Melanic morphs of Batrocera dorsalis, (Hendel) (Diptera: Tephritidae) possess distinct developmental time, weight, wing size and shape

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Abstract

The Scutum of Bactrocera dorsalis individuals shows a high degree of variability in their colouration, which goes from black to brown. Such variation depicts a different level of melanin production in these individuals. Here we asked whether the progeny produced by four melanic morphs of B. dorsalis would present a difference in their developmental time, weight, wing size and shapes. To address this, we followed eggs produced by gravid females of each B. dorsalis melanic morphs by recording their pupation time, emergence time, and larval, pupal and adult weights. Also using the landmark-based geometric morphometric analysis we assessed the variation in wing size and shape of the adults obtained from each parental melanic morphs of B. dorsalis. We found that larvae produced by adults with dark scutum exhibited faster development and weighed more than those produced by adults with brown scutum. At adult stages, individuals from parents with darker and brown scutum had a reduced weight and wing size (length, width, area and centroid size) as compared to those from the parents with moderate melanin production. We also found a significant wing shape variation across the four melanic morphs. Our study shows that melanisation in the scutum of B. dorsalis has a fitness gain in the preimaginal stages of this fly. While in the adult stage, the heavier or the lesser melanin production in the scutum has a fitness cost. This suggests that there is a trade-off between melanin production and other fitness parameters in B. dorsalis which could have implication on its flight and dispersal and consequently, its management.

Introduction

The oriental fruit fly*, Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is one of the most important horticultural pests comprising of nearly 100 morphologically similar taxa present in at least 75 countries (Drew and Romig 2013; Zeng et al. 2019). They are characteristically invasive and polyphagous, with a broad host range and therefore widely distributed (USDA-APHIS 2016; CABI 2020). Originally,*B. dorsalis* was endemic to the tropical and subtropical regions of Asia from where it is believed to have spread to other parts of the world (Wan et al. 2012). In sub-Saharan Africa (SSA), it was first detected in Kenya in 2003, rapidly spreading into East and West Africa, as well as central southern parts of Africa within a short period (Lux et al. 2003; Mwatawala et al. 2004; Goergen et al. 2011 ; Magagula et al. 2015; Manrakhan et al. 2015). The high global dispersal capacity, colonization and adaption of *B. dorsalis* to new habitats is largely attributed to several factors incuding increased international trade, change in land use and strong climate tolerance (Qin et al. 2019; Zeng et al. 2019; Zhao et al. 2020). Colonization of various regions by*B. dorsalis* has a significant impact on the production of fruits and vegetables, as well as the socio-economic aspects of the whole value chain in the affected countries (Ekesi et al. 2016; EPPO 2020).

Bactrocera dorsalis has a long and intricate taxonomic history which has significantly impacted pest management strategies and market access (Schutze et al. 2015a). Previously, the world's most important

horticultural pests namely the Asian Papaya fruit fly, *Bactrocera papayae* Drew & Hancock, the Philippine fruit fly, *Bactrocera philippinensis* Drew & Hancock and the invasive fruit fly,*Bactrocera invadens* Drew, Tsuruta & White, were considered separate and independent *Bactrocera* species from *B. dorsalis* . However, through various reliable approaches such as molecular genetics (Boykin et al. 2014), sexual compatibility (Schutze et al. 2013; Bo et al. 2014) chemoecology (Tan et al. 2010) Cytogenetics (Augustinos et al. 2014), and morphology and morphometric analysis (Krosch et al. 2013; Schutze et al. 2012; 2015b), it was determined that*B. papayae* , *B. philippinensis* and *B. invadens* are morphologically and genetically same biological species as *B. dorsalis* hence its junior synonyms (De Meyer et al. 2015; Schutze et al. 2015a). This synonymization has occasioned a broad intraspecific morphological variation between members of the *B. dorsalis*complex, including the existence of colour polymorphism.*Bactrocera dorsalis* has a high degree of intraspecific variation in their scutum colour that varies from entirely black pale to entirely red-brown, with the existence of variable lanceolate-patterned intermediates (Leblanc et al. 2013; Schutze et al. 2015a).

Melanism is one of the discernible phenotypic variations in conspecific and heterospecific insects (Ma et al. 2008). It commonly occurs through the existence of morphs that are incompletely or completely dark in pigmentation. This gradient of pigmentation can appear on the different parts of an insect body including thorax (e.g. *Gryllus firmus*(Roff and Fairbairn 2013), abdomen (e.g. *Drosophila polymorpha*(Brisson et al. 2005), and wings (e.g. *Harmonia axyridis* (Chen et al. 2019)). Melanin plays physiological and ecological roles including species recognition and communication, mimicry, warning, courtship/mate selection, resistance to temperature and prey-predator/parasite interactions (Wittkopp and Beldade 2009). Its synthesis which involves a complex of biochemical reactions (Futahashi et al. 2008; Arakane et al. 2009) can favour or detriment insect life-history traits in the function of its environmental conditions. Yin et al. (2016) found that in hot environments and under long photoperiods, *Saccharosydne procerus*(Matsummura), melanic morphs have greater fitness parameters (longevity, mating rate, fecundity, and egg hatchability); whereas non-melanic morphs can adapt more successfully to low temperatures and short photoperiods. In *Spodoptera littoralis* (Biosduval), dark larvae and pupae are heavier, and take a shorter time to emerge as adults compared to their pale counterparts (Cotter et al. 2008). To the best of our knowledge, no comprehensive studies have investigated the impact of melanisation on the life-history traits, wing size and shape of tephritids. Here, using the four melanic morphs of *B. dorsalis*reared at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya, we monitored the development of their preimaginal stages by recording their pupation time, emergence time, larval weight and pupal weight. Also, using the landmark-based geometric morphometric technique, we measured the wing size and shape of the adult flies.

Materials and methods

Insects

Bactrocera dorsalis used in this study were reared at the Animal Rearing and Quarantine Unit of *icipe* , following the procedures described by Ekesi and Mohammed (2011). For this experiment, adults with similar scutum colouration pattern (Fig. 1) were separated from the mass-reared colony, kept in Perspex cages (80 × 80 × 80 cm) and maintained at 25 ±1°C, 60 ±10% RH and a photoperiod of L12:D12 for more than 30 generations before the start of experiments. These adults were fed on an artificial diet consisting of a mixture of sugar and ultrapure grade enzymatic yeast hydrolysate (USB Corporation, Cleveland, Ohio, USA) at the ratio of 3:1 by volume and were provided with water in a Petri dish (8.6 cm diameter) with a layer of pumice granules to prevent drowning of the flies.

Study of the life-history traits of the four melanic morphs of*Bactrocera dorsalis*

Newly emerged individuals of each melanic morph of *B. dorsalis*were separated from the general population rearing cages and placed into two smaller cages (15×15×20 cm) for easier sorting. A couple of each morph (1 female: 1 male) was placed in separate cages (15×15×20 cm). Pieces of mango dome (with curved out exocarp) were placed in each of the 4 cages (this was done on the 10th day from the date of emergence). After 24 h , the mango domes were retrieved from the cages and from each separate dome at least 10 eggs from females of each morph were picked and inoculated in an artificial liquid diet. On the fifth day (from

the day when eggs were inoculated), 30 larvae were individually weighed using the Sartorius MC-1 AC210S Analytical (made in Germany; 0.0001 g precision) weighing scale. Also, soon after these larvae pupated, the pupation time was recorded and each pupa was weighed. In each morph, the time (days) taken by adult flies to emerge from the pupae was also recorded. The experiment was done in three replicates of 10 each. To see whether all the life-history traits recorded varied across the different morphs, we performed the Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls (SNK) posthoc tests using the R package called "agricolae" (de Mendiburu, 2021). All statistical comparisons were considered significant when $P < 0.05$.

Adult weight, wing size and shape of the four melanic morphs of*Bactrocera dorsalis*

After emergence, 30 males and 30 females of respective morphs were killed, individually weighed, and their right-wing excised from the thorax using a fine clamp. The insects were then preserved in in 70% ethanol. The removed wings were singly slide-mounted before image capture using a stereo microscope with LED and HD Camera Leica EZ4 HD (Leica Microsystems, Switzerland) at 16x magnification, to avoid deformation and enhance accuracy during photography and landmark collections.

The digital photographs were opened in the ImageJ software and Cartesian coordinates for 15 wing landmarks were generated (Fig. 1B). Using the same software, wing size parameters including (i) wing length (distance between the 5th and 15th landmark); (ii) wing width (distance between the 3rd and 13th landmark) (Fig. 1B); and (iii) wing area were generated. We used the PAST software V.3.09 (Hammer et al. 2001) to compute the centroid size parameter considered as a multidimensional measurement of wing size. It is calculated as the square root of the sum of squared Euclidean distances between each landmark and the wing centroid (Baleba et al. 2019). Also, based on the adult weight parameter, we calculated wing loading using the following formula:$wing\ loading\ (\frac{\text{kg}}{m^2}) = \frac{\text{mass}}{\text{wing area}}$.

To assess whether these wing size parameters were affected by the sex of the adults, the different level of melanin, and their interaction, we ran a two-way analysis of variance (ANOVA). The only significant effect that was conserved across the different wing size parameters was that related to the melanin level. For this, we only considered this factor in the 2 by 2 comparison, then performed an SNK posthoc test. All statistical comparisons were considered significant when$P < 0.05$. To identify correlations between centroid size, wing length, wing width, wing area, adult weight and wing loading, we performed separate principal component analysis (PCA) for four groups using the R packages called "FactoMineR" (Le et al. 2008) and "Factoextra" (Kassambara and Mundt 2020).

To determine the change of the wing shape across the different melanic morphs, we imported the raw landmark Cartesian coordinates into MorphoJ software (Klingenberg, 2011). We first performed a generalised Procrustes analysis to extract shape information from the data and eliminate differences in orientation, position and isometric size. Afterwards, using sex and level of melanin as factors, we executed a multivariate analysis of variance (MANOVA) to see whether these factors could impact the wing shapes of *B. dorsalis* . To visualise the wing shape deformations, we use the PAST software to generate the wing deformation grid of the wing of each sex and morph using the thin-plate spline analysis. Also, we used canonical variate analysis combined with discriminant analysis to analyse the relative similarities (or dissimilarities) of the different melanic morphs. To see the significance of pairwise differences in mean shapes, we performed permutation tests (10,000 rounds) with Procrustes distances.

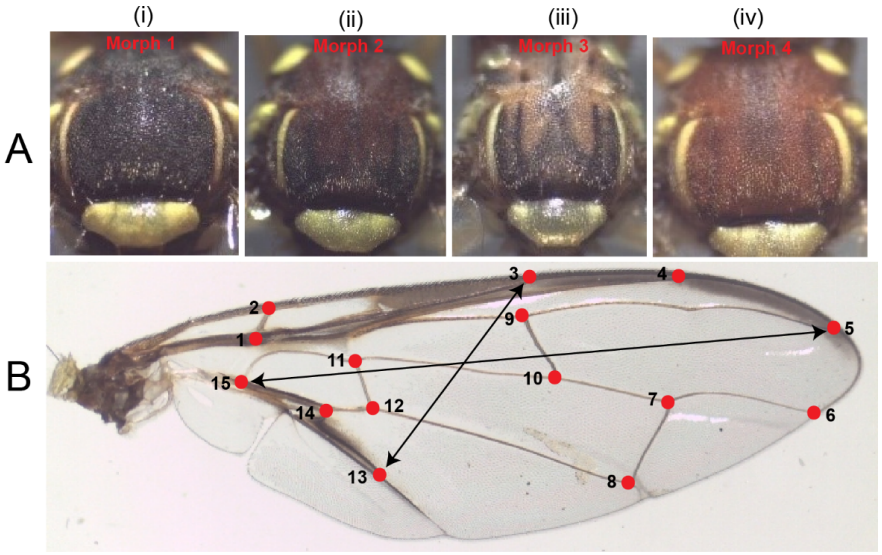

Figure 1. (A) Scutum colouration patterns of *Bactrocera dorsalis* individuals used in our study. Based on the dark intensity of their scutum, we named them morph 1 (i), morph 2 (ii), morph 3 (iii) and morph 4 (iv). (B) Dorsal view of the right-wing of *B. dorsalis* . The numbers indicate the location of the 15 landmarks targeted in our study.

Results

Change of life-history traits in the four melanic morphs of*Bactrocera dorsalis*

The preimaginal stages obtained from the four melanic morphs of *B. dorsalis* adults had significant different pupation times (ANOVA: $F_{3,\ 116}$ =164.3, *P<0.0001*), emergence time (ANOVA: $F_{3,\ 116}$ =118.7, *P<0.0001*), larval weight (ANOVA: $F_{3,\ 116}$ =13.97,*P<0.0001*) and pupal weight (ANOVA: $F_{3,\ 116}$ =19.45, *P<0.0001*). Larvae from morph 4 adults which has less melanin (pale) took a long time to reach the pupal stage as compared to those from the other morphs (Fig. 2A). Nonetheless, adults from this morph emerged faster from the pupae followed by adults from morph 1 (Fig. 2B). Larvae from morph 3 and 4 weighed less in comparison to those from morph 1 and 2 (Fig. 2 C). However, only pupae from morph 3 had a reduced weight; while pupae from morph 4 had the same weight as those from morph 1 and 2 (Fig. 2 D).

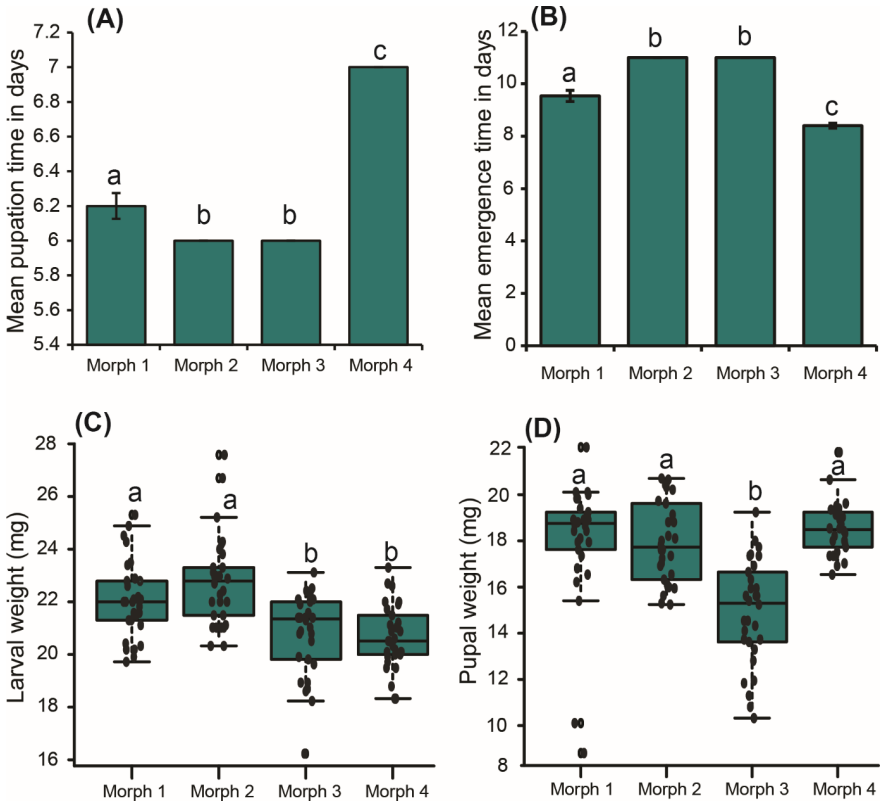

Figure 2. Some life-history traits in *B. dorsalis* change in function of the level melanisation of their scutum. Bar graphs showing the mean pupation time (A) and the mean emergence time (B) of the four melanic morphs studied. Boxplots depicting the larval (C) and pupal (D) weight of the four melanic morphs studied. Graphs with different letters illustrate significant differences across (ANOVA tests followed by the SNK posthoc test, $P<0.05$, n=30). On each bar graph, Error bars indicate the standard error of the mean. Each boxplot shows the median (lines in the box) with the interquartile range (whiskers). Dots on each boxplot represent the individual data point.

Variation of *B. dorsalis* wing size parameters across the melanic morphs

We found a significant variation of the wing length, wing width, wing area, wing centroid size and wing loading across the four melanic morphs of *B. dorsalis* (Table 1). The sex factor only affected the centroid size parameters. Flies from morph 2 and 3 had larger wing length (Fig. 3A.i), width (Fig. 3A.ii), area (Fig. 3A.iii) and centroid size (Fig. 3A.iv) as compared to those from morph 1 and 4. Flies from morph 4 possessed a smaller weight (Fig. 3A.v) and wing loading (Fig. 3A.vi). The first two dimensions that accounted for 95.9 % of the total wing size variation did not separate the four morph of *B. dorsalis* (Fig. 3B). Nonetheless, we observed a positive correlation between all the wing size parameters; with length, width, area, and centroid size being strongly related (Fig. 3B).

Table 1. Two factors analysis of variance table showing the variation of wing size and individual weight across the four groups of*B. dorsalis* studied.

	Factor	d.f.	Mean Sq	F value	*P value*
	Morphs	3	1.5492	15.954	<0.0001
Length	Sex	1	0.3693	3.804	0.052
	Morphs × Sex	3	0.0846	0.872	0.45

	Factor	d.f.	Mean Sq	F value	*P value*
	Morphs	3	0.4064	17.463	<0.0001
Width	Sex	1	0.0098	0.421	0.52
	Morphs × Sex	3	0.0166	0.715	0.54
	Morphs	3	18.42	16.412	<0.0001
Area	Sex	1	1.803	1.607	0.2
	Morphs × Sex	3	0.915	0.815	0.49
	Morphs	3	23247	13.856	<0.0001
Centroid Size	Sex	1	6823	4.067	**0.045**
	Morphs × Sex	3	1191	0.71	0.55
	Morphs	3	354.5	20.86	<0.0001
Adult Weight	Sex	1	0	0.002	0.96
	Morphs × Sex	3	11.2	0.661	0.58
	Morphs	3	3.348	23.395	<0.0001
Wing Loading	Sex	1	0.068	0.474	0.50
	Morphs × Sex	3	0.062	0.435	0.73

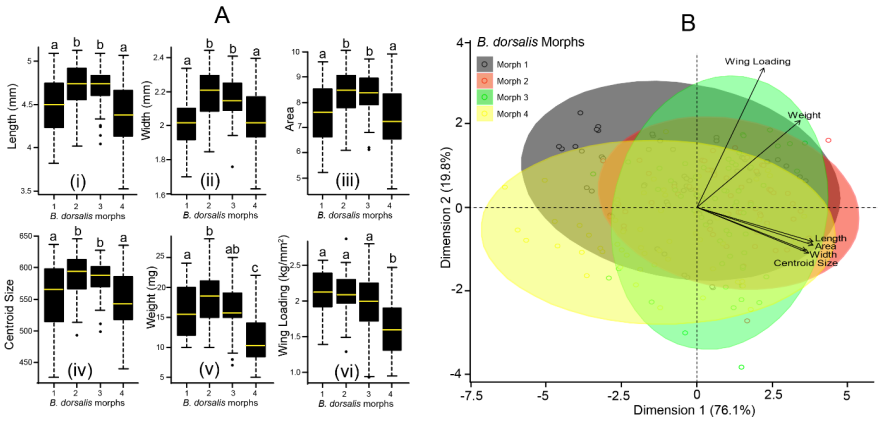

Figure 3. Adult weight and wing size vary across the four melanic morphs of *B. dorsalis* . **(A)** Boxplot showing the variation of wing length (i), wing width (ii), wing area (iii), wing centroid size (iv), adult weight (v) and wing loading (vi) across the four groups of *B. dorsalis* . Box plots show the median (horizontal lines) and the interquartile range (whiskers). Boxplot with different letters depict differences (ANOVA tests followed by the SNK posthoc test, *P<0.05* , n=30). **(B)** Principal component analysis biplot generated from the wing size parameters of all the four morph of *B. dorsalis* studied.

Wing shape variation across the four melanic morphs of *B. dorsalis*

We obtained a significant difference of *B. dorsalis* wing shape between the sexes and across the four melanic morphs (Table 2). The first two dimensions of the canonical variate analysis that explained 87.2 % of the total wing shape difference separated *B. dorsalis*based on their sex and morphs (Fig. 4A). In Male flies, all the pairwise permutation tests performed using the Procrustes distances showed a significant divergence of wing shape of the different male morphs (Fig. 4B.i). An illustration from the thin plate spline deformation grid depicted this deformation (Fig. 5A). For instance, while landmarks 11, 12, 13 and 14 were undergoing expansion movement in the morphs 1, 2 and 3, the same landmarks contracted in morph 4 (Fig. 5A). In females, except individuals from morphs 2 and 3 that showed no difference in their wing shape (*P=0.2*), all the other two-by-two comparisons were significant (Fig. 4Bii). For illustration, landmarks 4, 5, 6, 7 and 8 of morphs 1 and 2 were more contracted as compared to those of morphs 3 and 4 (Fig. 5B).

Table 2. MANOVA table illustrating the significant difference of *B. dorsalis* wing shape across the four groups and between the sexes.

Effect	SS	MS	d.f.	F value	*P value*	Pillai tr.	*P value*
Groups	0.01	0.00013	78	2.95	<.0001		
Sex	0.08	0.0031	26	71.63	<.0001		
Groups × Sex	0.0033	0	78	2.03	<.0001	0.59	<.0001

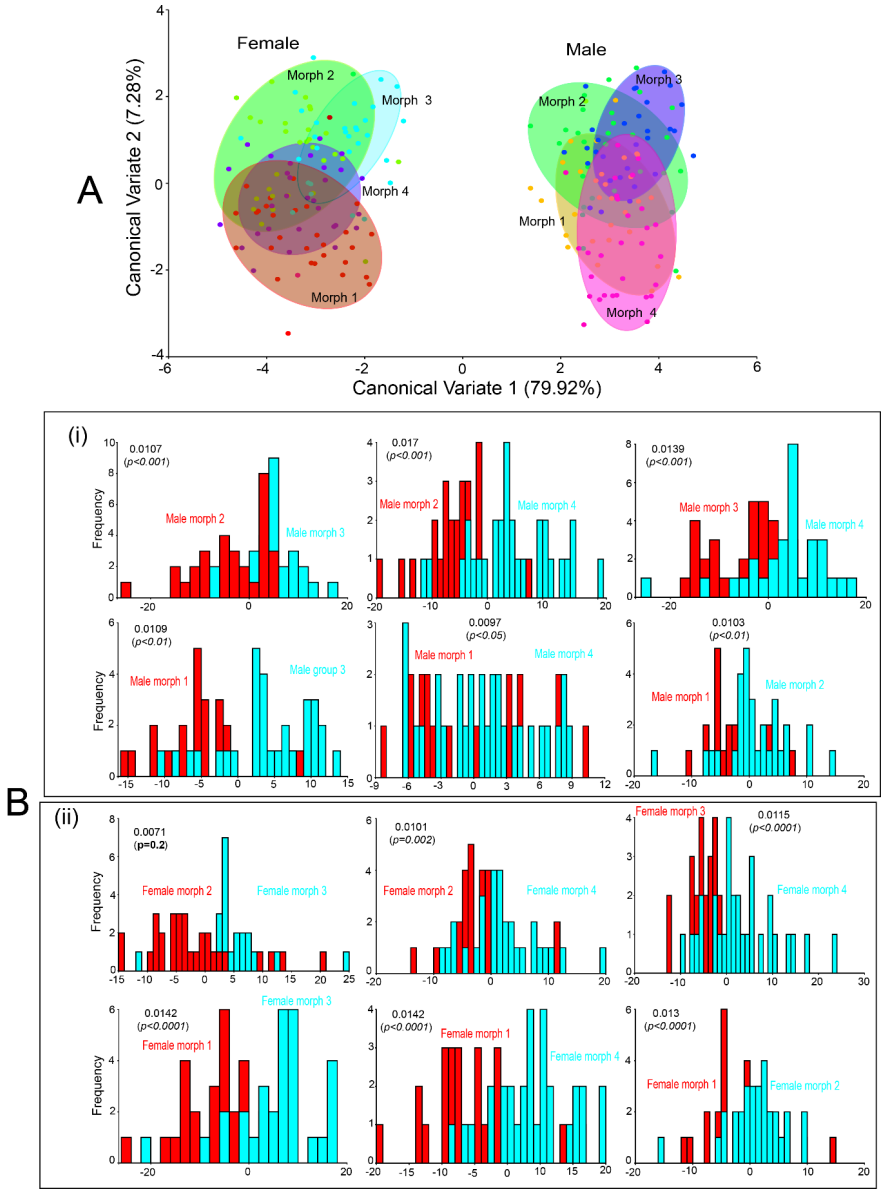

Figure 4. Wing shape of *B. dorsalis* varies across the four groups studied and between the sexes. **(A)** Graph from the canonical variate analysis clustering *B. dorsalis* individuals in function of their group and sex. **(B)** Histogram from the discriminant analysis depicting the difference of the wing shape in the different

groups of males (i) and females (ii) of *B. dorsalis* . Values above the parenthesis represent the Procrustes distance of two distinct groups. Values in the parenthesis show the significance level of the difference based on the permutation test. The differences are considered at $P<0.05$

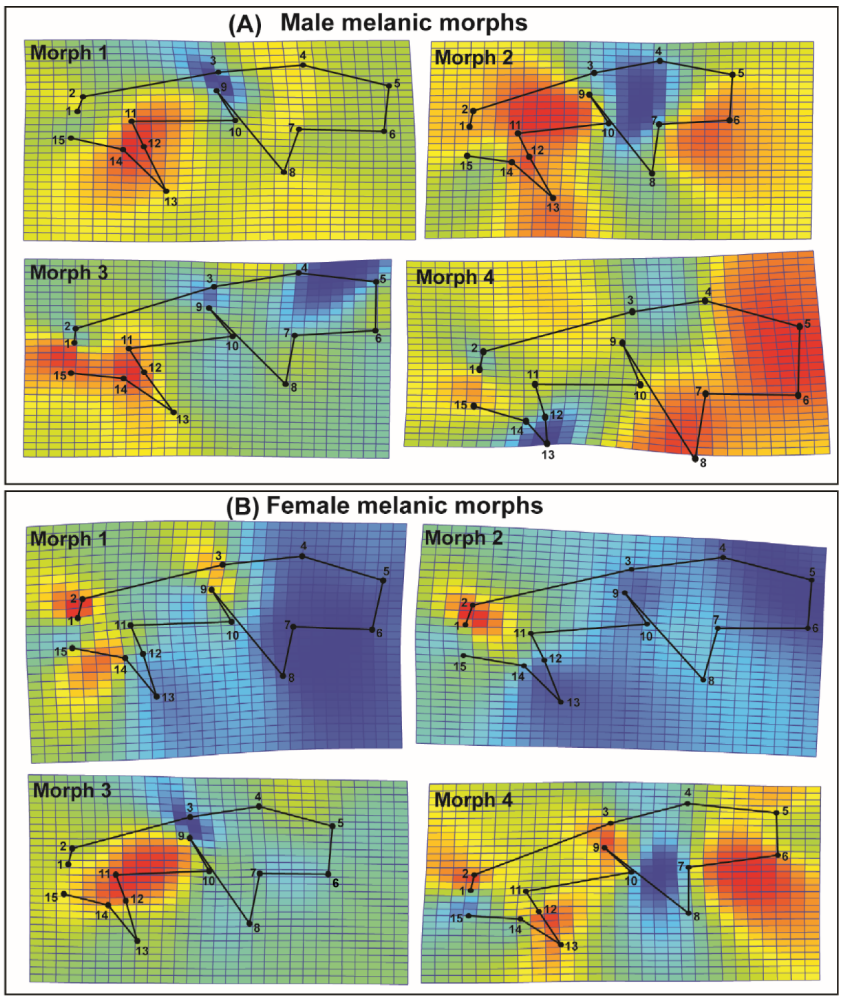

Figure 5. Thin plate spline illustrating the changes occurring in *B. dorsalis* wing shape between the sexes and across the different melanic morphs. The number displayed on each grid represents the landmark positions. Yellow to orange-red colours indicates landmark expansions, light to dark-blue indicates landmark contraction.

Discussion

In the present study, we aimed to see whether the plasticity existing in the colouration of *B. dorsalis* Scutum could persist in the developmental time, weight, wing size and shapes of this fly. We found a change of these fitness parameters in function of the level of melanisation of *B. dorsalis* Scutum. The offsprings from morph 1 and 2 which have an advanced level of melanisation had a shorter pupation time, heavier pupal and larval weight as compared to those produced by morph 3 and 4 characterized by a reduced level of melanisation (Fig. 2). This shows that melanin could possibly play an important role in the development of *B. dorsalis* preimaginal stages. To our knowledge, in Diptera, no studies have investigated the impact of adult melanisation on their progeny fitness. Few studies that exist are centred on the impact of melanin on adults. For instance, studies conducted on adults of *Drosophila polymopha* and *D. immigrans*

(Diptera: Drosophilidae) revealed that melanic morphs copulate longer, have higher fecundity and desiccation resistance (Brisson et al. 2005; Singh et al. 2009). Nonetheless, the fitness gained in preimaginal stages owing to melanic presence is found in other insect orders such as Lepidoptera. In the homozygous melanic strain of*Spodoptera exigua* (Lepidoptera: Noctuidae), Liu et al. (2015) showed that all the life stages of this moth have faster development and heavier weight than the brown strains. Also, faster development was found in the melanic morphs of *Mythimna separate* (Lepidoptera: Noctuidae) (Jiang et al. 2007), and *Biston betularia*(Lepidoptera: Geometridae) (Lorimer 1979). Our results are contrary to the trade-off hypothesis between melanism and fitness postulating that melanism is likely to have fitness cost than gain (Roff and Fairbairn 2013). Liu et al. (2015) further explain that the fitness change in response to melanin production should be case-specific. We argue that in*B. dorsalis* , adults with darker Scutum will give offspring with better life-history traits.

Adults obtained from the four parental morphs of *B. dorsalis* used in our study also presented differences in their weight, wing size and shape. We found that melanic morphs 2 and 3 had greater weight, wing length, wing width, wing area and centroid size as compared to melanic morphs 1 and 4 (Fig. 3A). This result on adults contrast those obtained on the preimaginal stages; where offspring's obtained from morph 1 had better fitness. This implies that in comparison to their preimaginal stages, adults of *B. dorsalis* will be more likely to benefit from a moderate production of melanin than its heavier or lesser secretion. We speculate that the fitness disadvantage found in darker adults (morph 1) could be the fact of the energy loss during the vast melanin production in their Scutum. In insects, the production and maintenance of melanin-based colouration are known to be energetically costly (Talloen et al. 2004; Stoehr 2006; González-Santoyo and Córdoba-Aguilar 2012; Roulin 2016). Therefore, in individuals such as morph 1 where melanin is massively produced, one could expect an energy allocation problem; solved by a distinctive energy investment in different biological processes. Therefore, it seems that in morph 1, most energy is allocated to melanisation; disadvantaging other fitness traits such as body weight and wing size. In morph 4, the lesser melanin production could explain the reduced body weight and wing size found. It is explained that heat penetrates faster the body of individuals with melanin than the one without melanin (Clusella-Trullas et al. 2008). This consequently can lead to variation in body temperatures, and such change may affect many life-history traits, e.g., development time, growth rate, and body weight (Su et al. 2013). We infer that in *B. dorsalis* adults, there is a need for a balance between melanin secretion and other biological processes. Studies involving various insect models have also observed a trade-off between melanin production and other fitness parameters. For illustration, Ma et al. (2008) found that in adults of *Helicoverpa armigera* (Hübner) melanism is associated with lower mating rate and fecundity, less mating time, and accordingly, lower net reproduction rate and population trend index.

In Pterygota, the flight capacity of an individual heavily relies on its wing size. According to Gidaszewski et al. (2009) there is a relation between flight behaviour, mating systems and variations in wing shape. Similarly, DeVries et al. (2010) demonstrates that long and large wings in insects are associated with long flight duration and high speed. Therefore, we predict that melanic morphs 2 and 3 of *B. dorsalis*are more likely to travel a long distance at a high speed to search for food, mates and appropriate oviposition substrates. This could render these morphs more aggressive in invading their host plants. Additionally, studies indicate that the ability of *B. dorsalis* to perform long-distance flights can enable this fly to disperse widely and infest more plants (Chen and Ye 2007; Froerer et al. 2010; Wan et al. 2011). Variation in size too can induce variations in shape (Debat et al. 2003). Based on the Procrustes ANOVA and the Canonical Variate analysis we confirmed this on the wing shapes in the four melanic morphs of *B. dorsalis* studied. We found that the wing shape of these morphs was both sex and colour morphs dependent. The variation of wing shape at the intraspecific and sex levels has also been reported in other insect species including *Drosophila melanogaster* (Meigen) (Reis et al. 2017), *Tongeia fischeri* (Eversman) (Jeratthitikul et al. 2014) and *Haematobosca aberrans* (Bezzi) (Changbunjong et al. 2020).

In conclusion, the study has underscored the existence of differences in developmental time, weight, wing size and shapes among the morphs of*B. dorsalis.* These subtle intraspecies morphological variations might have a significant influence on the overall performance of adult*B. dorsalis* including flight and dispersal and

consequently, management of this quarantine pest.

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Author contributions Conceptualization: N.L.M and S.B.S.B.; methodology: N.L.M and SBSB.; investigation: N.L.M.; data analysis: S.B.S.B.; funding acquisition: S.A.M.; project administration: S.N. and S.A.M.; supervision: S.N. and S.A.M.; original draft: N.L.M and S.B.S.B.; review and editing: S.N., S.A.M., N.L.M and S.B.S.B. All authors have read and agreed to the published version of the manuscript.

References

Arakane, Y. et al. 2009. Molecular and functional analyses of amino acid decarboxylases involved in cuticle tanning in Tribolium castaneum. - J. Biol. Chem. 284: 16584–16594.

Augustinos, A. A. et al. 2014. The Bactrocera dorsalis species complex: Comparative cytogenetic analysis in support of Sterile Insect Technique applications. - BMC Genet. 15: 1–10.

Baleba, S. B. S. et al. 2019. Effect of larval density and substrate quality on the wing geometry of Stomoxys calcitrans L. (Diptera: Muscidae). - Parasites and Vectors 12: 1–11.

Bo, W. et al. 2014. Mating compatibility between Bactrocera invadens and Bactrocera dorsalis (Diptera: Tephritidae). - J. Econ. Entomol. 107: 623–629.

Boykin, L. M. et al. 2014. Multi-gene phylogenetic analysis of south-east Asian pest members of the Bactrocera dorsalis species complex (Diptera: Tephritidae) does not support current taxonomy. - J. Appl. Entomol. 138: 235–253.

Brisson, J. A. et al. 2005. Abdominal pigmentation variation in Drosophila polymorpha: Geographic variation in the trait, and underlying phylogeography. - Evolution (N. Y). 59: 1046–1059.

CABI 2020. Bactrocera dorsalis (Oriental fruit fly) In: Invasive Species Compendium. - Wallingford, UK CAB Int.

Changbunjong, T. et al. 2020. Molecular identification and geometric morphometric analysis of haematobosca aberrans (Diptera: Muscidae). - Insects 11: 1–12.

Chen, P. and Ye, H. 2007. Population dynamics of Bactrocera dorsalis (Diptera: Tephritidae) and analysis of factors influencing populations in Baoshanba, Yunnan, China. - Entomol. Sci. 10: 141–147.

Chen, X. et al. 2019. The role of the dopamine melanin pathway in the ontogeny of elytral melanization in harmonia axyridis. - Front. Physiol. 10: 1–8.

Clusella-Trullas, S. et al. 2008. Testing the thermal melanism hypothesis: a macrophysiological approach. - Funct. Ecol. 22: 232–238.

Cotter, S. et al. 2008. Selection for cuticular melanism reveals immune function and life-history trade-offs in Spodoptera littoralis. - J. Evol. Biol. 21: 1744–1754.

de Mendiburu, F. 2021. Agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-5.

De Meyer, M. et al. 2015. Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. - Zookeys 2015: 1–3.

Debat, V. et al. 2003. Allometric and nonallometric components of Drosophila wing shape respond differently to developmental temperature. - Evolution (N. Y). 57: 2773–2784.

DeVries, P. J. et al. 2010. Vertical distribution, flight behaviour and evolution of wing morphology in Morpho butterflies. - J. Anim. Ecol. 79: 1077–1085.

Drew, R. and Romig, M. 2013. Tropical fruit flies (Tephritidae Dacinae) of South-East Asia: Indomalaya to North-West Australasia. - CABI.

Ekesi, S. and Mohammed, S. 2011. Mass Rearing and Quality Control Parameters for Tephritid Fruit Flies of Economic Importance in Africa. - Wide Spectra Qual. Control in press.

Ekesi, S. et al. 2016. Taxonomy, Ecology, and Management of Native and Exotic Fruit Fly Species in Africa. - Annu. Rev. Entomol. 61: 219–238.

EPPO 2020. PM1002(29) EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests. - EPPO Bull. 2: 1–19.

Froerer, K. M. et al. 2010. Long-distance movement of Bactrocera dorsalis (Diptera: Tephritidae) in Puna, Hawaii: How far can they go? - Am. Entomol. 56: 88–95.

Futahashi, R. et al. 2008. yellow and ebony are the responsible genes for the larval color mutants of the silkworm Bombyx mori. - Genetics 180: 1995–2005.

Gidaszewski, N. A. et al. 2009. Evolution of sexual dimorphism of wing shape in the Drosophila melanogaster subgroup. - BMC Evol. Biol. 9: 1–11.

Goergen, G. et al. 2011. Bactrocera invadens (diptera: Tephritidae), a new invasive fruit fly pest for the afrotropical Region: Host plant range and distribution in West and Central Africa. - Environ. Entomol. 40: 844–854.

González-Santoyo, I. and Córdoba-Aguilar, A. 2012. Phenoloxidase: a key component of the insect immune system. - Entomol. Exp. Appl. 142: 1–16.

Hammer, Ø. et al. 2001. PAST: Paleontological statistics software package for education and data analysis. - paleo.carleton.ca 4: 178.

Jeratthitikul, E. et al. 2014. Sexual dimorphism and intraspecific variation in wing size and shape of Tongeia fischeri (Lepidoptera: Lycaenidae). - Entomol. Sci. 17: 342–353.

Jiang, X. F. et al. 2007. Relative fitness of near isogenic lines for melanic and typical forms of the oriental armyworm, Mythimna separata (Walker). - Environ. Entomol. 36: 1296–1301.

Kassambara, A. and Mundt, F. 2020. factoextra: Extract and Visualize the Results of Multivariate Data Analyses version 1.0.7 from CRAN.

Klingenberg, C. 2011. MorphoJ: an integrated software package for geometric morphometrics. - Mol. Ecol. Resour. 11: 353–357.

Krosch, M. N. et al. 2013. Piecing together an integrative taxonomic puzzle: Microsatellite, wing shape and aedeagus length analyses of Bactrocera dorsalis s.l. (Diptera: Tephritidae) find no evidence of multiple lineages in a proposed contact zone along the Thai/Malay Peninsula. - Syst. Entomol. 38: 2–13.

Le, S. et al. 2008. FactoMineR: an R package for multivariate analysis. - J. Stat. Softw. 25: 1–8.

Leblanc, L. et al. 2013. A Preliminary Survey of the Fruit Flies (Diptera: Tephritidae: Dacinae) of Bangladesh. - Proc. Hawaiian Entomol. Soc. 45: 51–58.

Liu, S. et al. 2015. Pupal melanization is associated with higher fitness in Spodoptera exigua. - Sci. Rep. 5: 1–10.

Lorimer, N. 1979. The Genetics of Melanism in Malacosoma disstria Hübner (Lepidoptera: Lasiocampidae). - Genetics 92: 555–561.

Lux, S. A. et al. 2003. A New Invasive Fruit Fly Species from the Bactrocera dorsalis (Hendel) Group Detected in East Africa. - Int. J. Trop. Insect Sci. 23: 355–361.

Ma, W. et al. 2008. Trade-offs between melanisation and life-history traits in Helicoverpa armigera. - Ecol. Entomol. 33: 37–44.

Magagula, C. et al. 2015. Predicted regional and national distribution of Bactrocera dorsalis (syn. B. invadens) (Diptera : Tephritidae) in southern Africa and implications for its management. - African Entomol. 23: 427–437.

Manrakhan, A. et al. 2015. The progressive invasion of Bactrocera dorsalis (Diptera: Tephritidae) in South Africa. - Biol. Invasions 17: 2803–2809.

Mwatawala, M. et al. 2004. A new invasive Bactrocera species (Diptera : Tephritidae) in Tanzania. - African Entomol. 12: 154–156.

Qin, Y. et al. 2019. Climate change impacts on the global potential geographical distribution of the agricultural invasive pest, Bactrocera dorsalis (Hendel) (Diptera: Tephritidae). - Clim. Chang. 2019 1552 155: 145–156.

Roff, D. A. and Fairbairn, D. J. 2013. The costs of being dark: The genetic basis of melanism and its association with fitness-related traits in the sand cricket. - J. Evol. Biol. 26: 1406–1416.

Roulin, A. 2016. Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. - Biol. Rev. 91: 328–348.

Schutze, M. K. et al. 2012. Population structure of Bactrocera dorsalis s.s., B. papayae and B. philippinensis (diptera: Tephritidae) in southeast asia: Evidence for a single species hypothesis using mitochondrial DNA and wing-shape data. - BMC Evol. Biol. in press.

Schutze, M. K. et al. 2013. Mating compatibility among four pest members of the bactrocera dorsalis fruit fly species complex (Diptera: Tephritidae). - J. Econ. Entomol. 106: 695–707.

Schutze, M. K. et al. 2015a. Synonymization of key pest species within the Bactrocera dorsalis species complex (Diptera: Tephritidae): Taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. - Syst. Entomol. 40: 456–471.

Schutze, M. K. et al. 2015b. One and the same: integrative taxonomic evidence that Bactrocera invadens (Diptera: Tephritidae) is the same species as the Oriental fruit fly Bactrocera dorsalis. - Syst. Entomol. 40: 472–486.

Singh, S. et al. 2009. Fitness consequences of body melanization in Drosophila immigrans from montane habitats. - Entomol. Res. 39: 182–191.

Stoehr, A. M. 2006. Costly melanin ornaments: The importance of taxon? - Funct. Ecol. 20: 276–281.

Su, W. et al. 2013. Melanism in a Chinese Population of Harmonia axyridis (Coleoptera: Coccinellidae): A Criterion for Male Investment with Pleiotropic Effects on Behavior and Fertility. - J. Insect Behav. 26: 679–689.

Talloen, W. et al. 2004. The cost of melanization: butterfly wing coloration under environmental stress. - Evolution 58: 360–366.

Tan, K. H. et al. 2010. Comparison of phenylpropanoid volatiles in male rectal pheromone gland after methyl eugenol consumption, and molecular phylogenetic relationship of four global pest fruit fly species: Bactrocera invadens, B. dorsalis, B. correcta and B. zonata. - Chemoecology 2010 211 21: 25–33.

USDA-APHIS 2016. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Fruit Fly Host Lists and Host Assessments.

Wan, X. et al. 2011. The oriental fruit fly, bactrocera dorsalis, in china: Origin and gradual inland range expansion associated with population growth. - PLoS One 6: e25238.

Wan, X. et al. 2012. Invasion history of the oriental fruit fly, Bactrocera dorsalis, in the Pacific-Asia region: Two main invasion routes. - PLoS One 7: e36176.

Wittkopp, P. J. and Beldade, P. 2009. Development and evolution of insect pigmentation: Genetic mechanisms and the potential consequences of pleiotropy. - Semin. Cell Dev. Biol. 20: 65–71.

Yin, H. et al. 2016. The environmental plasticity of diverse body color caused by extremely long photoperiods and high temperature in Saccharosydne procerus (Homoptera: Delphacidae). - Front. Physiol. 7: 1–9.

Zeng, Y. et al. 2019. Global distribution and invasion pattern of oriental fruit fly, Bactrocera dorsalis (Diptera: Tephritidae). - J. Appl. Entomol. 143: 165–176.

Zhao, Z. et al. 2020. The synergy between climate change and transportation activities drives the propagation of an invasive fruit fly. - J. Pest Sci. (2004). 93: 615–625.