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FLIGHT ACTIVITY IN RELATION TO FLIGHT MUSCLE AND WING AREA IN THREE INSECT SPECIES

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ABSTRACT: Factors like larger wings, greater thorax mass, a higher thorax-abdomen ratio, more stored reserves, and lower wing loading enhance flight performance, movement and dispersal. This study assessed the flight activity of three species—*Melanitis leda* (both male and female), *Apis dorsata* (worker female), and *Calliphora* sp. (both male and female)—and evaluated their dorsolongitudinal flight muscle (DLM) and forewing area. Tethered technique was used to observe the flight activity. *A. dorsata* showed the longest mean flight duration (4.14 min), followed by male *M. leda* (2.30 min), female *M. leda* (1.42 min), female *Calliphora* sp. (1.35 min), and male *Calliphora* sp. (0.19 min). In *M. leda*, male flight activity correlated positively with forewing volume (r = 0.565) and negatively with DLM volume (r = -0.91), while female showed strong positive correlation with forewing volume (r = 0.925) and weak positive correlation with DLM volume (r = 0.274). In *A. dorsata*, flight activity slightly decreased with larger wings (r = -0.07) and DLM (r = -0.560). In *Calliphora* sp., male showed negative correlations with forewing (r = -0.328) and DLM volumes (r = -0.567), while female showed positive (r = 0.316) and weak negative (r = -0.089) correlations, respectively. These findings highlight the complex interplay between flight morphology and activity, offering new insights into the conservation strategies of different insect species.

Key words: Flight muscle, Flight activity, *Melanitis leda, Apis dorsata, Calliphora* sp.

INTRODUCTION

The mechanics of insect flight rely extensively on wings and flight muscles. Flight muscles serve as the power engine, while the wings generate the aerodynamic forces essential for flight (Lu *et al.* 2020). The structure and kinematics of insect wings are highly varied, with some species possessing a single pair of wings, others two pairs of connected fore- and hind wings, and yet others two pairs of unconnected wings performing different strokes (Grodnitsky 1995). This diversity in wing morphology and flight mechanics enables insects to adapt to a wide range of ecological niches and environmental conditions.

Traits such as increased wing size, thorax mass, thorax-abdomen ratio, and

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stored reserves, as well as decreased wing loading, have been shown to enhance flight performance, thereby facilitating movement and dispersal. Sexual dimorphism in these traits suggests differential selection pressures on flight-related characteristics between male and female. Additionally, behavioral traits like exploration, territoriality, aggression, and sociability, which may vary between sexes, can impact the intrinsic motivation for movement and dispersal (Reim *et al.* 2019). Body size and wing loading (body mass/wing area) are also positively correlated with flight speed in neotropical butterflies (Dudley and Srygley 1994).

Long-distance airborne migrations enable insects to escape degrading habitats and seek more favorable conditions. Accurately assessing flight behavior and capacity across a range of economically beneficial and non-beneficial migratory insects is of great interest because these migratory trips influence pathogen dissemination, the invasion of major agricultural and public health pests, the seasonal cycling of substantial biomass, and the distribution of pesticide resistance alleles (Altizer *et al.* 2011, Chapman *et al.* 2015, Dingle 2014, Hu *et al.* 2016, Minter *et al.* 2018). The study of insect dispersal not only enhances our understanding of insect populations but also aids in developing pest invasion forecasting systems for farmers and foresters.

The three insect species selected for this study *Melanitis leda* (Lepidoptera: Nymphalidae), *Apis dorsata* (Hymenoptera: Apidae), and *Calliphora* sp. (Diptera: Calliphoridae) represent diverse taxa with distinct flight behaviors, ecological significance, and physiological adaptations. *M. leda* is a common tropical butterfly that serves as a pollinator, environmental indicator, and food source in ecosystems, and is sensitive to climatic fluctuations and habitat changes (Ghazanfar *et al.* 2016). Its moderate flight ability and large wings make it ideal for examining dispersal in lepidopterans. *A. dorsata*, the giant honeybee, is an ecologically and economically important species in tropical regions due to its long-distance foraging capabilities, strong flight muscles, and role in pollination and honey production (Batra 1997, Neupane *et al.* 2006, Raju and Rao 2004). It uses indirect flight muscles, particularly the dorsolongitudinal (DL) and dorsoventral (DV) muscles, for various behaviors such as flight, thermoregulation, and hive ventilation (Esch and Goller 1991). *Calliphora* sp. is a widely distributed blowfly known for its rapid, agile flight and ecological roles in pollination, decomposition, and forensic investigations (Heath 1982). Its selection allows for the comparison of flight performance in a dipteran species with different flight mechanics. Including these species provides a broad perspective on how variation in flight-related morphology affects movement across ecologically relevant insect groups. Therefore, this study aimed to assess the flight activity of three insect species—*M. leda*, *A. dorsata* and *Calliphora* sp. by examining the relationship between flight muscle and wing area.

MATERIAL AND METHODS

The experiment was conducted in the Entomology Research Laboratory, Department of Zoology, University of Dhaka, from September 2021 to February 2022. Specimens were collected from the Curzon Hall area, Animal Garden, and Botanical Garden of the University of Dhaka. Each parameter was assessed using five replicates. In this study, both male and female individuals of *M. leda* and *Calliphora* sp. were included to evaluate potential sex-based differences in flight performance. For *A. dorsata*, only female worker bees were used, as they are the primary foragers responsible for flight-based activities such as nectar collection, pollination, and hive thermoregulation, whereas male have limited flight activity (Gary 1967, Raju and Rao 2004).

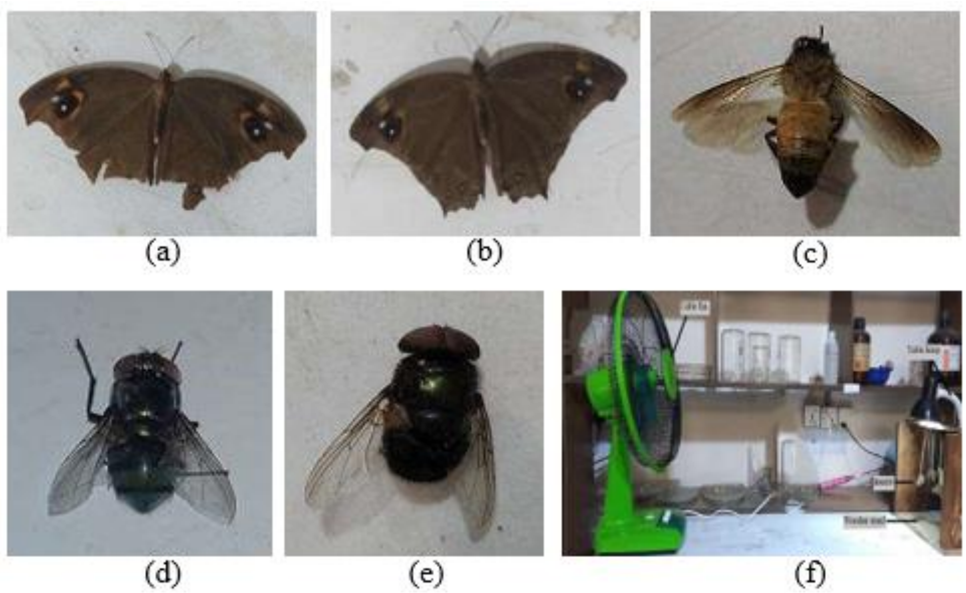

Fig.1. Biological materials and experimental setup. (a) *Melanitis leda* (male), (b) *Melanitis leda* (female), (c) *Apis dorsata* (worker), (d) *Calliphora* sp. (female), (e) *Calliphora* sp. (male), (f) Tethered flight settings.

Observation of flight activity: Flight activity was observed through tethered technique which involved attaching the insect to a fixed support and recording wing beating duration (Dudley and Ellington 1990, Ferdousi *et al.* 2021, Sane 2003). A table fan at 1200 RPM at a distance of one meter and a table lamp were used as stimuli. Each species was collected using a sweeping net and identified morphologically (Chapman 1998, Kabir *et al.* 2009a,b). The insects were anesthetized at -4°C, then tethered to a wooden stand. After regaining consciousness, wing movement duration was recorded within one hour using a stopwatch. The duration of flight activity for each specimen was recorded and expressed in minutes.

Measurement of forewing: Forewing measurements were taken, and bodies were preserved in 70% alcohol. Forewing volume was determined using the following formula, $V = \frac{1}{3}\pi r^2 h$, Where, V = forewing volume, π = 3.1416, r = radius (half of breadth of forewing), h = length of forewing.

Measurement of flight muscle: Preserved insects were dissected in a waxed plate and observed through microscope. The measurements were taken using 'ImageFocus' software. Flight muscle volume was determined using following formula, $V = \pi r^2 h$, Where V = flight muscle volume, π = 3.1416, r = radius (half of breadth of flight muscle), h = length of flight muscle.

Data analysis: All statistical analyses were conducted using Excel 2013, including calculations of mean, standard error, t-tests, and correlation.

RESULTS AND DISCUSSION

Observation of flight activity: The flight activity of male and female *M. leda*, *Calliphora* sp. and worker *A. dorsata* are presented in Table 1. Male *M. leda* had an average flight duration of 2.30 minutes, while female averaged 1.42 minutes. Male *Calliphora* sp. had a flight duration of 0.19 minutes, and female averaged 1.35 minutes. *A. dorsata* had the longest flight duration at 4.14 minutes. Male *M. leda* and *Calliphora* sp. exhibited longer flight durations than their female counterparts. *A. dorsata* demonstrated the highest flight endurance among the studied species, aligning with its ecological role as a long-distance forager (Neupane *et al.* 2006). *Calliphora* sp. had the lowest flight activity, possibly due to lower wingbeat frequencies relative to their body mass compared to honeybees (Unwin and Corbet 1984). The study also found that male *M. leda* was faster flyers than female, despite female having stronger flight muscles. Male had lighter abdomens, reducing wing loading and energy expenditure, while female's heavier abdomens increase wing loading and energy requirements, limiting their speed (Wickman and Karlsson 1989).

Table 1. The flight activity of *M. leda*, *Calliphora* sp. and *A. dorsata* (in minutes)

	M. leda **[Mean (SE)]**	***Calliphora* sp. [Mean (SE)]**	***A. dorsata*** **[Mean (SE)]**
Male	2.30 (1.02)	0.19 (0.10)	
Female	1.42 (1.19)	1.35 (0.79)	4.14 (2.54)

Measurement of forewing: Table 2 shows the measurements of length, breadth, and volume of the forewing for male and female *M. leda*, *Calliphora* sp., and female *A. dorsata*. Male *M. leda* had a mean length of 37.4 mm, breadth of 24.4

mm, and volume of 5834.8 mm³, while female had a mean length of 38.0 mm, breadth of 24.2 mm, and volume of 5860.5 mm³. The mean length and volume of forewing of female *M. leda* was greater than male but the mean breadth of forewing of male was slightly higher than female. The wing of *A. dorsata* was a smaller than *M. leda*. The mean of length, breadth and volume of forewing of *A. dorsata* were 13.3 mm, 4.5 mm and 70.5 mm³. The wing measurements of *Calliphora* sp. were smaller than those of both *M. leda* and *A. dorsata*. In male *Calliphora* sp., the mean forewing length, breadth, and volume were 7.9 mm, 3.1 mm, and 20.1 mm³, respectively, while in female, these values were 7.7 mm, 3.1 mm, and 19.6 mm³, respectively.

Table 2. The measurement of forewing of *M. leda*, *Calliphora* sp. and *A. dorsata* in millimeter (mm)

Species	Gender	Mean (SE) length in mm	Mean (SE) breadth in mm	Mean (SE) volume in mm³
M. leda	Male	37.4 (0.40)	24.4 (0.25)	5834.8 (164.68)
	Female	38.0 (0.49)	24.2 (0.20)	5860.5 (146.65)
Calliphora sp.	Male	7.9 (0.19)	3.1 (0.10)	20.1 (1.82)
	Female	7.7 (0.20)	3.1 (0.10)	19.6 (1.92)
A. dorsata	Female	13.3 (0.17)	4.5 (0.11)	70.5 (3.14)

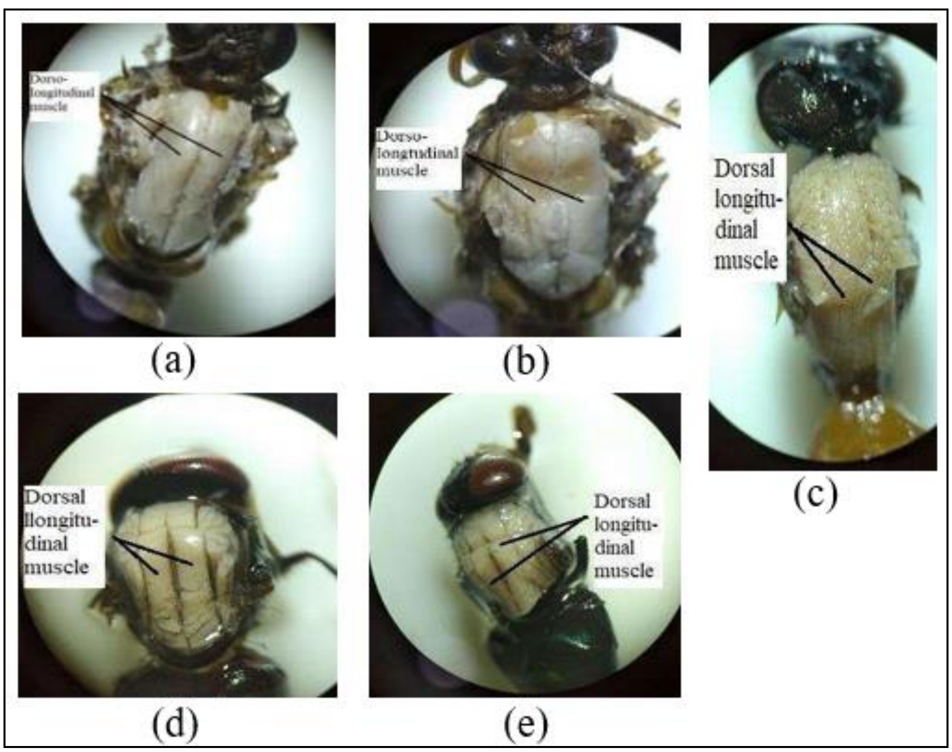

Fig. 2. The DLM of *M. leda* [(a) male, (b) female], female *A. dorsata* [(c)] and *Calliphora* sp. [(d) male, (e) female].

Measurement of flight muscle: Flight muscle was observed to understand their flight capacity. The main flight muscle, dorso-longitudinal muscle (DLM) was observed (Fig 2). The analysis of DLM showed that female *M. leda* exhibited larger DLM dimensions, with mean values of 6.2 mm in length, 3.3 mm in breadth, and a volume of 53.1 mm^3, compared to male with 5.7 mm in length, 2.9 mm in breadth, and a volume of 37.5 mm^3. These differences in muscle size were statistically significant for breadth and volume ($P<0.05$). In *Calliphora* sp., sexual dimorphism in DLM breadth was minimal. However, male exhibited a slightly longer DLM (4.5 mm) and a marginally greater volume (43.9 mm^3) compared to female (4.2 mm length, 40.4 mm^3 volume). In *A. dorsata*, the DLM had an average length of 5.1 mm, breadth of 3.0 mm, and a volume of 36.8 mm^3 (Table 3).

Table 3. The measurement of DLM of *M. leda*, *Calliphora* sp. and *A. dorsata* in millimeter (mm).

Species	Gender	Mean (SE) length of DLM (in mm)	Mean (SE) breadth of DLM (in mm)	Mean (SE) volume of DLM (in mm^3)
M. leda	Male	5.7 (0.20)	2.9 (0.13)	37.5 (1.39)
	Female	6.2 (0.12)	3.3 (0.15)	53.1 (4.01)
Calliphora sp.	Male	4.5 (0.16)	3.5 (0.16)	43.9 (5.17)
	Female	4.2 (0.12)	3.5 (0)	40.4 (1.18)
A. dorsata	Female	5.1 (0.10)	3.0 (0.11)	36.8 (3.16)

Flight activity and wing-muscle correlation: In *M. leda*, male showed a moderate positive correlation between flight activity and forewing volume ($r = 0.565$) and a strong negative correlation with DLM volume ($r = -0.91$), suggesting that as forewing size increased, flight activity tended to increase, while higher DLM volume was associated with reduced flight activity. In female, a very strong positive correlation was observed between flight activity and forewing volume ($r = 0.925$) and a weak positive correlation with DLM volume ($r = 0.274$). Overall, the relationship between flight activity and forewing volume appeared stronger in female than in male (Fig. 3).

In female *A. dorsata*, flight activity decreased slightly as forewing area increased ($r=-0.07$) and decreased moderately with increasing DLM volume ($r=-0.560$). (Fig 4). In *Calliphora* sp., male individuals showed a negative correlation between flight activity and both forewing volume ($r=-0.328$) and DLM volume ($r=-0.567$), suggesting that as forewing or DLM size increased, flight activity tended to decrease, with a stronger relationship observed for DLM volume. In contrast, female exhibited a positive correlation with forewing volume ($r = 0.316$) and a negative correlation with DLM volume ($r=-0.089$). Overall, the relationship between flight activity and morphological traits in *Calliphora* sp. appeared to be more pronounced in male than in female (Fig 5).

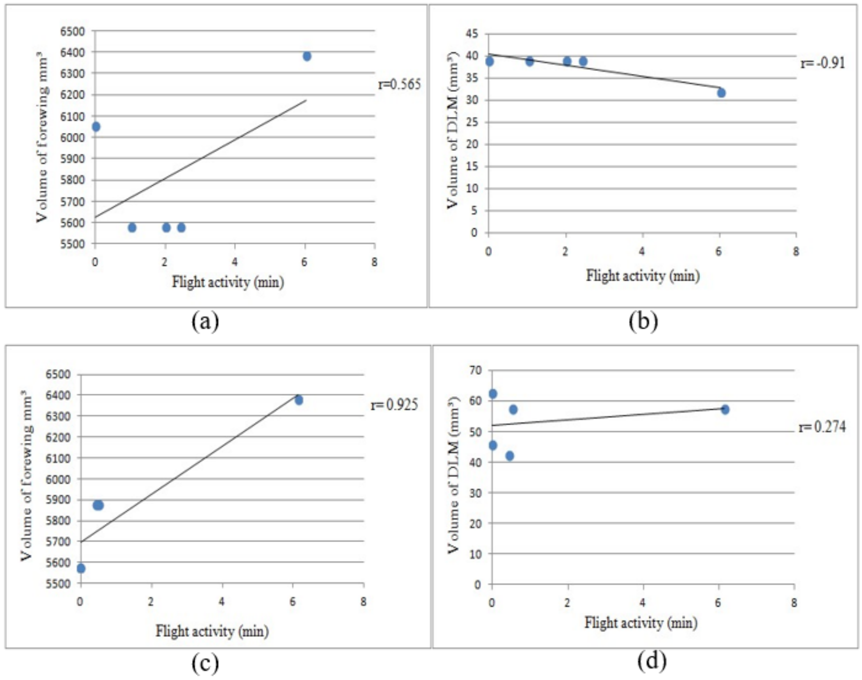

Fig.3. Correlation between flight activity and the volume of forewings and DLM in (a, b) male and (c, d) female *M. leda*.

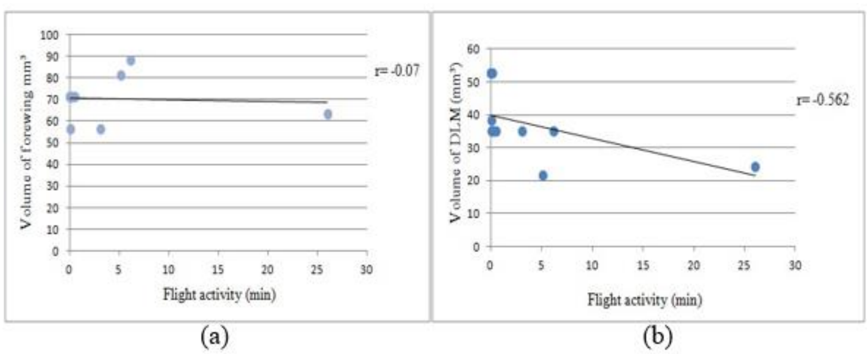

Figure 4. Correlation between flight activity and the volume of (a) forewing and (b) DLM in female *A. dorsata*.

These findings align with previous research, which suggests that flight muscle size and wing morphology significantly influence flight performance. For example, in an earlier study, male *Junonia almana* butterflies were found to be weaker fliers than female, who had stronger flight muscles, thereby improving their pollination efficiency (Roy *et al.* 2020). However, the current study found that female *M. leda*, despite having larger thoraxes, were weaker fliers than male, highlighting species-specific differences. This discrepancy may also be influenced by factors like the oogenesis-flight syndrome, which posits a trade-off between reproductive and flight capabilities. Moreover, earlier studies support

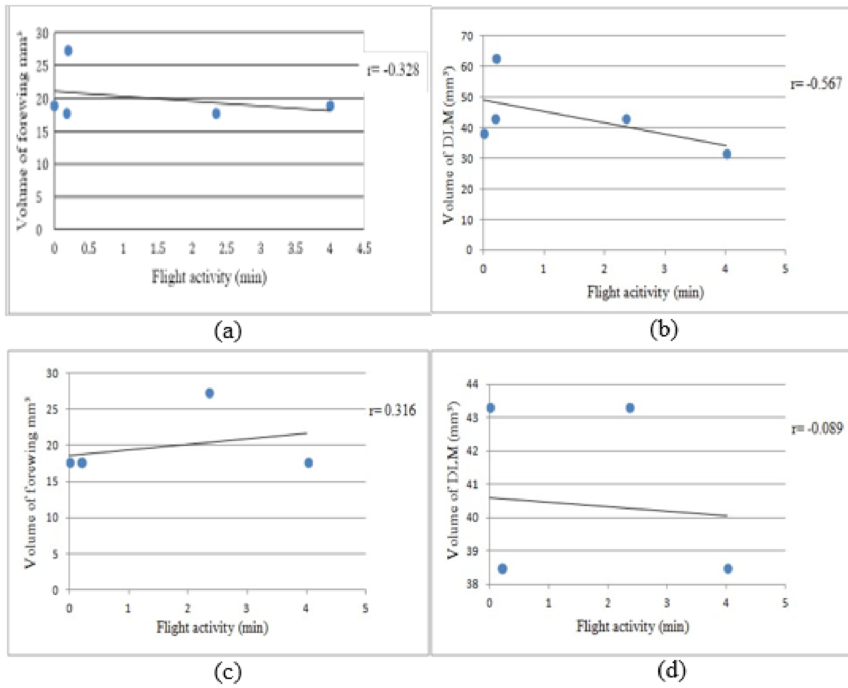

Fig.5. Correlation between flight activity and the volume of forewing and DLM in (a, b) male and (c, d) female *Calliphora* sp.

the idea that egg production and flight efficiency are mutually exclusive to some extent (Almbro and Kullberg 2012, Schumacher *et al.* 1997). Larger thoraxes generally offer better flight performance due to increased muscle power and thrust, but morphological efficiency varies (Mena *et al.* 2020). For example, a study on *Danaus plexippus* butterflies found that while both female and male had similar relative thorax sizes (wing muscle), female, despite being smaller, had thicker wings with greater mechanical strength and lower wing loading, which contributed to their higher migratory success (Davis and Holden 2015). Long and narrow wings are more efficient in reducing drag and conserving energy during flight compared to short and wide wings (Mena *et al.* 2020).

However, the present study found that female *M. leda* had higher thorax volume and wing area than male, which might explain the observed higher flight rate in male *M. leda*. For *Calliphora* sp., male had higher wing area and thorax volume, but female had greater flight activity. The observed differences in flight activity between sexes may be attributed to variations in muscle-to-body ratio and resource allocation strategies (Gibbs *et al.* 2010, Gibbs and Van Dyck 2010). Overall, the study underscores the complexity of flight dynamics influenced by morphological traits and suggests that further research is needed to understand the implications for pollination efficiency and flight adaptation in these species.

CONCLUSION

The present study aimed to investigate the relationship between flight activity, wing morphology, and DLM dimensions across three ecologically diverse insect species: *M. leda*, *A. dorsata*, and *Calliphora* sp. Our findings demonstrated that flight performance is not solely dependent on larger wings or greater flight muscle volume. Although female *M. leda* exhibited larger DLM and wing size compared to male, their flight activity was lower, suggesting that structural size does not always translate to superior flight ability. Similarly, *A. dorsata* worker, despite having smaller wings and moderate DLM volumes, achieved the longest flight durations, reflecting high flight efficiency adapted for foraging. Correlations between flight traits and activity varied across species and sexes, with *M. leda* male showing a positive association with wing volume but a negative one with DLM volume, while female showed strong positive correlations with both. In *Calliphora* sp., the relationships were weaker or negative, indicating different ecological or physiological strategies influencing flight performance. These results highlight the complex interplay between morphology and flight behavior and emphasize that flight capacity is shaped by a combination of wing architecture, muscle development, and ecological demands. The study provides valuable insights into the adaptive significance of flight-related traits and lays a foundation for further research on insect dispersal, evolution, and conservation strategies.

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