



Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

Comparative morphology of the prothoracic leg in heliconian butterflies: Tracing size allometry, podite fusions and losses in ontogeny and phylogeny

Gilson R.P. Moreira ^{a, *}, Denis S. Silva ^b, Gislene L. Gonçalves ^{c, d}

^a Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Prédio 43435, Avenida Bento Gonçalves, 9500, Agronomia, 91501-970, Porto Alegre, RS, Brazil

^b Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Prédio 43435, Avenida Bento Gonçalves, 9500, Agronomia, 91501-970, Porto Alegre, RS, Brazil

^c Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Prédio 43423, Av. Bento Gonçalves, 9500, Agronomia, 91501-970, Porto Alegre, RS, Brazil

^d Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Casilla 6-D, Arica, Chile

ARTICLE INFO

Article history:

Received 26 October 2016

Received in revised form

17 February 2017

Accepted 28 March 2017

Available online xxx

Keywords:

Insect legs

Allometric growth

Evolutionary losses

Drumming behavior

Host plant selection

Heliconian butterflies

ABSTRACT

Prothoracic legs of heliconian butterflies (Nymphalidae, Heliconiinae, Heliconiini) are reduced in size compared to mesothoracic and metathoracic legs. They have no apparent function in males, but are used by females for drumming on host plants, a behavior related to oviposition site selection. Here, taking into account all recognized lineages of heliconian butterflies, we described their tarsi using optical and scanning electron microscopy and searched for podite fusions and losses, and analyzed allometry at the static, ontogenetic and phylogenetic levels. Female tarsi were similar, club-shaped, showing from four to five tarsomeres, each bearing sensilla chaetica and trichodea. Male tarsi were cylindrical, formed from five (early diverging lineages) to one (descendant lineages) either partially or totally fused tarsomeres, all deprived of sensilla. Pretarsi were reduced in both sexes, in some species being either vestigial or absent. Tarsal lengths were smaller for males in almost all species. An abrupt decrease in size was detected for the prothoracic legs during molting to the last larval instar at both histological and morphometric levels. In both sexes, most allometric coefficients found at the population level for the prothoracic legs were negative compared to the mesothoracic leg and also to wings. Prothoracic tarsi decreased proportionally in size over evolutionary time; the largest and smallest values being found for nodes of the oldest and youngest lineages, respectively. Our results demonstrate that evolution of the prothoracic leg in heliconian butterflies has been based on losses and fusions of podites, in association with negative size allometry at static, ontogenetic and phylogenetic levels. These processes have been more pronounced in males. Our study provided further support to the hypothesis that evolution of these leg structures is driven by females, by changing their use from walking to drumming during oviposition site selection. In males the leg would have been selected against due to absence of function and thus progressively reduced in size, in association with podites fusions and lost.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Positive allometric growth (*sensu* Huxley, 1950; Gould, 1966) has been recurrently evoked in the literature as an important process to explain the evolution of exaggerated traits in a variety of animals, as

for example, deer antlers, toucan bills and beetle horns (Gould, 1977; Stern and Emlen, 1999; Hughes, 2014). Establishment of allometric growth has led to comprehension of adaptations in these structures, since many of these patterns are adaptive, being under either natural or sexual selection. Conversely, the underlying mechanisms involved in evolutionary losses of useless body structures have been rarely studied from this perspective, although they are very likely associated with negative allometric growth. Here, by using this approach as a case study, we search for explanation of the underlying processes involved in evolution of the extremely small size of

* Corresponding author.

E-mail addresses: gilson.moreira@ufrgs.br (G.R.P. Moreira), denissantossilva@yahoo.com.br (D.S. Silva), gislene.ufrgs@gmail.com (G.L. Gonçalves).

the prothoracic legs of passion vine butterflies (Lepidoptera, Nymphalidae, Heliconiini). After describing respective morphological variation among exemplar species of extant lineages, we explore the growth of prothoracic legs from an allometric perspective, at the static, ontogenetic and phylogenetic levels (*sensu* Cock, 1966; Klingenberg and Zimmermann, 1992; Stern and Emlen, 1999).

The prothoracic legs do not serve for walking in passion vine butterflies. They apparently have no function in males, but are used by females to drum on the host plant prior to oviposition (Renou, 1983; Menna-Barreto and Araújo, 1985; Thiele et al., 2016). By scraping and lacerating the plant surface during drumming, they provoke the release of exudates that are identified by specialized chemosensory sensilla present on their prothoracic legs. This is a very fast decision process, always performed preceding oviposition (Thiele et al., 2016). Meso- and metathoracic legs of heliconian butterflies have similar chemosensory sensilla. However, they are not used for drumming, but might be involved in both sexes in the identification of sugar (Silva et al., 2017). Heliconian butterflies are generally oligophagous, using several host plants (Passifloraceae) throughout their range, but few in a given locality (Benson et al., 1976; Brown, 1979, 1981; Gilbert, 1991). Host plant choices are in fact tied to oviposition in this case, since their larvae are relatively sedentary, using a single passion vine shoot for development. Several experiments have demonstrated they are very selective, taking into account inter- and intraspecific attributes of host plants during oviposition, as for example regarding host plant species, presence of conspecifics, size and density of shoots, age, damage and nutrient level of leaves, among others. Choices in this case have important life history consequences, including effects on survivorship and development rates of immature stages, and the size, fecundity and shape of adults (e.g. Menna-Barreto and Araújo, 1985; Périco and Araújo, 1991; Rodrigues and Moreira, 2002; Bianchi and Moreira, 2005; Kerpel and Moreira, 2005; Kerpel et al., 2006; Jorge et al., 2011; Silva et al., 2014). Drumming is supposedly an essential behavior in evaluating host plant quality in all such cases, since the isolation of chemosensory sensilla present on prothoracic legs precludes egg-laying in these butterflies (Silva et al., 2017).

It has been known for a long time that the prothoracic legs of all heliconian butterflies are reduced in size compared to mesothoracic and metathoracic legs (Emsley, 1963). There is, however, no comparative morphological study of these legs at the ultrastructural level among the different heliconian lineages, except for *Heliconius charitonius* Linnaeus and *Heliconius erato* Linnaeus (Renou, 1983; Silva et al., 2017). Furthermore, Emsley (1963) did not examine males carefully in his analysis, assuming that all have prothoracic tarsi reduced to one tarsomere, which is not true. Reduced prothoracic legs are not unique to heliconian butterflies; this is a character found in nearly all butterflies of the family Nymphalidae in which heliconians are nested, and modified prothoracic legs are also present in Riodinidae and male Hedyliidae (Ackery et al., 1998). Phylogenetic allometry in size has been suggested as a mechanism involved in the evolution of these structures for nymphalid butterflies and related lineages, but in a broader scenario within Papilionoidea (Wolfe et al., 2011). Again, these authors did not take into account variation in podite fusions among lineages. Also, they did not search for information about the corresponding importance of static and ontogenetic allometry, if any. Here, all these aspects are taken into account together. We first conducted a comparative study of tarsal morphology using optical and scanning electron microscopy, including exemplar members of the ten recognized genera of heliconian butterflies (Lamas, 2004). Then, we performed histological sectioning on the fifth instar of *Dione juno* (Cramer), and measured comparatively leg size during development and at the population level for the other species, thus searching for ontogenetic and static allometry, respectively. We

also looked for the existence of phylogenetic allometry in tarsal development by reconstructing the ancestral states of the estimated ratios between prothoracic and mesothoracic tarsal lengths, using phylogeny based on molecular data as a reference.

2. Material and methods

2.1. Tarsal descriptions and measurements

The material used for studies carried out with the adult stage came from dried specimens deposited in the collection of the *Laboratório de Morfologia de Insetos* (LMCI), Zoology Department, Campus do Vale, Federal University of Rio Grande do Sul State (UFRGS), Porto Alegre, RS, and the *Coleção Padre Jesus S. Moure* (DZUP), Zoology Department, Federal University of Paraná State, Curitiba, Brazil. Males and females of at least one species from all recognized heliconian genera (Lamas, 2004) were included in the analyses: *Agraulis vanillae maculosa* (Stichel), *Dione juno juno* (Cramer), *Podotricha telesiphe* (Hewitson), *Philaethria wernickei* (Röber), *Dryas iulia alcionea* (Cramer), *Dryadula phaetusa* (Linnaeus), *Eueides isabella dianasa* (Hübner), *Laparus doris doris* (Linnaeus), *Neruda aoede aoede* (Hübner), *Heliconius sara apseudes* (Hübner), *Heliconius erato phyllis* (Fabricius), *Heliconius ethilla narcaea* Godart, and *Heliconius melpomene nanna* Stichel. For comparison, specimens of either *Ascia monuste* (Linnaeus) (Pieridae) or *Danaus erippus* (Cramer) (Nymphalidae, Danainae) were used as outgroups (for phylogeny of Papilionoidea butterflies, see Heikkilä et al., 2012). Females of additional species belonging to *Dione* Hübner and *Eueides* Hübner were included to check further the existence of reduction in prothoracic pretarsi, as mentioned by Emsley (1963): *D. glycera* (C. & R. Felder), *D. moneta moneta* Hübner, *Eueides lybia lybia* (Fabricius), *Eueides tales tales* (Cramer), *Eueides pavana* Ménétré, and *Eueides alipha alipha* (Godart).

For measurements under light microscopy, specimens ($n = 10$ to 15/sex/species) had their legs dissected under a stereomicroscope and fixed in Dietrich's fluid. Thereafter, they were cleared with 10% KOH solution and stained with silver nitrate to facilitate visualization of tarsomeres and corresponding sensilla (Nagy, 1978; Silva et al., 2017). Then the detached legs were washed with distilled water, immersed in glycerin and slide-mounted in glycerin jelly. A Leica M125 stereomicroscope was used to perform observations of the gross morphology and to photograph legs with an attached Sony® DSC-H10 digital camera. Measurements were performed on corresponding digital images using the AxioVision® software (Carl Zeiss). Tarsus and tibia lengths were used as a surrogate of leg length since only these podites are seen externally in the pupal stage, thus allowing comparison from an ontogenetic perspective. Linear measurements adopted in this study are schematically represented in Supplementary Material (Fig. 1S).

For characterization of male tarsi, additional high-resolution light microscopy micrographs were obtained using a Nikon®DS-U3 digital camera mounted on a Nikon®AZ100 M microscope, with the aid of the imaging software NIS-Elements. The external tegument ultrastructure of both male and female tarsi was studied at the *Centro de Microscopia Eletrônica* of UFRGS. For these analyses, tarsi of additional specimens were dehydrated in a Baltec® CPD030 critical-point dryer, mounted with double-sided tape on metal stubs and coated with gold in a Bal-tec®SCD050 sputter coater. Specimens were examined and photographed in a JEOL® JSM6060 scanning electron microscope.

2.2. Ontogenetic and static allometry

To compare growth rates among legs throughout ontogeny, specimens were reared on their respective host-plants as follows:

Download English Version:

<https://daneshyari.com/en/article/5585026>

Download Persian Version:

<https://daneshyari.com/article/5585026>

[Daneshyari.com](https://daneshyari.com)