [58]. Species, temperature treatments and interaction of species and temperature were fixed effects while isofemale lines, number of replicates per isofemale line and variation at the individual fly level (right and left wing) were treated as random effects. Since temperature and wing size are correlated, i.e. higher temperatures are correlated with smaller wings and vice versa we divided the response variable of mean wing pigmentation by the total wing area. Correlated variables can change outcome and hence model interpretation [4] and hence, wing size was not included as co-variate with other independent variables. We ran the random intercept model which included an interaction of the fixed effects along with random factors as the base model. Subsequent models were derived by removing each factor from the base model. Upon removing a factor, the original and reduced models were compared with likelihood ratio tests (Chi-square statistic). Retaining a factor in the model was based on the significance of the Chi-square statistic. Further, the best model accounting largest variation of the data was the one with the lowest Akaike information criterion (AIC) [4]. Post-hoc analyses were conducted using the *emmeans* package [40] to understand variation in wing pigmentation across species as well wing pigmentation across temperatures for both species.

Results

Wing size and mean wing pigmentation independently exhibit considerable variation across isofemale lines for both species (see Supplementary Sheet 1 and Supplementary Figure 1). In both species, right and left wings were similar in size (*D. biarmipes*, ANOVA: F=0.1657, P>0.05; *D. suzukii*, ANOVA: F=0.34, P>0.05) and so was the mean wing pigmentation (grayscale) (*D. biarmipes*, ANOVA: F=0.13, P>0.05; *D. suzukii*, ANOVA: F=0.13, P>0.05) (Fig. 4). Raman spectroscopic analyses revealed carbon (C=C and C=O) to be a major component of wing pigmentation.

Wing spot pigmentation across three developmental temperatures

Wing size is correlated with temperature (D. biarmipes r = -0.73, P < 0.001 and D. suzukii, r = -0.88, P < 0.001). We therefore corrected for wing size by using a ratio: mean wing pigmentation divided by wing size/ area as response variable in our analyses. We noted that adult flies did not eclose with similar frequencies in all isofemale lines across temperatures in both species (Supplementary sheet 1). While D. biarmipes isofemale lines had successful adult eclosions at all tested temperatures this was not the case for all isofemale lines of D. suzukii. Hence, for statistical comparison, we randomly chose 10 isofemale lines for both species which not only had eclosions across all tested temperatures but had a minimum of 3 adult male eclosions per isofemale line (highlighted in Supplementary sheet 1). We found the most complex model had the lowest AIC (Akaike information criterion) and best explained variation in wing pigmentation (Tables 1 and 2). Thus, temperature $(\chi^2 = 554.09, df (4), P < 0.001), species (\chi^2 = 183.91, df$ (3), P < 0.001) and their interaction ($\chi^2 = 38.154$, df (2), P < 0.001) jointly influence wing pigmentation (Tables 1 and 2). Specifically, wing pigmentation decreased with increase in temperature (Figs. 4 and 5). In D. biarmipes; wing pigmentation was the darkest $(6.69 \times 10^{-3} \text{ arbi})$ trary units/ au) (inverse of mean values since darkness and pigmentation scores are inversely correlated) at the lowest temperature (18°C) compared to the intermediate $(6.36 \times 10^{-3} \text{ au})$ (23°C) (Tukey's Post-hoc: F = 10.58, P < 0.01) and the highest (5.29 × 10⁻³ au) (28°C) (Tukey's Post-hoc: F = 201.13, P < 0.01) temperatures. Also, wing pigmentation at intermediate temperature (23°C) was darker $(6.36 \times 10^{-3} \text{ au})$ compared to the highest



Fig. 4 The bar graphs represent the average wing pigmentation values corrected for wing size of 10 isofemale lines for both *D. biarmipes* and *D. suzukii* at three different growth temperatures (18°C, 23°C and 28°C). The wing pigmentation values for the left and right wings are shown in panel **AC** and **BD**, respectively. The trends indicate a linear decrease in the degree of wing pigmentation with increasing temperature. The differences in wing pigmentation for the left and right wing were found to be non-significant (*D. biarmipes*, *p* > 0.05; *D. suzukii*, *p* > 0.05). However, variation in the wing-spot pigmentation across temperatures was highly significant (*p* < 0.001). AU: arbitrary units. In *D. biarmipes*; wing pigmentation was the darkest (6.69×10^{-3} arbitrary units/ au) at the lowest temperature (18°C) compared to the intermediate (6.36×10^{-3} au) (23°C) and the highest (5.29×10^{-3} au) (28°C) temperatures. Similarly, in *D. suzukii* wing pigmentation was darkest (1.13×10^{-2} au) at the lowest temperature (18°C) compared to the intermediate (9.16×10^{-3} au) (23°C) and the highest (6.79×10^{-3} au) (28°C) temperature. Therefore, wing pigmentation exhibits a negative correlation with temperatures in both *D. biarmipes* and *D. suzukii*

temperature $(5.25 \times 10^{-3} \text{ au})$ (28°C) (Tukey's Posthoc: F = 153.95, P < 0.01). Similarly, in *D. suzukii* wing pigmentation was darkest $(1.13 \times 10^{-2} \text{au})$ at the lowest temperature (18°C) compared to the intermediate (9.16 × 10⁻³ au) (23°C) (Tukey's Post-hoc: F = 193.28, P < 0.01) and the highest (6.79 × 10⁻³ au) (28°C) (Tukey's Post-hoc: F = 460.97, P < 0.01) temperatures. However, wing pigmentation at intermediate temperature was darker (9.16 × 10⁻³ au) (23°C) (Tukey's Post-hoc: F = 460.97, P < 0.01) temperatures. However, wing pigmentation at intermediate temperature was darker (9.16 × 10⁻³ au) (23°C) than the highest temperatures (6.79 × 10⁻³ au) (28°C) (Tukey's Post-hoc: F = 176.67, P < 0.01). Wing pigmentation exhibits a negative correlation with temperatures in both *D. biarmipes* and *D. suzukii*.

Raman spectral analyses of wing spots

Raman spectral analyses was performed for two different areas of wings (unspotted area, and spotted area, Fig. 3).

Analyses revealed significant variation in the two different wing-areas (See Fig. 6). The Raman spectroscopy highlighted two different peaks for the spotted region (i.e., 1550 cm^{-1} and 1350 cm^{-1}) indicating the presence of C=Cand C=O (Fig. 6A and B). The spectral analysis of the second region (i.e., the unspotted or the transparent region) did not show any clear peaks (Fig. 6C and D). Raman spectral analyses of the spotted region of *D. biarmipes* raised at 23°C showed a sharp peak which was close to 1550 cm^{-1} (presence of graphitic carbon). The wing spot region of *D. suzukii* was also analyzed under Raman spectroscopy and the peaks obtained were in the same range as were seen in the case of *D. biarmipes* (Fig. 6B).

Discussion

Wing pigmentation patterns are known for several species from the Drosophilidae family. However, the origin and diversification of wing pigmentation across the **Table 1** Model comparison. The table compares the global model and the derived models in terms of the Akaike criterion, log-likelihood, and ANOVA values. Wing pigmentation is a ratio: the mean pigmentation of the wing divided by the total wing area. The base model is model 1 which is the most complex model. Subsequent models are subsets of the base model. As a single variable term gets dropped, both the earlier and the reduced model are compared with the likelihood ratio test statistic. A *p*-value < 0.05 indicates the variable dropped contributed significantly to the model and needs to be retained. Also, an AIC value greater than 2 of the base model suggests that the derived model has not a good fit

Model	Comparison	AIC	Loglik	Chisq	df	P-value
Model 1: Wing pigmentation ~ Species + Temperature + Species: Temperature + (1 Iso-female lines) + (1 Replicates) + (1 Symmetry)		4373.4	-2176.7			
Model 2: Wing pigmentation ~ Species + Temperature + (1 Iso-female lines) + (1 Replicates) + (1 Symmetry)	Model 2, Model 1	4407.6	-2195.8	38.154	2	< 0.001
Model 3: Wing pigmentation ~ Species + (1 Iso-female lines) + (1 Replicates) + (1 Symmetry)	Model 3, Model 1	4919.5	-2453.8	554.09	4	< 0.001
Model 4: Wing pigmentation ~ Temperature + (1 Iso-female lines) + (1 Replicates) + (1 Symmetry)	Model 4, Model 1	4551.3	-2268.7	183.91	3	< 0.001
Model 5: Wing pigmentation ~ 1 + (1 Iso-female lines) + (1 Replicates) + (1 Symmetry)	Model 5, Model 1	4996.7	-2493.4	633.34	5	< 0.001

Table 2 Coefficients of linear regression. Model constructed was mixed effect type with degree of pigmentation (corrected for wing size) being the response variable and "Species" (*D. biarmipes* and *D. suzukii*), "Temperature" treatments (18 °C, 23 °C, and 28 °C) as fixed effects. "Iso-female line number" and number of replicates per isofemale lines (sample replicates) and right and left wing measurements (symmetry) were random effects. Coefficients represented are for the most basal but complex model. All fixed effect terms in the basal model were found to be significantly important in predicting wing pigmentation. Species and temperature also interact with each other and hence wing pigmentation differs across two species as well as within a species across temperatures. Fixed effect coefficients are estimates, standard error (SE), degrees of freedom (df). t-statistic, and *p*-value. Random effect coefficients are variance and standard deviation

Fixed Effects						
	Estimate	Standard error	df	t-value	P-value	
Intercept	148.688	3.020	38.907	49.227	< 0.001	
Species (<i>D. suzukii</i>)	-59.171	3.570	150.264	-16.573	< 0.001	
Temperature (23 ⁰ C)	7.870	2.194	501.102	3.587	< 0.001	
Temperature (28 ⁰ C)	39.118	2.244	501.545	17.431	< 0.001	
Species (<i>D. suzukii</i>): Temperature (23 ⁰ C)	13.097	3.166	501.481	4.137	< 0.001	
Species (<i>D. suzukii</i>): Temperature (28 ⁰ C)	19.944	3.223	501.748	6.188	< 0.001	
Random Effects						
Groups	Name	Variance	Standard Deviation			
Iso-female lines	Intercept	82.55	9.085			
Sample replicates	Intercept	1.57	1.253			
Symmetry	Intercept	< 0.001	< 0.001			
Residual		220.73	14.857			

D. biarmipes N = 271, D. suzukii N = 252

Drosophilid phylogeny remains largely unknown. The random distribution of the spot in the *melanogaster* subgroup raises the possibility that wing spots have been gained and lost several times [34]. Alternatively, wing pigmentation in the *melanogaster* subgroup could be predicted due to gain and then lost several times [50]. Molecular studies indicate *distal-less* enhances activity of *yellow* regulating melanin expression in the



Fig. 5 The line graph illustrates the variation in wing pigmentation across three growth temperatures for two species: (**A**) *D. biarmipes* (**B**) *D. suzukii.* Each line in the graph represents one isofemale line within a population. The graph indicates a clear trend, as the temperature increases from 18° C to 28° C, there is a decrease in the wing pigmentation values for both the species. The trend is consistent across multiple isofemale lines suggesting that the response to temperature is a general characteristic within each species. These trends across temperatures from a natural population strongly indicate a genotype by environment (G x E) interaction for wing pigmentation

antero-distal region of the wing in *D. biarmipes* [1]. Modulation at the gene regulatory level could have possibly created the diversity of wing spot pigmentation patterns [1]. Insights could be derived from pigmentation regulation in abdominal segments from the model organism *D. melanogaster* [12, 22]. While trends observed in laboratory reared strains could be associated with more controlled growth conditions, these traits could be roughly extrapolated to those occurring in natural populations which could be more diverse and ecologically relevant.

In this study we find two species occupying different niches (Fig. 1) exhibit distinct patterns of wing pigmentation in response to temperature. Darker spots were associated with the lowest temperature treatment in both species (Figs. 4 and 5). Both D. biarmipes and D. suzukii share common ancestry of the suzukii sub-group and are phylogenetically related [35, 47]. However, wing pigmentation darkness differs greatly across the two species (Fig. 5; Table 1). D. suzukii exhibits darker pigmentation than D. biarmipes at all temperatures studied (see Table 2, slope for gray scale is -48.2, indicating D. suzukii wings are generally around 48-fold darker than D. biarmipes (Supplementary Table 3). Differences in wing pigmentation across the two species could likely reflect differences in the geographic zones in which the two species occur.

The impact of temperature on insect body pigmentation is well documented in both natural populations [11, 28, 52, 54, 65, 69] and under laboratory experimentation [30, 60]. Insects living at higher latitudes and altitudes are generally darker in coloration due to thermoregulatory advantages [3, 53, 62]. For instance, *D. melanogaster* and some other species from the same genus develop darker abdominal tergite pigmentation when reared at low temperatures compared to the flies reared at higher temperatures [9, 19–21]. Darker pigmentation at lower temperatures could be justified by the 'thermal-melanism' hypothesis which states that a darker body could warm up and cool down faster than a lighter/ paler body [3, 41, 62, 74]. Thus, darker bodies could be at an advantage in colder habitats (i.e. higher latitudes and higher altitudes) wherein a darker insect warms up in short intervals of light intensity meeting energy requirements [7, 49]. Contrarily, lighter pigmentation could be adaptive in warmer habitats and prevent overheating [7, 74]. Considering the fact that wing spot in both *D. biarmipes* and D. suzukii does not cover a major portion of the total wing area, it is unlikely that the darker wing spots could significantly affect the overall thermal budget or interfere with the thermo-regulation in spotted male flies. Alternately, the observed pigmentation patterns in wing spot could simply be due to the expression of the common pigmentation genes such as yellow, tan, distal-less, and *wingless* in the wing areas [1, 23, 51].

While wing pigmentation was plastic in response to temperature for both *D. biarmipes* (Fig. 4A) and *D. suzukii* (Fig. 4B), we found a substantial variation for wing pigmentation within a temperature treatment (Supplementary Figure 1). Variation represented at the individual female lineages is a likely indicator of genetic variation found in the wild. However, our results from natural populations strongly indicate a genotype by environment (G x E) interaction for the wing pigmentation (Fig. 5), not previously reported in both *D. biarmipes and D. suzukii*. A significant interaction of species and



Fig. 6 Raman spectral analysis of spotted (S) and unspotted regions (US) of wings of *D. biarmipes* and *D. suzukii* males. The analysis revealed distinct differences in the chemical composition between these two areas. The peaks indicate C = C richness in the spotted area of the wings; however, the same was not observed in the unspotted region of the same wing (data not shown) suggesting a chemical variation between the two different areas of the wings. The Raman spectroscopy results highlighted two different peaks for the spotted region (i.e., 1550 cm^{-1} and 1350 cm^{-1}) indicating the presence of C = C and C = O respectively. The spectral analysis of the second region (i.e., the unspotted or the transparent region) did not show any clear peaks indicating an absence or lower concentration of the compound, further indicating a chemical heterogeneity between the spotted and the unspotted areas of the wings

temperature in the model (Tables 1 & 2) supports the speculations that although wing pigmentation is plastic trait in response to temperature, but sensitivity of the trait is also influenced by the geographic distribution. An earlier study [70] attempting wing spot size variation did not measure the wing pigmentation, but instead measured wing spot size and wing size in D. suzukii. Both wing spot and wing size were found to be correlated with each other and both traits were inversely correlated with temperature. However, at higher temperature of 28°C, this correlation was uncoupled. Higher temperatures could disrupt the tight correlation between wing spot size and wing size in the cold adapted *D. suzukii* [32, 63]. In our case, we consider the overall wing pigmentation to be a proxy of the spot pigmentation. We observe a further decrease in the wing pigmentation as we move from intermediate (23°C) to higher temperatures (28°C). Wing pigmentation (Fig. 4; Table 1) significantly differed in response to temperatures and changed drastically from lowest to the highest temperatures, similar to the wing size (see Supplementary Table 2). In fact, wing size reduced drastically from 2.4 pixels/ mm² at 18°C to 1.90 pixels/ mm² (23°C) and 1.42 pixels/ mm² (28°C) (refer Supplementary Table 2). Thus, wing size reduced by 1.2X at 23°C and 1.7X at 28°C in D. suzukii. D. biarmipes also exhibited correlated plastic changes in both pigmentation and size of wing. Wing size reduced from 1.4 pixels/ mm² at 18°C to 1.32 pixels/ mm² (23°C) and 1.06 pixels/ mm² (28°C) (refer Supplementary Table 2). Thus, wing size reduced by 1.08X at 23°C and 1.33X at 28°C in D. biarmipes. Fold change of wing size reduction was more drastic for D. suzukii than D. biarmipes. Wing size-wing pigmentation response followed similar trend in both D. biarmipes and D. suzukii. Clearly, geographic segregation of D. biarmipes and D. suzukii across warm and cold habitats respectively is reflected in wing pigmentation variation across two species. It is surprising that despite both species inhabiting different geographic zones differing in temperature regimes, their response to temperature is conserved with respect to wing pigmentation. This observation raises several interesting questions about the evolutionary pressures and mechanisms underlying wing pigmentation in these species. Understanding the multifaceted selection pressures that influence wing pigmentation in *D. biarmipes* and *D. suzukii* could provide broader insights into the evolutionary processes that shape phenotypic diversity in natural populations.

Plastic responses are hypothesized not to be costly since they are largely assumed to be adaptive. However trade-offs also associate with plasticity. In the case of bark beetles it has been demonstrated that, with increase in temperature the developmental rate increases. Faster development trades-off with the next generation facing harsh colder environment [13]. In another study linking trade-offs with plasticity [64] higher temperatures led to an early termination of diapause at the cost lower pupal weight and reduced forewings. However, this wasn't the case in other two temperature regimes i.e. moderate and cold treatments [64]. Wing pigmentation has been shown to be adaptive in D. suzukii and spotless males seem to have a trade-off in terms of reproductive strategies. A recent study on D. suzukii reported pigmentation of the wing spot to be advantageous in mating [73]. Spotted and spotless males were grown across a series of temperatures (15, 21, 25, and 28°C) and then checked for the traits associated with reproductive fitness. Spotted males clearly exhibited lower mating latency and higher copulation period compared to spotless males, in addition to higher mating success since female preferred spotted males over spotless males. Further, spotted males were also desiccation and cold stress tolerant compared to the spotless males. Hence, plasticity of the wing spot pigmentation trait could be associated with adaptive functions [73] and the effect of fluctuating temperature conditions on plasticity needs to be addressed across diverse spotted Drosophilid species and other animal taxa [59].

A relatively recent study [16] adds further clarity to thermal plasticity in spot size regulation. In *D. guttifera* wing size and wing spot size are independently regulated [16]. That is the sensitivity period for both these traits differ and hence wing size and wing spot size develop independently in response to thermal variation. In our case wing size and wing pigmentation co-vary with temperature (for *D. biarmipes* and *D. suzukii*). Reaction norms for wing spots differ across wing locations in *D. guttifera* which has multiple spots. On the other hand, *D. biarmipes* and *D. suzukii* have pigmentation restricted to a single wing spot only in males and at a defined location. It could be interesting to find how conserved are concerted response patterns of both wing size and wing pigmentation to temperature in other species and if spot size pigmentation develops in temperature-independent manner in other Drosophilid species.

The role of wing spots is not clearly known. While a possibility of wing display leading to successful mating has been reported in *D. biarmipes* [29], removal of spots from males did not alter mating success [56]. Further, wing displays are known to be a part of mating rituals even in species with spot monomorphism (i.e. spots present in both sexes) [35, 61]. Sexual selection of wing spots could be expected in species exhibiting sexual dimorphism for wing spot pigmentation. However, the role of temperature in mate selection through wing spot pigmentation requires further investigation.

Raman spectral analysis of wing spot areas (i.e. spotted area) showed two major peaks at 1550 cm⁻¹ and 1350 cm⁻¹ (Fig. 6) corresponds to C=C enrichment [14, 72]. The abundance of C=C in the spotted regions of the wings of *D. biarmipes* and *D. suzukii* raises several questions around chemical evolution of traits (e.g. spot) involved in visually guided behaviours. It is not clear if pigmentation observed in the species arises from a common developmental pathway or whether alternate genetic networks are involved needs exploration. The role of *Dll* and *yellow* genes expression has been explored [1]. Further work on thermal properties of *Dll* and *yellow* interactions, and C=C product quantification along with behavioural analyses will shed more light on sexually driven properties of the wing spot pigmentation trait.

Conclusion

We integrated morphology, thermal plasticity, G*E interactions, and biophysical approaches to understand wing pigmentation in two spotted Drosophila species. The presence of G*E interactions in wing spot clearly demonstrated within species complexity for this morphological trait. Wing pigmentation is a phenotypically plastic trait and responds to thermal variation in accordance with the native range of species. Functional relevance of wing spots in Drosophilids is primarily linked to sexual selection. This could have fitness implications across individual and across habitats. However, if differential wing pigmentation in response to thermal variation has alternate functions, it needs further testing. The abundance of C=C in the spotted region of the wings indicate the presence of additional players at the molecular level controlling pigmentation patterns and these could be jointly explored further on other insect appendages as well.

Supplementary Information

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Supplementary Material 1. Supplementary Material 2. Supplementary Material 3.

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Authors' contributions

Conceptualization: Subhash Rajpurohit. Experimentation: Divita Garg. Fieldwork: Divita Garg, Subhash Rajpurohit. Data curation: Divita Garg. Funding acquisition: Subhash Rajpurohit. Methodology: Divita Garg, Monalisa Mishra, Subhash Rajpurohit. Data analysis: Divita Garg, Harshad Mayekar, Subhash Rajpurohit. Project administration: Subhash Rajpurohit. Resources: Subhash Rajpurohit. Software: Divita Garg, Sanjeev Paikra, Monalisa Mishra. Supervision: Subhash Rajpurohit. Validation: Subhash Rajpurohit. Visualization: Divita Garg, Monalisa Mishra, Subhash Rajpurohit. Writing – original draft: Divita Garg, Subhash Rajpurohit. Writing – review & editing: Divita Garg, Harshad Mayekar, Monalisa Mishra, Subhash Rajpurohit.

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Data availability

All data relating to this manuscrip has been provided as supplementary files.

Declarations

Ethics approval and consent to participate

Collecting wild populations of *Drosophila* species from orchards does not require ethical approval.

Competing interests

The authors declare no competing interests.

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References

- Arnoult L, Su KFY, Manoel D, Minervino C, Magriña J, Gompel N, Prud'homme B. Emergence and diversification of fly pigmentation through evolution of a gene regulatory module. Science. 2013;339(6126):1423–6. https://doi.org/10.1126/science.1233749.
- Bates D, Maechler M, Bolker B. Walker S. Fitting linear mixed-effects models using Ime4. J Stat Softw. 2015;67(1):1–48.
- Bishop TR, Robertson MP, Gibb H, van Rensburg BJ, Braschler B, Chown SL, Foord SH, Munyai TC, Okey I, Tshivhandekano PG, Werenkraut V, Parr CL. Ant assemblages have darker and larger members in cold environments. Glob Ecol Biogeogr. 2016;25(12):1489–99. https://doi.org/10.1111/geb.12516.
- Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC in model selection. Sociol Methods Res. 2004;33(2):261–304.
- Caro T, Mallarino R. Coloration in mammals. Trends Ecol Evol. 2020;35(4):357–66. https://doi.org/10.1016/j.tree.2019.12.008.
- Casiraghi C, Ferrari A, Robertson J. Raman spectroscopy of hydrogenated amorphous carbons. Phys Rev B. 2005;72(8):085401.
- Clusella-Trullas S, Terblanche JS, Blackburn TM, Chown SL. Testing the thermal melanism hypothesis: a macrophysiological approach. Funct Ecol. 2008;22:232–8.

- Cook L, Saccheri IJ. The peppered moth and industrial melanism: evolution of a natural selection case study. Heredity. 2013;110(3):207–12.
- David JR, Capy P, Gauthier JP. Abdominal pigmentation and growth temperature in Drosophila melanogaster: similarities and differences in the norms of reaction of successive segments. J Evol Biol. 1990;3(5–6):429–45.
- Deans AR, Lewis SE, Huala E, Anzaldo SS, Ashburner M, Balhoff JP, Blackburn DC, Blake JA, Burleigh JG, Chanet B. Finding our way through phenotypes. PLoS Biol. 2015;13(1):e1002033.
- 11. Delhey K. A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. Biol Rev. 2019;94(4):1294–316.
- Dembeck LM, Huang W, Magwire MM, Lawrence F, Lyman RF, Mackay TF. Genetic architecture of abdominal pigmentation in Drosophila melanogaster. PLoS Genet. 2015;11(5):e1005163.
- Dworschak K, Gruppe A, Schopf R. Survivability and post-diapause fitness in a scolytid beetle as a function of overwintering developmental stage and the implications for population dynamics. Ecol Entomol. 2014;39(4):519–26.
- 14. Ferrari AC, Robertson J. Interpretation of Raman spectra of disordered and amorphous carbon. Phys Rev B. 2000;61(20):14095.
- Ferrari AC, Robertson J. Raman spectroscopy of amorphous, nanostructured, diamond–like carbon, and nanodiamond. Philos Trans A Math Phys Eng Sci. 2004;362(1824):2477–512.
- 16. Fukutomi Y, Takahashi A, Koshikawa S. Thermal plasticity of wing size and wing spot size in Drosophila guttifera. Dev Genes Evol. 2023;2023:1–13.
- Futahashi R, Fujiwara H. Juvenile hormone regulates butterfly larval pattern switches. Science. 2008;319(5866):1061–1061.
- Galván I, Rodríguez-Martínez S, Carrascal LM. Dark pigmentation limits thermal niche position in birds. Funct Ecol. 2018;32(6):1531–40. https:// doi.org/10.1111/1365-2435.13094.
- Gibert P, Moreteau B, David JR. Phenotypic plasticity of abdominal pigmentation in Drosophila kikkawai: multiple interactions between a major gene, sex, abdomen segment and growth temperature. Genetica. 1999;105(2):165–76.
- Gibert P, Moreteau B, David JR. Developmental constraints on an adaptive plasticity: reaction norms of pigmentation in adult segments of Drosophila melanogaster. Evol Dev. 2000;2(5):249–60. https://doi.org/10. 1046/j.1525-142x.2000.00064.x.
- Gibert J-M, Peronnet F, Schlötterer C. Phenotypic plasticity in Drosophila pigmentation caused by temperature sensitivity of a chromatin regulator network. PLoS Genet. 2007;3(2):e30.
- Gibert J-M, Mouchel-Vielh E, Peronnet F. Modulation of yellow expression contributes to thermal plasticity of female abdominal pigmentation in Drosophila melanogaster. Sci Rep. 2017;7(1):43370.
- 23. Gompel N, Prud'homme B, Wittkopp PJ, Kassner VA, Carroll SB. Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in Drosophila. Nature. 2005;433(7025):481–7.
- 24. Guillermo-Ferreira R, Therézio EM, Gehlen MH, Bispo PC, Marletta A. The role of wing pigmentation, UV and fluorescence as signals in a neotropical damselfly. J Insect Behav. 2014;27(1):67–80.
- Grimaldi DA. Phylogenetics and taxonomy of Zygothrica(Diptera: Drosophilidae). Bull Am Mus Nat Hist. 1987;186:104–268.
- Gygax M, Rentsch AK, Rudman SM, Rennison DJ. Differential predation alters pigmentation in threespine stickleback (Gasterosteus aculeatus). J Evol Biol. 2018;31(10):1589–98. https://doi.org/10.1111/jeb.13354.
- Halperin T, Carmel L, Hawlena D. Movement correlates of lizards' dorsal pigmentation patterns. Funct Ecol. 2017;31(2):370–6. https://doi.org/10. 1111/1365-2435.12700.
- Harris RM, McQuillan P, Hughes L. A test of the thermal melanism hypothesis in the wingless grass-hopper Phaulacridium Vittatum. J Insect Sci. 2013;13(1):51.
- Hegde S, Chethan B, Krishna M. Mating success of males with and without wing patch in Drosophila biarmipes. Indian. J Exp Biol. 2005;43:902–9.
- Heinrich B. Thermoregulation in endothermic insects: body temperature is closely attuned to activity and energy supplies. Science. 1974;185(4153):747–56.
- Houle D, Diddahally R, Omholt S. Phenomics: the next challenge. Nat Rev Genet. 2010;11(12):855–66.
- Jakobs R, Gariepy TD, Sinclair BJ. Adult plasticity of cold tolerance in a continental-temperate population of Drosophila suzukii. J Insect Physiol. 2015;79:1–9. https://doi.org/10.1016/j.jinsphys.2015.05.003.

- Johnson CA, Patrick JW, Thomas KM. Characterization of coal chars by Raman spectroscopy, X-ray diffraction and reflectance measurements. Fuel. 1986;65(9):1284–90.
- 34. Kopp A, True JR. Evolution of male sexual characters in the oriental Drosophila melanogaster species group. Evol Dev. 2002;4(4):278–91.
- Koshikawa S. Evolution of wing pigmentation in Drosophila: diversity, physiological regulation, and cis-regulatory evolution. Dev Growth Differ. 2020;62(5):269–78.
- Kronforst MR, Barsh GS, Kopp A, Mallet J, Monteiro A, Mullen SP, Protas M, Rosenblum EB, Schneider CJ, Hoekstra HE. Unraveling the thread of nature's tapestry: the genetics of diversity and convergence in animal pigmentation. Pigment cell Melanoma Res. 2012;25(4):411–33.
- Kühl HS, Burghardt T. Animal biometrics: quantifying and detecting phenotypic appearance. Trends Ecol Evol. 2013;28 7:432–41.
- Lafuente E, Alves F, King JG, Peralta CM, Beldade P. Many ways to make darker flies: intra-and interspecific variation in Drosophila body pigmentation components. Ecol Evol. 2021;11(12):8136–55.
- Laughlin DC, Messier J. Fitness of multidimensional phenotypes in dynamic adaptive landscapes. Trends Ecol Evol. 2015;30(8):487–96. https://doi.org/10.1016/j.tree.2015.06.003.
- Lenth R, Singmann H, Love J, Buerkner P, Herve M. Emmeans: estimated marginal means, aka leastsquares means.–R package ver. 1.6. 1. In. 2020.
- Martínez-Freiría F, Toyama KS, Freitas I, Kaliontzopoulou A. Thermal melanism explains macroevolutionary variation of dorsal pigmentation in eurasian vipers. Sci Rep. 2020;10(1):16122. https://doi.org/10.1038/ s41598-020-72871-1.
- Massey J, Wittkopp PJ. The genetic basis of pigmentation differences within and between Drosophila species. Curr Top Dev Biol. 2016;119:27–61.
- Massey JH, Rice GR, Firdaus AS, Chen CY, Yeh SD, Stern DL, Wittkopp PJ. Co-evolving wing spots and mating displays are genetically separable traits in Drosophila. Evolution. 2020;74(6):1098–111.
- 44. Markow TA, O'Grady P. A guide to species identification and use. Entomol Ber. Elsevier. 2006;66:157–58.
- Mills MG, Patterson LB. Not just black and white: pigment pattern development and evolution in vertebrates. Semin Cell Dev Biol. 2009;20(1):72– 81. https://doi.org/10.1016/j.semcdb.2008.11.012.
- Nair RR, Peterson AT. Mapping the global distribution of invasive pest Drosophila suzukii and parasitoid Leptopilina Japonica: implications for biological control. PeerJ. 2023;11:e15222. https://doi.org/10.7717/peerj. 15222.
- Ørsted IV, Ørsted M. Species distribution models of the Spotted Wing Drosophila (Drosophila suzukii, Diptera: Drosophilidae) in its native and invasive range reveal an ecological niche shift. J Appl Ecol. 2019;56(2):423–35.
- Pool JE, Aquadro CF. The genetic basis of adaptive pigmentation variation in Drosophila melanogaster. Mol Ecol. 2007;16(14):2844–51. https://doi. org/10.1111/j.1365-294X.2007.03324.x.
- Porter W. Heat Balances in Ecological Contexts. In (pp. 49-87). Cambridge University Press; 2016. https://doi.org/10.1017/CBO9781107110632.005.
- Prud'homme B, Gompel N, Rokas A, Kassner VA, Williams TM, Yeh S-D, True JR, Carroll SB. Repeated morphological evolution through cis-regulatory changes in a pleiotropic gene. Nature. 2006;440(7087):1050–3.
- 51. Raja KK, Bachman EA, Fernholz CE, Trine DS, Hobmeier RE, Maki NJ, Massoglia TJ, Werner T. The genetic mechanisms underlying the concerted expression of the yellow and tan genes in complex patterns on the Abdomen and wings of Drosophila guttifera. Genes. 2023;14(2):304.
- Rajpurohit S, Schmidt PS. Latitudinal pigmentation variation contradicts ultraviolet radiation exposure: a case study in tropical Indian Drosophila melanogaster. Front Physiol. 2019;10:84.
- Rajpurohit S, Parkash R, Ramniwas S. Body melanization and its adaptive role in thermoregulation and tolerance against desiccating conditions in drosophilids. Entomol Res. 2008a;38:49–60. https://doi.org/10.1111/j. 1748-5967.2008.00129.x.
- Rajpurohit S, Parkash R, Ramniwas S, Singh S. Variations in body melanisation, ovariole number and fecundity in highland and lowland populations of Drosophila melanogaster from the Indian subcontinent. Insect Sci. 2008b;15(6):553–61.
- Reichstein Tv, Von Euw J, Parsons J, Rothschild M. Heart poisons in the Monarch Butterfly: some aposematic butterflies obtain protection from cardenolides present in their food plants. Science. 1968;161(3844):861–6.

- Roy PR, Gleason JM. Assessing the use of wing ornamentation and visual display in female choice sexual selection. Behav Processes. 2019;158:89– 96. https://doi.org/10.1016/j.beproc.2018.10.010.
- R Core Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing; 2023. https://www.Rproject.org.
- RStudio Team. RStudio: Integrated Development for R. Boston: RStudio, PBC; 2023. http://www.rstudio.com/.
- Sgrò CM, Terblanche JS, Hoffmann AA. What can plasticity contribute to insect responses to climate change? Ann Rev Entomol. 2016;61(1):433–51.
- Sibilia CD, Brosko KA, Hickling CH, Thompson LM, Grayson KL, Olson JR. Thermal physiology and developmental plasticity of pigmentation in the Harlequin bug (Hemiptera: Pentatomidae). J Insect Sci. 2018;18(4). https://doi.org/10.1093/jisesa/iey066.
- 61. Spieth HT. Drosophilid mating behaviour: the behaviour of decapitated females. Anim Behav. 1966;14(2–3):226–35.
- Stelbrink P, Pinkert S, Brunzel S, Kerr J, Wheat CW, Brandl R, Zeuss D. Colour lightness of butterfly assemblages across North America and Europe. Sci Rep. 2019;9(1):1760–1760. https://doi.org/10.1038/ s41598-018-36761-x.
- Stephens AR, Asplen MK, Hutchison WD, Venette RC. Cold hardiness of winter-acclimated Drosophila suzukii (Diptera: Drosophilidae) adults. Environ Entomol. 2015;44(6):1619–26. https://doi.org/10.1093/ee/nvv134.
- Stuhldreher G, Hermann G, Fartmann T. Cold-adapted species in a warming world–an explorative study on the impact of high winter temperatures on a continental butterfly. Entomol Exp Appl. 2014;151(3):270–9.
- Svensson El, Waller JT. Ecology and sexual selection: evolution of wing pigmentation in calopterygid damselflies in relation to latitude, sexual dimorphism, and speciation. Am Nat. 2013;182(5):E174–95.
- Takahashi A. Pigmentation and behavior: potential association through pleiotropic genes in Drosophila. Genes Genet Syst. 2013;88(3):165–74. https://doi.org/10.1266/ggs.88.165.
- 67. True JR. Insect melanism: the molecules matter. Trends Ecol Evol. 2003;18(12):640–7.
- True JR, Edwards KA, Yamamoto D, Carroll SB. Drosophila wing melanin patterns form by vein-dependent elaboration of enzymatic prepatterns. Curr Biol. 1999;9(23):1382–91. https://doi.org/10.1016/s0960-9822(00) 80083-4.
- Tseng M, Bevanda C, Bhatti SS, Black EN, Chang E, Chiang J, Dhaliwal H, Dimitriou A, Gong SY, Halbe E. Effects of temperature on monarch caterpillar pigment variation in nature. Insect Conserv Divers. 2023;16(1):164–71.
- Varón-González C, Fraimout A, Debat V. Drosophila suzukii wing spot size is robust to developmental temperature. Ecol Evol. 2020;10(7):3178–88.
- Wittkopp PJ, True JR, Carroll SB. Reciprocal functions of the Drosophila yellow and ebony proteins in the development and evolution of pigment patterns. Development. 2002;129(8):1849–58. https://doi.org/10.1242/ dev.129.8.1849.
- 72. Xie Y, You J, Lu L, Wang M, Wang J. Raman spectroscopic study of coal samples during heating. Appl Sci. 2019;9(21):4699.
- Yadav M, Khandia R, Parkash R. Divergence in climate adaptations of seasonally variable wing spot dimorphism in Drosophila suzukii indicus, a Himalayan species. entomoljournal.com. 2024;12(2):121-6. https://doi. org/10.22271/j.ento.2024.v12.i2b.9302.
- Zeuss D, Brandl R, Brändle M, Rahbek C, Brunzel S. Global warming favours light-coloured insects in Europe. Nat Commun. 2014;5(1):1–9.

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