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Divergence in climate adaptations of seasonally variable wing spot dimorphism in *Drosophila suzukii indicus*, a Himalayan species

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Abstract

Drosophila suzukii indicus, exhibits sexual dimorphism for wing melanisation but its ecological significance is not clear. We explored the effect of temperature and humidity on the mating behavior and success of *D. suzukii indicus*. The numbers of *D. suzukii indicus*, eclosing in winter and autumn differ significantly in accordance to wing melanisation. Our results of field and laboratory studies showed that the frequency of spotted and spotless males is different in different seasons i.e. cold and dry season. *D. suzukii indicus* is better in their mating success and fitness than those of wet and dry season because there are differences in mated pairs with spotted and spotless males which correlate significantly with winter and autumn climatic conditions. The degree of seasonal variation in wing melanisation in wild caught *D. suzukii indicus* was quantified to determine if it shows patterns of plasticity and male showed the characteristic seasonal adaptive plasticity for wing melanisation. Sexual dimorphism of wing melanisation may results from, and / or affects, sexual dimorphism of behavior and physiology of *D. suzukii indicus*. Mating with a high-quality male (spotted wing) results in offspring with high performance. To the best of our knowledge, this is the first report on the ecological of seasonally varying wing color dimorphism in *D. suzukii indicus*.

Keywords: Divergence, adaptations, seasonally, *Drosophila suzukii indicus*

Introduction

The very diverse wing melanization patterns of insects have been shown to be significant for thermoregulation, mate selection, predator defense, and mimicry in a number of butterfly species (Watt 1968; Roland 1982; Kingsolver 1987; Wiernasz 1989; Ellers & Boggs 2002, 2003) [40, 33, 18, 41, 7]. Wing melanization has been linked to improved flight capabilities in alpine (*Colias* species) and copper butterflies in colder climates (Watt 1969; Roland 1982; Guppy 1986; Ellers & Boggs 2004; Karl *et al.* 2009) [38, 33, 11, 8, 16]. Studies on butterflies (Wiernasz & Kingsolver 1992; Jiggins *et al.* 2001) [42, 15]; calypterygid damselfly (Siva-Jothy 1999) [36] have demonstrated the significance of wing patterns in mate recognition. On the other hand, a number of studies have demonstrated that wing spots directly affect mated pairings in *Drosophila suzukii* (Fuyama 1979) [9] and *Drosophila biarmipes* (Singh & Chatterjee 1987) [35]. The ecological importance of wing spot dimorphism for *Drosophila* species is still mostly unclear. The patterns of melanin in wings have been studied extensively. Males belonging to the *takahashii*, *suzukii*, and *elegans* species subgroups have been reported to exhibit melanization of the anterior-distal wing (Bock 1980, Toda 1991) [4, 37]. In contrast, no melanization is observed in the *ananassae*, *montium* subgroups, or the nearby *melanogaster* species group. In *D. prostipennis* and *D. pseudotakahashii* species, sexual dimorphism is more prominent, with females exhibiting hardly noticeable darkening. Only males have wing spots in *D. suzukii* and *D. biarmipes* (Kopp & True 2002) [19].

The evolution of adaptations that have enabled species to increase in abundance and/or dispersion is a key objective of ecology (Andrewartha and Birch 1954) [2]. Extreme seasonal and geographic changes in ambient temperature and solar radiation are two major elements that control seasonal activity and restrict the dispersal of all species, but especially small poikilotherms.

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While the effects of various abiotic factors have considered less study, insect taxa from different regions of the world are widely documented to exhibit thermal adaptations (Lee & Denlinger 1991; Loeschcke *et al.* 1994; Addo-Bediako *et al.* 2000; Hoffmann *et al.* 2003; Overgaard & Sorensen 2008) [20, 21, 1, 13, 29]. Temperatures in the tropics are generally constant, there are notable seasonal differences in precipitation. Moreover, distinct species exhibit seasonal variations in their wet and dry forms. On the other hand, numerous studies have demonstrated the direct impact of wing spot on mated pairs in *Drosophila biarmipes* (Singh and Chatterjee, 1987; Hegde *et al.*, 2005) [35, 12] and *Drosophila sukukii* (Fuyama, 1979) [9]. Male *D. biarmipes* with an apical dark wing patch had higher mating success than males without one, according to female choice trials (Singh and Chatterjee, 1987) [35]. Another study then revealed that in both competitive and non-competitive circumstances, spotted male *D. biarmipes* showed higher frequency of courtship behaviors (tapping, scissoring, vibration, licking, and twist dance) than non-spotted males (Hegde *et al.*, 2005) [12]. These investigations, however, did not take into account potential variations in the mating advantage of wing spot morphs in various environmental circumstances. The ecological importance of wing spot dimorphism is still mostly unclear for many *Drosophila* species.

The temperate species *Drosophila sukukii indicus* is found in the Oriental region of the Indian subcontinent. (Parshad and Paika, 1964) [31]. *D. sukukii indicus* was categorized into the melanogaster species groups *sukukii* subgroup based on its physical characteristics. The melanin pattern in *D. sukukii indicus* resembles an intense spot on the wings and very mild melanization is infrequently observed in females of this species, this pattern is exclusive to males. The habitat of this species is found in northern India, where the climate varies periodically. In addition, the northern winter and autumn seasons have very different temperatures and humidity levels. Therefore, in populations living in subtropics, we would anticipate evolutionary reactions to sexual selection on characteristics linked to desiccation and cold stress. Therefore, the role of wing spot in mating success under varying climate conditions could contribute to the understanding of *D. sukukii indicus* varying behavioral response. As a result, we believed *D. sukukii indicus* to be appropriate for addressing the following queries. (i) Does wing spot have any flexibility? (ii) Does wing spot play a part in mating success (length of copulation, mating latency)? (iii) How do seasonal fluctuations affect the fitness of spotted and spotless males differently? The current research on how WSA affects mating success is intriguing in a number of ways. (b) After desiccation and cold stress, the likelihood of spotted males to mate increased significantly, but the propensity of spotless males to mate decreased. Our findings demonstrate how male wing spot dimorphism affects mating success in *D. sukukii indicus* under varying environmental conditions.

Materials and Methods

Collections and cultures

Using the net sweeping approach, wild *D. indicus sukukii* (n = 110-150 per population) were gathered from four lowland localities (~300 – 600 m; Rohtak, Chandigarh, Mandi, and Kalka). *D. indicus sukukii* females lack wing spots, whereas males exhibit dimorphism in their wing spots. Isofemale lines (20 lines per population) were started using females that were captured in the wild. Every culture was started with a 12-hour

egg-laying time and kept on a cornmeal-yeast-agar medium at 21°C with a low density of eggs per vial. To minimize the effects of laboratory adaptation, all studies were conducted with G1 and G2 Generations 1 and 2 shortly after collections. The flies used for all experiments were 7 days old and sexed shortly after eclosion. Laboratory-raised *D. indicus sukukii* individuals were examined for wing spot for each isofemale line. This was followed by evaluations of mating latency (ML), copulation duration (CD), Fecundity, resistance to desiccation and cold stress.

Analysis of eco physiological traits

To study the effects of temperature developmental changes on *D. sukukii indicus* wing spot, 15–20 pairs of each isofemale line were allowed to lay eggs in 20 replicate vials at 21 °C. After that, five of these vials were moved to growth temperatures of 15, 21, 25, and 28 °C. Therefore, at these growth temperatures, we examined the thermal plastic effects for the wing spot area. Flies that were six days old and from varying developmental temperatures were examined for a range of eco physiological characteristics. Twenty is female lines per group were employed for each attribute, and ten randomly selected individuals from each is female line were studied. Soon after exclusion, the flies were sexed and kept as virgins on a cornmeal medium. As the desiccation stress tests began, all flies were 6–8 days post-eclosion. Ten virgin males of each morph were placed in a dry plastic vial with a foam piece covering it and two grams of silica gel at the bottom in order to measure the desiccation stress. These foam-plugged vials were kept in a desiccation chamber (Secador electronic desiccator cabinet), which keeps the relative humidity at about 8%. Until half of the flies (LT50) perished, the mortality from desiccation stress was observed every hour. After that, observations were taken every 30 minutes. Spotted and spotless males were divided into groups of ten and placed in 10-milliliter glass vials that were cooled to 0 degrees Celsius with a 10% glycol solution to test their resistance to cold. After varied stress levels for one to ten hours, the vials were taken out, and the mortality was recorded. When the flies could not stand on their legs, it was thought that they had died.

Calculation of Wing spot area (WSA; mm²)

We used image analysis to determine the percentage of wing spot area per fly. To do this, each male fly wing was put on a slide and run through the Biowizard image processing program from Dewinter Optical Inc. (www.dewinterindia.com). The extraordinarily significant correlation ($r = 0.99$) between the results from the image analysis approach and the visual method supports its validity.

Mating propensity under stressful condition

Separate groups of 50 laboratory-raised virgin males and females (spotted and spotless) were exposed to stressful conditions (desiccation and cold) for five hours in a desiccating environment (~10% RH) or for two day at 10 °C. Batches of virgin male and female flies were not acclimated for the control group. Ten virgin females, five virgin males with spots and five virgin males without spots were placed in each mating room. Observations were conducted on ten pairings of these flies under the condition of female choice for sixty minutes. The percentage of mated pairs (MP), copulation duration (CD; the period of time from the start of mating to the separation of mated couples), and mating

latency (ML; the time from fly introduction to the start of copulation time) were all recorded for every observed mating. Mating was therefore seen for twenty is female lines, each with ten replications.

Fecundity

Each mated pair was aspirated, the male was removed, and the pair was then placed in an oviposition chamber for a whole day in order to estimate fecundity. Every day, the number of eggs laid on the food that was placed at the oviposition chamber replaceable bottom plate was counted. Every day, the flies were moved to fresh food vials, and each female's 24-hour egg lay record was kept track. Since the 7th to the 31st fell inside the window of optimum egg production, this was done for 15 consecutive days, with the data displayed as daily fecundity. Everyday fecundity was evaluated in both desiccating and controlled environments.

Statistical analyses

For the purpose of creating tabular data and graphs, means ($n = 20$ lines \times 10 replicates \times 10 individuals) were combined with standard error (SE) for each trait. The study employed the contingency χ^2 test to evaluate statistical differences among various mating types in a female choice experiment conducted in conditions that were either cold or desiccating or controlled. Both the statistical computations and the drawings were performed using the Statistica 7.

Results

Percent mated pairs for Spotted and Spotless males in wild:

The basic statistics of percentage frequency for spotted and spotless males in field circumstances over seasons are provided in (Table 1; Fig. 1a). These verify that the frequency of spotted males is higher in the winter (cold and dry) than it is during the autumn (dry and wet). Further, we found no plastic effects between three growth temperatures (15, 21 and 30 °C) for spotted and spotless wing males. Thus, the data across growth temperature were pooled for all the traits i.e. stress related traits and mating propensity.

Further, spotted males showed significantly higher desiccation resistance as well as cold stress survival (data not shown). Under desiccation conditions, spotted males had significantly higher fecundity per day, longer copulation duration, higher mated pair frequency (Fig. 1b, c, d).

Alternatively, spotless males exhibit a substantially longer mating latency and lower fecundity (Fig. 1B).

We conducted female choice mating studies to see if spotted and spotless males in *D. sukuzii indicus* had different preferences when it comes to mating with females. The results of these experiments are shown in Figure 1. Spotted males displayed a higher percentage of mated pairs (MP) in all types of mating than spotless males (Figure 1, Table 2). We examined the mating tendency of spotted and spotless wing males under control vs. stress conditions (desiccating or wet), based on female-choice mating tests. The results are displayed in Table 2 and Figure 1. We employed data from desiccation stress for analysis because we could not detect any noticeable distinctions between desiccation and cold stress. There were significant differences in the mated pairs and mating latency under control as well as desiccation circumstances. The mating preferences of spotted or spotless males were calculated based on two aspects of the mating process (mating latency and copulation duration) (Table 2). Therefore, the males with spotted wings performed better than spotless wings in low humidity (desiccating) circumstances.

Table 2 displays the results of our investigation into whether the mating tendency of spotted and spotless males changes in cold environments. After two days of acclimating virgin males and females to a low temperature (~ 10 °C), we conducted studies measuring mating latency, copulation duration, and mated pairings under female choice. The propensity of spotted and spotless males to mate was shown to differ significantly; in colder climates, spotted males mated more frequently than spotless males (Table 2). Significant differences were also seen between the spotted and spotless male flies that were acclimated to a cold environment. Specifically, the spotted males mated earlier due to their lower mating latency compared to the spotless males longer mating latency. Lastly, there were notable variations in the length of the copulation duration; for example, spotted males used to cold environments clearly displayed a longer copulation period than spotless males (Table 2). As a result, in cold environments, the likelihood of spotted males to mate was much higher. Furthermore, fecundity for mating with spotted vs spotless males varies significantly under desiccating conditions (Figure 1), which is consistent with sexual selection for fitness benefits.

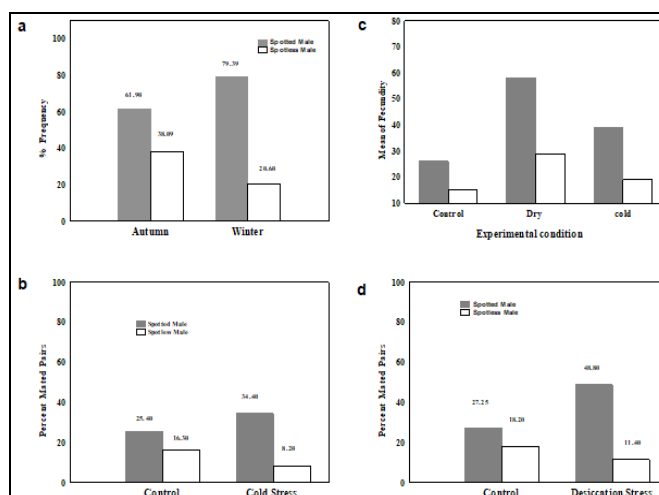


Fig 1: Percent frequency of spotted and spotless male in two different seasons (a) percent mated pairs of spotted and spotless males under control and cold stress (b) mean of fecundity under different conditions with control group (c) changes in percent mated pairs of spotted and spotless males in *D. sukuzii indicus* in control vs. flies exposed to desiccation stress (d).

Table 1: The frequency of both spotted and spotless wild males captured in different seasons (Autumn and winter).

Seasons	Autumn		Winter	
	Spotted male	Spotless male	Spotted male	Spotless male
No. of individuals	78	48	158	41
Frequency	61.90%	38.09%	79.39%	20.60%

Table 2: Data (mean \pm S.E.) on the basis of female choice method for percent mated pairs (MP), for mating latency (ML), and copulation duration (CD) under control and stressful conditions for *D. sukuzii indicus*. For each experiment, there were twenty replicates. *** $p < 0.001$; S = spotted males; SL = spotless males.

Experiment/ Mated pairs	MP (%)	ML (min) $m \pm SE$	CD (min) $m \pm SE$
(A) Control			
1. ♀ X S ♂	25.40	08.50 \pm 2.33	22.40 \pm 2.02
2. ♀ X SL ♂	16.30	17.40 \pm 3.05	16.60 \pm 3.12
Contingency χ^2	***	***	***
(B) Desiccation stress			
1. ♀ X S ♂	48.80	09.40 \pm 1.10	26.30 \pm 2.11
2. ♀ X SL ♂	11.40	18.45 \pm 2.33	15.30 \pm 1.40
Contingency χ^2	***	***	***
(C) Cold stress			
1. ♀ X S ♂	34.40	10.30 \pm 1.44	27.22 \pm 1.38
2. ♀ X SL ♂	08.20	23.30 \pm 2.89	12.40 \pm 1.56
Contingency χ^2	***	***	***

Discussion

Ectothermic insects live in harsh environments, are predicted to develop adaptive traits (Hoffmann & Parsons 1997; Gibbs & Matzkin 2001; Matzkin *et al.* 2007) [14, 10, 26]. The difference in the thermal characteristics of the dark and light morphs causes thermal melanism in two-spot ladybirds and arctic/alpine butterflies (Majerus 1994; Brakefield & Willmer 1985; Berry & Willmer 1986) [5, 43, 25]. Seasonal variations in body color variation for temperate populations of *Pieris* butterflies (Kingsolver and Wiernasz 1991) [17] and *D. putrida* (Sabath *et al.* 1973) [34] also correlate with thermal melanism. Timofeeff-Ressovsky demonstrated that the incidence of melanics decreased in the spring and increased in the fall based on field investigations on *Adalia bipunctata* conducted over a ten-year period (1929 to 1940) in Berlin (Timofeeff-Ressovsky 1940) [38]. The data on variations in humidity were not taken into account in this investigation, and these changes were explained in terms of the thermal characteristics of dark versus light morphs. In contrast to thermal melanism, there are reports of a higher frequency of melanic morphs in a number of species, including *Adalia bipunctata* (Luis 1973) [22], *Coccinella septempunctata* (Rhamhalinghan 1988) [32], *Harmonia axyridis* (Osawa & Nishida 1992) [28], and the current data on *D. biarmipes* from India (Parkash *et al.* 2013) [30]. Therefore, the reason why melanic variants arise in the tropics is unclear.

The effects of humidity variations on body color polymorphism in the wild and in laboratories are poorly studied. In Brazilian populations of *D. polymorpha*, Brisson and colleagues have shown evidence of relationships between body melanism and habitat types; that is, dark morph occur in open areas, whereas light morph exist in forested environments (Brisson *et al.* 2005) [6]. Nevertheless, the habitat preference of wing spot males was not examined in the current research. The impacts of humidity variations have been revealed in *D. sukuzii indicus* due to the lack of temperature effects on either spotted or spotless adults. On the Indian subcontinent, relative humidity varies with latitude and longitude; that is, locations in the south and east show

noticeably greater relative humidity levels. According to field data, there is a noticeable seasonal difference in the percentage of spotted and spotless adults; that is, there are more spotted adults in the winter than in the autumn (Table 1; Fig. 1A). According to our statistics, the winter seasons dryer and colder conditions prefer spotted flies, while the autumn seasons dry and wet conditions favour spotless flies.

An organism's fitness depends on how its shape and behavior interact. Majerus and colleagues have conducted in-depth research on the color morphs of two-spot ladybirds that they prefer to mate with (Majerus 1994) [25]. In two-spot ladybirds, selection tests aimed at varying the level of mating preference revealed that this feature is genetically regulated (Majerus 1986) [24]. On the other hand, it has been demonstrated that no environmental condition affects the two-spot ladybird's ability to maintain melanic polymorphism (O' Donald & Muggleton 1979) [27]. In contrast, the preference for mating in *Harmonia axyridis* differed depending on the season. For example, in the spring, both melanic and non-melanic individuals favored mating with non-melanic males, but in the summer, melanic individuals were overrepresented in matings (Osawa & Nishida 1992) [28]. Nevertheless, we are not aware of any comparable field research using *Drosophila* species exhibiting variation in wing color. According to the current findings, various wing-spotted males exposed to varying environmental stresses exhibit preferences for mating; specifically, under conditions of desiccation and cold stress, the percentage of spotted males that mate increases (Table 2). Seasonal variations in the percent frequencies of spotted males in the field have been explained by these observations.

Two noteworthy characteristics characterize a phenotypic manifestation of wing melanization in *D. sukuzii indicus*: (i) The influence of temperature on the melanization of wings in both laboratory-grown flies and in the field; (ii) The preference of spotted males over spotless males during mating. The frequency of these wing melanism traits in various insect taxa found in tropical regions is unknown. Two similar cases that we are aware of are: (a) The Brazilian population of *D. polymorpha* does not exhibit heat plastic effects (Brisson *et al.*, 2005) [6]; (b) The dominant allele in populations of ten-spot ladybirds is non-melanic, and there is no change in the morphs based on location (Majerus 1994; Brakefield 1985) [25]. There should be adaptive importance to the widespread prevalence of such genetic methods among insect groups. The spotted male's superiority over the spotless male during mating may aid *D. sukuzii indicus* in adapting to the cold, dry, and rainy conditions found in the tropics.

Though it is widespread in insect species, melanism has a wide range of evolutionary origins. Thermal melanism is favorable due to the ecological significance of melanism in cold-adapted temperate insect species, such as cosmopolitan and temperate endemic drosophilids (Majerus 1998; Sabath *et al.* 1973) [23, 34]. On the other hand, seasonal variations in precipitation in tropical and subtropical areas result in desiccation stress in the fall. Through assortative matings, tropical species like *D. sukuzii indicus* have modified the frequencies of wing spot males in line with cold and dry (winter) and dry and wet (autumn) seasons in order to adapt to

environmental pressures. Precipitation and temperature are therefore the main factors influencing variations in the frequency of spotted and spotless male *D. suzukii indicus*. The melanism-desiccation theory is directly supported by field and lab data on *D. suzukii indicus*. Many tropical endemic species of the *suzukii* group and other melanogaster groups are anticipated to offer comparable evidence to *D. suzukii indicus*. We can draw the conclusion that seasonal variations in the tropics contribute to the persistence of spotted and spotless male *D. suzukii indicus* populations.

Conclusion

Our study provides comprehensive insights into the adaptive significance of wing melanization in *Drosophila suzukii indicus*, particularly focusing on the differences between spotted and spotless males in terms of mating propensity and stress resistance. Our results demonstrate that spotted males exhibit superior desiccation resistance, cold stress survival, copulation duration, and mating frequency compared to spotless males. Female choice experiments also revealed a higher percentage of mated pairs with spotted males across different mating scenarios. Moreover, in colder environments, spotted males showed significantly higher mating success, indicating an adaptive advantage under low-temperature conditions.

The observed seasonal variations in the frequency of spotted and spotless males in the field, with higher frequencies of spotted males during colder and drier seasons, suggest a correlation between wing melanization and environmental factors such as temperature and humidity. This phenomenon aligns with the thermal melanism hypothesis and supports the idea that melanization in *D. suzukii indicus* is adaptive, potentially providing fitness benefits in response to seasonal changes.

Our findings contribute to the understanding of melanism in tropical insect populations, highlighting the complex interplay between environmental conditions, stress resistance, mating behavior, and phenotypic traits. Further research exploring the genetic basis of wing melanization and its evolutionary implications in *D. suzukii indicus* and related species would provide valuable insights into the mechanisms driving adaptive evolution in tropical insects.

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