Trade-off between mass allocation and perching behavior in the pierid butterfly *Archonias brassolis tereas* (Godart, 1819) (Lepidoptera: Pieridae: Pierinae)

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Abstract

We tested the hypothesis that males of the perching butterfly *Archonias brassolis tereas* (Godart, 1819) (Lepidoptera: Pieridae: Pierinae) allocate more body mass to the thorax than patrolling pierid species. Butterflies were collected from January to November of 2009 on 59 different sampling occasions along the right bank of river Quilombo, Santos, SP, Brazil. Weights of abdomen, thorax and wings were recorded to estimate the aspect ratio of the wings and wing loading. Wing status was used to place specimens in the three different age classes. Analysis showed four groups of individuals with perchers being light and agile and patrollers heavy and slow. This study supports the hypothesis that the *A. b. tereas* have morphological traits associated with higher acceleration ability and speed. Some individuals which do not have these morphological traits to defend a territory might adopt the patrolling strategy.

Keywords: Mating behavior, perching strategy, territoriality, wing loading, wing aspect ratio

1. Introduction

The balance between morphological and physiological traits on a species’ natural history is important [1]. In flying insects, traits related to flight capacity are decisive to their survival. Wing aspect ratio (AR) is the ratio of its length to its breadth (chord). A high aspect ratio indicates long, narrow wings, whereas a low aspect ratio indicates short, stubby wing. In fixed wings, wing loading (WL) is the ratio between body mass and wing area that has an important influence on minimum speed. An increase in the wing area should reduce the value of W/S, and so reduce the minimum velocity at which level flight is possible [2].

Take-off is the behavior performed when a butterfly can quickly escape from a predator or attacks a rival who enter its territory and it is dependent of both AR and WL. However, the ability to fly for a longer time is important to search for food, host plants for oviposition and for finding partner for mating. These skills are different for each sex. Males of butterflies of some species can actively seek for females using the strategy of patrolling; where they actively search for females [3-5], otherwise, in perching species males await the passage of females. Consequently, there are species where the male defends limited areas (territories) to obtain females for mating purposes [6-8].

Alcock [8] studied the association of territoriality with mating systems in butterflies of three territorial species in Costa Rica. He also [9] found a scramble competition between males for access to emerging virgin females in two species of Australian butterflies, *Anaphaetes java teutonia* (Pierinae) and *Acraea andromacha andromacha* (Acraeinae). He stated also that this behavior was derived of the moderately to highly density of females in some populations. In the species, *Chlosyne californica* (Nymphalinae), males can be patrollers when the population density is high and perchers when the density is low [10]. In the European speckled wood butterfly, *Pararge aegeria*, these strategies are derived from the body design of males [11].

There are territories defended by males in the same spot during the same period of the day. These temporarily ephemeral territories were studied in species *Heliconius sara*, *H. leucadia* and *Eueides tales* [12], in Brazilian Amazonia. By the other hand, small size is advantageous to males of *Heliconius sara* during territorial fights and, in the satyrini butterfly *Hermeuptychia fallax*, there is evidence that heavy individuals have more chances to maintain territories [14-15]. In some species, as *Actinote pellenea*, males get physical contact in territorial disputes [16]. However, some authors [17] could not find any evidence that two prerequisites for
contestants in butterflies are filled: the capacity to inflict substantial costs on opponents and capacity to discriminate the sex of flying conspecifics. Body mass in relation to wing area and/or weather conditions interferes on flight maneuvers [18-19] causing some may be perchers and other patrollers [20-23]. Therefore, it is expected that perching males must allocate more mass to thorax musculature (ratio: thorax mass/body mass) and higher wing loading (ratio: fresh body mass/wing area) than patrollers [24, 19]. These changes should also promote a marked sexual dimorphism because females of this species would not necessarily need the same trade-off.

Encounter sites of Pereute, Leodonta, Catasticta, and Archonias butterflies are used as non-resource-based encounter sites to locate mates [25]. In Catasticta and Archonias, males adopt a perching strategy while waiting at these and other landmarks. Braby & Nishida [26] suggested a phylogenetic hypothesis for the Aporina where patrolling is ancestral state and evolved in the common ancestor of the subtribe, whereas perching is derived and evolved relatively recently in the immediate common ancestor of Catasticta + (Archonias + Charonias).

The butterfly Archonias brassolis teresa (Godart, 1819) (Pieridae) is resident in some sites of the coastal area of the state of São Paulo, South eastern Brazil, and particularly abundant at the river Quilombo, Santos, SP [27]. Ecological or behavioral information on Archonias are from [28] focusing on A. b. approximata (=A. tereas approximata), and A. b. eurytelle (=A. eurytelle) from Costa Rica. Concerning Brazilian A. b. tereas, [29] commented the male territorial behavior, observing that it defends territories formed along edges of roads against other intruding males. Braby & Nishida [25], described several aspects of the larval and adult behavior, including territoriality of these butterflies. Later [26], they hypothesized that the perching genera Catasticta and Archonias are predicted to have morphological traits associated with higher acceleration ability and speed, such as higher thorax/body mass ratio, higher wing loadings and higher aspect ratios (narrower wings) compared with other obligatory patrolling genera. Our objective was to test the hypothesis that males of the butterfly Archonias brassolis teresa allocate more mass to thorax than other body parts due to their behaviour to defend territorial areas where females are present.

2. Material and Methods
2.1. Study area
We made the observations and samplings along a dirt road on the right bank of the Quilombo’s river, near Santos, São Paulo, Brazil (center coordinates 23.51°S and 46.21°W). More details of the study site can be found in [27].

2.2. Sampling procedure
Male butterflies were collected from January to November of 2009 on 59 different sampling occasions when defending its territory (Figure 1) or flying along the road. Sex determination was done observing the terminal portion of abdomen.

Collected material was placed in plastic zip-loc envelopes tagged with adequate data. In the same day, butterflies were killed in a freezer at -10 °C for 24 hours, after which they were held at room temperature for at least one hour before determining its body wet mass, using a Sartorius analytical balance (model BP 221S with 0.0001 g precision). Then they were dehydrated by holding in a hot air oven at 60°C for one week to determine and record the dried body mass (dried) of each individual specimen. We carefully separated each part of dehydrated specimens to determine and record the weight of the abdomen, thorax and wings individually. We scanned the wings at resolution of 600 DPI in a HP G2710 scanner to measure the forewing length (FWL) and surface areas of the forewings and hindwings. Aspect ratio of the wings (AR = 4 * wing length2 / wing area) followed [30]. Wing loading in N/m2 (WL = M * g / S; where M = fresh body mass in kg, g = 9.80665 m/s, and S = wing area, in m2) followed [20], with our original data (g and mm2) being converted to these units. We obtained total wing area by superposing about 10% of the FW and the HW and multiplying the result by two, because this was the pattern roughly equivalent to that observed in photographed specimens in the field. The analysis of the photographs of the wings was implemented using ImageJ software [31] which permitted the recording of the lengths and integration of the surface areas. Using wing status, each sampled individual was placed in the class 1 when new, in class 2 when intermediate, and in class 3 when old.

2.3. Data organization and analysis
We used R software v. 3.02 win [32] using routines Shapiro Wilk (shapiro.test) to detect data normality, and Kruskall-Walis (kruskal.test) and Wilcox test (pairwise.wilcox.test) to detect differences between samples. Regressions used function lm() of package MASS [33].

3. Results
Males of the butterfly A. b. tereas had wet mass ranging from 0.04050 to 0.10110 g (mean = 0.0729 g; sd = 0.0128 g; n = 53; Figure 2A) and dry mass ranging from 0.0154 to 0.0429 g (mean = 0.03108 g; sd = 0.0060 g; n = 91; Figure 2B).
Fig 2: (A) Mass of fresh body of males and females of the butterfly *A. b. tereas* in the study area during 2009 (Welch Two Sample t-test = -2.4692; df = 7.104; p = 0.04239). (B) Mass of dried bodies of males and females of the butterfly *A. b. tereas* in the study area during 2009 (Welch Two Sample t-test = 0.9409; df = 5.984; p = 0.3832).

Percentages of water content of males ranged from 34.0% to 47.8% (n = 50). Values of FWL were not normally distributed (Shapiro-Wilk normality test W = 0.9660; p < 0.00007; Figure 5A). Length of forewing ranged from 24.0 to 34.0 mm (median = 30.00 mm; n = 206; Figure 3A). Wing area ranged from 779 to 1371 mm² (mean = 1137 mm²; sd = 131.5224 mm²; n = 109; Figure 3B).

In males, dry mass of abdomen declined with age (adjusted R-squared: 0.8363; F = 481.2; df = 93; p < 0.00001; Figure 4A). Percentages of mass allocation to thorax of dry bodies of males were not normally distributed (Shapiro-Wilk normality test W = 0.8171; p < 0.00001; Figure 5B). Dry body mass allocation to thorax was significant greater than to the abdomen or wings (Kruskal-Wallis chi-squared = 183.5299; df = 43, p < 0.00001; Figure 4B). Post-hoc pairwise Wilcoxon test was significant for all combinations (p < 0.00001).
Fig 4: (A) Decrease of dry mass of abdomen with increasing age in males of *A. b. tereas* ($R = -0.5476; t = -5.1524; df = 62; p = 0.00001836$). (B) Percentage of dry mass allocation to thorax, abdomen and wings of males of the butterfly *A. b. tereas* in the study area during 2009 (Kruskal-Wallis $H = 222.2782$, df = 2, $p < 0.0001$) with post-hoc Dunn test showing $p < 0.05$ for all combinations.

As the differences in FWL reaches 29.41% and mass allocation to thorax differences of 31.04%, this is an indication that some males could adopt the strategy of patrol because costs of perching are too high to pay.

Fig 5: (A) Plot of FWL size of males of the butterfly *A. b. tereas* in the study area during 2009 using qqplot() function to demonstrate non-normality of data distribution (mean = 29.89; s. d. = 1.7385; Shapiro-Wilkinson normality test $W = 0.9454$; $p = 0.00002$). (B) Plot of mass allocation to thorax of males of the butterfly *A. b. tereas* in the study area during 2009 using qqplot() function to demonstrate non-normality of data (mean = 29.96; s. d. = 1.71; Shapiro-Wilk normality test $W = 0.8171$; $p < 0.000001$).

The AR ratio ranged from 2.21 to 4.52 ($n = 109$; Figure 6A). WL ranged from 0.73 to 1.79 N/m² ($n = 91$; Figure 6B).
Fig 6: Wing traits of males of the butterfly A. b. tereas in the study area during 2009. (A) wing aspect ratio and (B) wing loading.

Plotting the dispersion of body dry mass against percentage of mass allocation to thorax of males (Figure 7) showed the formation of four groups of individuals: LA = light and agile; LS = light and slow; HA = heavy and agile, and HS = heavy and slow. Perchers would be type LA and patrollers HS. Only 20 (22%) are LS or HA. When the male copulates its rate between thorax mass/body mass will increase together with its flight capacity changing their status from LS or HS in direction to LA. However, its mating capacity is now lower due to depletion of his gonads.

Fig 7: Dispersion plot of body dry mass (g) versus wing loading (N/m²) of males of the butterfly A. b. tereas in the study area during 2009 showing a linear fit (y = -43.276x + 3.5597; R² = 0.67; R = 0.82; p < 0.0001). Shaded central rectangular area groups central quartiles from the medians of data. Four quadrants are created showing groups: LA = light and agile; LS = light and slow; HA = heavy and agile, and HS = heavy and slow. Perchers would be type LA and patrollers HS. Only 2 (22%) are LS or HA.

4. Discussion
The mass of emerged butterflies is dependent on the mass acquired by the larva during its development and this is dependent on the type and quantity of available food. By example, mass of males of the orange-tip butterfly (Anthocharis cardamines; Pieridae), a European species, differs between hostplants and males reared on Alliaria petiolata (Brassicaceae) were bigger and emerged earlier than males reared on Cardamine pratensis (Brassicaceae) [34]. As WL is a composite of mass and wing area, it will vary with age and physiological state of the butterfly because water content is not constant along its life. Due to the dependency of butterflies to liquid alimentation and/or water loss by behavioral or environmental conditions the fresh body mass vary along time. However, she will lose substantial part of her body mass after oviposition. Different from body mass, wing area cannot increase with age but can decrease when pieces of wings are lost by predator attacks or during aerial contests between males. After eclosion, the bulk mass of an adult consists of reproductive reserves stored in the abdomen and flight muscles [35, 36, 37, 38, 39]. Moreover, the ratios of abdomen mass to total body mass also change with age since abdomen mass decreases with age in both wild and captive populations of some butterfly species [36, 40, 41]. This could affect an individual’s flight ability over time [42].

Our data showed that old male butterflies had a mean body dry mass which was 2.3 times lesser than that one of the new butterflies. Svärd & Wiklund [43] showed that the mass of ejaculates of males of species of genera Aporia, Pieris, Colias and Leptidea (Pieridae) from Europe, could reach to 16% of male weight. If this rule is applicable on other pierid butterflies, we can state that during their adult life both sexes will have a considerable variation in their WL values. Berwaerts et al. [44] showed in adults of both sexes of the butterfly Pararge aegeria (Nymphalidae) that the acceleration capacity was positively correlated with all phenotypic traits measured (total body mass, thorax mass, forewing area, forewing length, wing loading, aspect ratio and center of forewing area). These relationships were stronger in males due to differential mass allocation to the thorax than females, which supported the assumed mechanism behind the relationship between flight morphology and mate-locating behavior. Van Dyck & Wiklund [45] assumed the hypothesis that there is a decrease in male territoriality of the butterfly Pararge aegeria from late spring to summer, a decline in ‘territorial perching’ morphology (i.e. relative thorax mass,
In the butterfly *A. b. tereus*, the variation of 31.0% of the percentage of dry mass allocation to thorax (32.9% to 63.9%) could be an indication that light males could adopt patrolling strategies because they could not afford to maintain a territory. In Europe, Van Dyck [19] found only 3.3% of this variation in males of *Pararge aegeria* (Nymphalidae: Satyrinae) which can adopt both strategies: patrolling or perching. Nevertheless, its capacity of to maintain a territory could decrease together to its age. If the focus is only WL, we also could hope changes because both body mass and wing area will vary along time. The first for the reasons above stated and the second because aerial contents with other males could produce wing damages. In this situation, the scenarios are not foreseeable.

Zheng et al. [46] found AR = 3.18 for *Vanessa cardui* (Nymphalidae), Dudley [21] found AR = 3.86 for *Aphrissa boisduvalii*, 2.47 for *Itaballia demophile*, (both Pieridae), and 5.09 for *Dryas iulia* (Nymphalidae). In spite of the variation of WL found along the life of an adult butterfly it is a good index of characteristics of perching. The range of WL in males of *A. b. tereus*, between 0.73 to 1.79 N/m², shows some coherence to phylogenetic traits due that these values were in the range of variation of European species of Pieridae. Wickman [34], showed variation of WL ranging from 0.40 to 1.00 N/m² in seven species of Pieridae and 18 of Nymphalidae, and from 1.00 to 1.20 N/m² in 19 species of Hesperidae, Papilionidae and Lycaenidae. Of the 15 species with higher WL values (> 1.50 N/m²), 11 were perchers and four patrollers. Corbet [47] found WL values from 0.56 to 0.83 N/m² in four European species of Pieridae and from 1.20 to 1.80 N/m² in five species of Nymphalidae, three being perchers. Fric et al. [48] (2006) discussed AR and WL but did not show raw data. However, several values were in the range of species of other families which males are perchers.

5. Conclusion

Concluding, we can agree with Braby & Nishida [26] hypothesis that the *Archonias* species have morphological traits associated with higher acceleration ability and speed, when compared with other pierid patrolling species. On the other hand, our data showed that some individuals of *A. b. tereus* do not have these morphological traits and, therefore could adopt the patrolling strategy.

6. Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

7. Author Contribution

RBF conceived and designed the study, collected the data, analysed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper. TS-S collected the data, reviewed drafts of the paper.

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9. References

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