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DIPLOMARBEIT

Proboscis musculature in pollen feeding and non pollen feeding Heliconiini (Lepidoptera: Nymphalidae)

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1. Introduction

1. 1. *Evolution of pollen feeding*

Lepidoptera belong to the class of insects and represent an enormous order with over 150 000 species (DETTNER & PETERS 1999). Except for three families, i. e., Micropterigidae, Heterobathmiidae and Agathiphagidae, all remaining taxa of moths and butterflies possess a coilable, long and very flexible proboscis to feed on fluids by suction (SCOBLE 1992, for review see STEKOLNIKOV & KORZEEV 2007). The organ is coiled up and stored under the head during rest and can be uncoiled rapidly for feeding (Figure 1 - A, B). The proboscis functions like a drinking straw through which different kinds of liquids (most commonly floral nectar, but also liquids from rotten fruit, dung, carrion, urine and fungi) are sucked from the tip into the head and the digestive tract (KINGSOLVER & DANIEL 1995, KRENN 2008). Fluid enters the food canal of the proboscis through slits in a particular region near the tip (PAULUS & KRENN 1996, KRENN 1998). A sucking pump located in the head produces a pressure gradient to suck fluid and transport it to the esophagus (EBERHARD & KRENN 2003, 2005).
Apart from liquid food sources, pollen can be used for nutrition. Non-glossatan moths of the family Micropterigidae feed on pollen grains or spores of ferns by chewing on them with their mandibles (SCOBLE 1992). Only some species of the tribe Heliconiini feed on pollen using their proboscis. These Neotropical passion-vine butterflies take centre stage in my study.

1. 2. *Description of mouthparts in butterflies*

The proboscis is the most prominent feature of the lepidopteran mouthparts (Figure 1 – A) and consists of two elongated maxillary galeae joined ventrally by exocuticular, toothed hooks and dorsally by overlapping plates (Figure 1 - C). These coilable tubes form a food canal between them. The mandibles are reduced to fixed vestiges incorporated into the head capsule, also the maxillary palps are reduced to minute vestiges (EASTHAM & EASSA 1955). The labium bears two large labial palps, each consisting of three segments (KRISTENSEN 2003). The galeae of the proboscis are basally joined to the distal

end of the paired stipes, the latter are connected to the cardo on each side (EASTHAM & EASSA 1955). The labrum is reduced to a pair of bristled lobes termed pilifers (SCOBLE 1992).

1. 2. 1. *Functional regions of the proboscis*

The proboscis looks relatively uniform from the outside and tapers from its base towards the tip. However, five functional areas can be distinguished (Figure 1 - B). The proboscis consists of a short basal, uncoilable region that is connected to the stipites and a longer distal coilable region, which is composed of four different regions. At about one third of its length from its base the proboscis can be bent downward at variable angles. This region, termed knee bend (EASTHAM & EASSA 1955, BÄNZIGER 1971), separates the proximal and the distal region from each other. The distal end of the proboscis, the tip region, bears numerous sensilla styloconica and slits for nectar-uptake (PAULUS & KRENN 1996).

1. 2. 2. *Anatomy of the galea*

The outer wall of each galea is formed by a series of exocuticular ribs embedded in flexible endocuticle. The lining of the food channel is composed of exocuticular bars, which themselves consist of tightly packed laminae (SCOBLE 1992). Each galea contains a nerve, a trachea, one or two longitudinal septa and numerous muscles (KRENN 1990). In higher Lepidoptera both extrinsic and intrinsic musculature occur (SCOBLE 1992), whereas the latter are considered to belong to the autapomorphic groundplan of the Myoglossata (KRISTENSEN 2003). Higher Ditrysia usually have two sets of intrinsic galeal muscles termed the lateral intrinsic and the median intrinsic galeal muscles (KRENN & MÜHLBERGER 2002, KRISTENSEN 2003), formerly called primary oblique and secondary oblique galeal muscles, respectively (EASTHAM & EASSA 1955). The two series can be classified according to their position in the galeal lumen and their particular courses. The lateral intrinsic galeal muscles overlap each other in the lateral half of the cross-section extending in an oblique direction along the lateral wall. They originate either on the lateral galeal wall, on the

dorso-lateral, or on the dorsal wall. All insertions lie in the middle of the ventral wall. The median intrinsic galeal muscles are located in the medial half of the cross-sections and run ventrally to the nerve and trachea. They originate on the medio-ventral wall below the food groove and extend to the middle of the ventral wall where they insert. The lateral intrinsic muscles extend obliquely from the dorso-lateral wall to the ventral wall, while the median intrinsic muscles slant only slightly from the ventro-median wall to the ventral wall (EASTHAM & EASSA 1955, Figure 1 - C).

1. 2. 3. *Functional aspects of proboscis movement*

Proboscis extension is initiated by an elevation of the galeal base caused by contraction of the basal galeal muscles (KRENN 1990, WANNENMACHER & WASSERTHAL 2003). The actual uncoiling of the proboscis spiral is accompanied by rapid compressions of the stipites which are caused by actions of the stipital muscles (WANNENMACHER & WASSERTHAL 2003). Uncoiling is brought about by an increase of hemolymph pressure since the stipites force hemolymph into the galeae. Recoiling is caused by the contraction of both sets of intrinsic galeal muscles supported by elasticity of the galeal cuticle (BÄNZINGER 1971, KRENN 1990, WANNENMACHER & WASSERTHAL 2003). Another stipital muscle pulls down the galea base which brings the coiled proboscis back to its resting position where it is held in some species in a U-shaped groove of the labium without further muscle activity (WANNENMACHER & WASSERTHAL 2003). After complete coiling, the spiral of the proboscis widens passively because of its elastic properties. The combination of the spring-loaded proboscis and the cuticular processes that interlock the coils prevents further uncoiling and maintains the resting position without further muscle action (KRENN 1990).

1. 3. *Biology and life-history of passion-vine butterflies*

Neotropical butterflies of the genus *Heliconius* possess the unique ability to collect and gather pollen on their proboscides. These butterflies are able to extract and ingest amino acids from pollen that adheres to their proboscides (GILBERT 1972, 1991). Pollen feeding in *Heliconius* is considered as a key

adaptation that promoted diversification of this group (BROWN 1981, GILBERT 1991) and is not known in any other taxa of butterflies (PENZ & KRENN 2000).
Both larvae and adults use the ingested amino acids to produce cyanogenic glycosides (NAHRSTEDT & DAVIS 1983). These substances are employed as chemical defence against predators and cause unpalatability of *Heliconius* species (GILBERT 1991). Furthermore, this nitrogen source allows for nuptial gifts (BOGGS & GILBERT 1979), prolonged reproductive longevity and permanent egg production in females (DUNLAP-PIANKA ET AL. 1977). Other trends in *Heliconius* evolution which are associated with the innovation of pollen feeding include an increase of adult behavioural sophistication and enhanced capacity of memory (GILBERT 1975). *Heliconius charitonia* has 2.5 - 7 times the mushroom body volume to brain volume ratio of other analysed nymphalides, including the non-pollen feeding heliconiines *Dryas* and *Agraulis*. The mushroom body in insects seems to be connected to memory function (SIVINSKI 1989).

1. 3. 1. *Adaptations to pollen-feeding*

The mouthparts as well as the behaviour during flower visitation show adaptations to pollen feeding and the extra-oral extraction of amino acids.

1. 3. 1. 1. *Behavioural adaptations*

During pollen gathering, butterflies remain at a single flower longer than during nectar feeding (up to 10 minutes, GILBERT 1972). They repeatedly scrape the proboscis tip over the anthers with short jerky thrusts (GILBERT 1972). The proboscis often then remains motionless for several seconds (KRENN 2008). When the proboscis is extracted from the narrow corolla, pollen grains adhere to the outer surface of the proboscis. Most of the pollen grains accumulate ventrally in the basal third of the proboscis (BOGGS ET AL. 1981). Pollen is then soaked with saliva, which is excreted from the tip of the proboscis and contains proteases for the degradation of amino acids (EBERHARD ET AL. 2007). Through repeated coiling and uncoiling of the proboscis the pollen lump is kneaded and finally amino acids are extracted and ingested

(BOGGS ET AL. 1981, KRENN 2008, Figure 1 - E). This pollen processing behaviour lasts several hours (GILBERT 1972).
In behavioural experiments with Heliconiini, it has been observed that the proboscis tip is extremely flexible and can be moved backwards, forwards and sideways (KRENN, personal communication).

1. 3. 1. 2. *Morphological adaptations of mouthparts*

Within the Heliconiini there are genera that use both nectar and pollen for nutrition ("pollen feeding species": *Heliconius*) and species that solely subsist on nectar ("non-pollen feeding species": *Agraulis vanillae*, *Dryas julia*, *Neruda aoede, Eueides lybia*, etc.). These two guilds differ in some morphological traits. Pollen feeding species have a significantly longer proboscis than non-pollen feeding relatives without elongation of the proboscis tip. In the proximal and mid regions of the proboscis in pollen feeding Heliconiini are numerous, exceptionally long sensilla trichodea. Furthermore, the labial palpi are significantly shorter and narrower in pollen feeding species than in non-pollen feeding Heliconiini (KRENN & PENZ 1998).

1. 4. *Musculature in the proboscis of Heliconiini*

The internal composition of the two galeae, especially the arrangement of intrinsic galeal musculature differs among the butterfly species within the superfamily Papilionoidea. The longitudinal arrangement of muscles in the proboscides of the two Heliconiini, *Heliconius melpomene* and *Dryas julia*, is unique compared to other members of Nymphalidae. The median intrinsic muscles are present in the proximal region (10 - 20 % of total proboscis length), the knee bend (30 - 35 % of total proboscis length) and the tip (85 - 95 % of total proboscis length). However, these muscles are absent in the distal region (50 - 70 % of total proboscis length). This apomorphic condition probably resulted from a loss of median muscles in the distal region rather than from the evolution of new muscles of this type in the proboscis tip (KRENN & MÜHLBERGER 2002).

1. 5. *Hypothesis*

The pollen processing behaviour of Heliconiini is characterised by repeated uncoiling and coiling of the proboscis (GILBERT 1972, KRENN 2008), where intrinsic galeal muscles are crucially involved (BÄNZIGER 1971, KRENN 1990, 2000; SCHMITT 1938, WANNENMACHER & WASSERTHAL 2003). Some species of Heliconiinae are known to have a derived muscle arrangement in the distal half of the proboscis (KRENN & MÜHLBERGER 2002). Both the unique muscle arrangement in the proboscis and the extraordinary flexibility of its tip can be considered as functional adaptations, which presumably evolved in context with pollen feeding. It is hypothesized that the arrangement of median intrinsic galeal musculature and/or the number of muscles differs among pollen feeding and non-pollen feeding species of Heliconiini.

The aim of this study is to give a detailed reconstruction of the occurrence and arrangement of the various series of intrinsic galeal muscles in the distal half of the proboscis. The number, course and insertion point of these muscles is compared in various species of pollen feeding and related non-pollen feeding Heliconiinae.

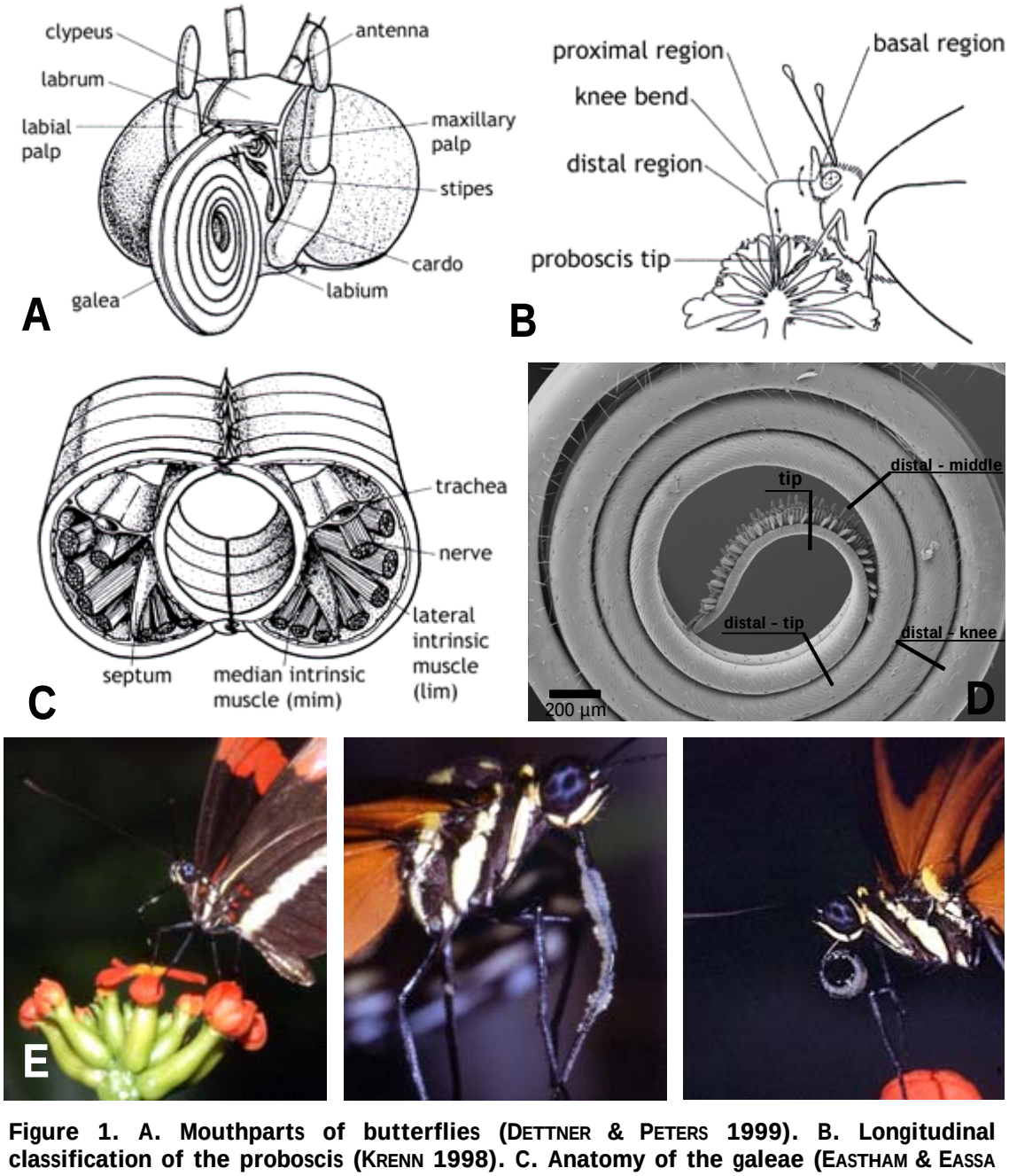

Figure 1. A. Mouthparts of butterflies (DETTNER & PETERS 1999). B. Longitudinal classification of the proboscis (KRENN 1998). C. Anatomy of the galeae (EASTHAM & EASSA 1955, modified after MÜHLBERGER & KRENN 2005). D. Various regions selected for sectioning, SEM micrograph of the proboscis of *H. melpomene*. E. Pollen feeding behaviour. *H. erato* visits an inflorescence of *Psiguria tabascensis* (Cucurbitaceae). Probing behaviour with flexed proboscis is similar to nectar feeding behaviour. *H. hecale* collects pollen on the proboscis by repeatedly poking it into a single flower. Using saliva, *H. hecale* extracts amino acids from the pollen by partly uncoiling and coiling the recoiled proboscis (KRENN 2008).

2. Material and Methods

2. 1. *Studied species*

The proboscides were studied in several species (Table 1) of the tribe Heliconiini SWAINSON 1822 and in one species of the tribe Argynnini DUPONCHEL 1835.

Table 1. Studied species and feeding guild, systematic arrangement after http://tolweb.org/Heliconiini/70208 (BELTRÁN ET AL. 2008, last view on 28.01.2009).

Species	Feeding guild
Heliconiinae	
Argynnini	
Argynnis paphia (LINNEAUS 1758)	Non-pollen feeding
Heliconiini	
Neruda aoede (HUEBNER 1813)	Non-pollen feeding
Agraulis vanillae (LINNAEUS 1758)	Non-pollen feeding
Dryas julia (FABRICIUS 1775)	Non-pollen feeding
Heliconius melpomene (LINNAEUS 1758)	Pollen feeding
Heliconius pardalinus BATES 1862	Pollen feeding
Heliconius hecale (FABRICIUS 1776)	Pollen feeding
Heliconius doris (LINNAEUS 1771)	Pollen feeding
Heliconius sara (FABRICIUS 1793)	Pollen feeding
Heliconius charitonia (LINNAEUS 1767)	Pollen feeding

2. 2. *Semithin Sections*

Serial semithin section technique for light microscopy was used for the examination of the internal anatomy of the distal proboscis region and the proboscis tip (see BLUMER ET AL. 2002). Specimens were fixed in 70 % alcohol or Duboscq-Brasil (confer ROMEIS 1989) and stored in 70 % alcohol (Table 2). The proboscides were separated from the head prior to embedding.

Table 2. Origin, fixation and storage of specimen.

Individuals	Origin	Fixation
Argynnini		
Argynnis paphia	NÖ Weißenbach	70 % Ethanol
Argynnis paphia	NÖ Weißenbach	Duboscq-Brasil
Heliconiini		
Neruda aoede	Peru, Matthieu Joron	70 % Ethanol
Neruda aoede	Peru, Matthieu Joron	70 % Ethanol
Agraulis vanillae	London Pupae Supply	70 % Ethanol
Agraulis vanillae	London Pupae Supply	Duboscq-Brasil
Dryas julia	London Pupae Supply	Duboscq-Brasil
Dryas julia	London Pupae Supply	Duboscq-Brasil
Heliconius melpomene	London Pupae Supply	Duboscq-Brasil
Heliconius melpomene	London Pupae Supply	Duboscq-Brasil
Heliconius melpomene	London Pupae Supply	Duboscq-Brasil
Heliconius pardalinus	Peru, Matthieu Joron	70 % Ethanol
Heliconius pardalinus	Peru, Matthieu Joron	70 % Ethanol
Heliconius hecale	London Pupae Supply	Duboscq-Brasil
Heliconius hecale	La Gamba, Costa Rica	70 % Ethanol
Heliconius doris	London Pupae Supply	70 % Ethanol
Heliconius doris	London Pupae Supply	70 % Ethanol
Heliconius sara	London Pupae Supply	Duboscq-Brasil
Heliconius sara	La Gamba, Costa Rica	70 % Ethanol
Heliconius charitonia	London Pupae Supply	Duboscq-Brasil
Heliconius charitonia	Butterfly Breeding G. B.	Duboscq-Brasil

Drawings of the alcohol-preserved proboscides were made under the binocular with the aid of a drawing tube to measure the whole length of the proboscis (Table 3). The individual error of measurement was also determined (Table 4). To determine the length, measurement was made from the apex of the proboscis to the proximal part connected to the head. Accordingly, the proboscis tip amounts to 5 - 15 % of overall proboscis length and the distal region to 30 - 50% of the galeal length.

Table 3. Length of different proboscis regions, NPF…non-pollen feeding, PF…pollen feeding.

Individuals	Feeding guild	Length of different proboscis regions [mm]		
		Tip	Distal	Overall
Argynnini				
Argynnis paphia	NPF	1.161	5.105	12.532
Argynnis paphia	NPF	1.226	5.919	14.29
Heliconiini				
Neruda aoede	NPF	0.903	7.017	15.839
Neruda aoede	NPF	1.016	5.871	13.774
Agraulis vanillae	NPF	0.936	8.306	18.484
Agraulis vanillae	NPF	0.919	8.476	18.79
Dryas julia	NPF	1.5	5.557	14.113
Dryas julia	NPF	2.016	5.304	14.64
Heliconius melpomene	PF	0.94	8.512	18.903
Heliconius melpomene	PF	1.113	7.960	18.145
Heliconius melpomene	PF	1.323	7.516	17.677
Heliconius pardalinus	PF	1.113	8.694	19.613
Heliconius pardalinus	PF	1.339	9.532	21.742
Heliconius hecale	PF	1.371	8.774	20.29
Heliconius hecale	PF	1.129	6.887	16.032
Heliconius doris	PF	1.71	7.508	18.436
Heliconius doris	PF	1.548	7.404	17.903
Heliconius sara	PF	0.968	6.355	14.645
Heliconius sara	PF	0.984	6.129	14.226
Heliconius charitonia	PF	0.71	6.802	15.024
Heliconius charitonia	PF	0.84	5.676	13.032

Table 4. Individual error of measurement.

Object number	True length of proboscis [mm]
484	12.58
	12.60
	12.58
	12.63
	12.58
	12.56
	12.53
	12.60
	12.63
	12.58

n = 10, mean = 12.59, standard deviation = 0.029

individual error of measurement = 0.2 %

The proboscides were cut at the middle of their length to separate the distal region from the proximal parts. Additionally, the tip was clipped off to ensure efficient resin impregnation in the apical, slender parts of the proboscis. The galeae were dehydrated in an ascending alcohol series and embedded in Agar Low Viscosity Resin under vacuum impregnation. Polymerisation took place in an oven at 65 - 70 °C within 24 hours (see PERNSTICH ET AL. 2003).

The embedded parts of the proboscides were drawn under the binocular with the aid of a drawing tube to find the most suitable cutting plane. Serial sectioning was carried out on a microtome (Leica EM UC6) with a diamond knife (Histo Jumbo Diatome) at a thickness of 1 µm. Series of cross sections for a span of 70 - 90 µm were taken from the proboscis tip and three different positions in the distal region: near the transition to the tip, in the middle of the distal region and near the transition to the knee bend (figure 1-D). To locate muscle insertion in the proboscis tip, the resin block was trimmed with a razor-blade or metal microtome blade until the apical end of the tip was reached. Then, further trimming was accomplished with the aid of the microtome for a range of 300 – 500 µm depending on the investigated species.

This was possible, as explained later, because at the apical end of the tip, musculature is absent. Empirically it is known that during trimming and sectioning approximately 10 – 20 µm of sections may become lost, and this contributes to a certain margin of inaccuracy. Sections were stained with a mixture of 1 % azure II and 1 % methylene blue in an aqueous 1 % borax solution (confer ROMEIS 1989) at 60 - 70 °C.

At each cutting site, four sections were dislodged from each other at intervals of 20 µm and selected to count the number of muscles in different regions of the proboscis. From the four sections, a mean value (n = 4) for the number of muscles per galea was calculated. One muscle unit resembles the cross section of a single muscle strand. Micrographs were taken with an Olympus CX41 microscope equipped with an Olympus E330 digital camera.

2. 3. *Statistics*

Statistical analyses were performed with SPSS 16.0. The tests included only data from members of the tribe Heliconiini. To compare the two feeding guilds, the nonparametric Mann-Whitney-U-Test was performed due to low sample size. The significant level was set at $p < 0.05$.

3. Results

3. 1. *Proboscis musculature arrangement*

Pollen feeding and non-pollen feeding butterflies have, in principle, a similar musculature arrangement in the proboscis, including series of lateral and median intrinsic galeal muscles (Figure 2). The muscle arrangement in the distal half of the proboscis is given in table 6. Variation occurs among the studied species and in the various individuals.

The intrinsic galeal muscles of the proboscis tip could not be assigned to a distinct series because of the very small triangular cross sectional area in this region, which made differentiation into distinct series impossible. Therefore, the muscles located in the proboscis tip are termed intrinsic galeal muscles.

Near the transition to the proboscis tip (at 7 – 18 % of the galeal length), the distal region contains both muscle series (lateral and median intrinsic galeal muscles) in all examined individuals of all species.

In the middle of the distal region (at 26 – 36 % of the galeal length) median intrinsic musculature is present to varying degrees. Lateral intrinsic musculature occurs in all investigated specimen, whereas median intrinsic musculature is absent in some individuals.

In the distal region near the transition to the knee bend (at 39 – 51 % of the galeal length), the majority of the examined individuals possess lateral intrinsic musculature alone. Median intrinsic muscles are present only in 7 of 21 studied specimens.

Comparing non-pollen feeding and pollen feeding species, it is apparent that the main difference between these two feeding guilds concerns the occurrence of median intrinsic muscles in the middle of the distal region and near the knee bend.

The non-pollen feeding Heliconiini possess both muscle series merely in the distal region near the transition to tip. In *Agraulis vanillae* and one individual of *Dryas julia* the median intrinsic musculature terminates before the middle of the distal region. In *Neruda aoede* and one individual of *Dryas julia* these muscles terminate before the transition to the knee bend.

Also, in some pollen feeding species, the median intrinsic musculature ends in the middle of the distal region (one individual each of *Heliconius melpomene* and *Heliconius pardalinus*) or at the transition to the knee bend (one individual each of *Heliconius hecale*, *Heliconius melpomene*, *Heliconius pardalinus* and *Heliconius doris*, as well as *Heliconius sara*).

Specimens that possess both muscle series throughout the distal region were *Argynnis paphia*, *Heliconius charitonia* and one individual each of *Heliconius hecale*, *Heliconius melpomene* and *Heliconius doris*.

Table 6. Proboscis musculature arrangement in different regions. igm…intrinsic galeal musculature, lim…lateral intrinsic musculature, mim…median intrinsic musculature, x…missing data, NPF…non-pollen feeding, PF…pollen feeding.

		Muscles in the various regions of the proboscis			
Individuals	**Feeding guild**	**Tip**	**Distal, tip**	**Distal, middle**	**Distal, knee bend**
Argynnini					
A. paphia	NPF	igm	lim+mim	lim+mim	lim+mim
A. paphia	NPF	igm	lim+mim	lim+mim	lim+mim
Heliconiini					
N. aoede	NPF	igm	lim+mim	lim+mim	lim
N. aoede	NPF	x	lim+mim	lim+mim	lim
A. vanillae	NPF	igm	lim+mim	lim	lim
A. vanillae	NPF	igm	lim+mim	lim	lim
D. julia	NPF	x	lim+mim	lim	lim
D. julia	NPF	igm	lim+mim	lim+mim	lim
H. melpomene	PF	igm	lim+mim	lim	lim
H. melpomene	PF	igm	lim+mim	lim+mim	lim+mim
H. melpomene	PF	igm	lim+mim	lim+mim	lim
H. pardalinus	PF	igm	lim+mim	lim	lim
H. pardalinus	PF	x	lim+mim	lim+mim	lim
H. hecale	PF	igm	lim+mim	lim+mim	lim
H. hecale	PF	igm	lim+mim	lim+mim	lim+mim
H. doris	PF	igm	lim+mim	lim+mim	lim
H. doris	PF	igm	lim+mim	lim+mim	lim+mim
H. sara	PF	igm	lim+mim	lim+mim	lim
H. sara	PF	igm	lim+mim	lim+mim	lim
H. charitonia	PF	igm	lim+mim	lim+mim	lim+mim
H. charitonia	PF	igm	lim+mim	lim+mim	lim+mim

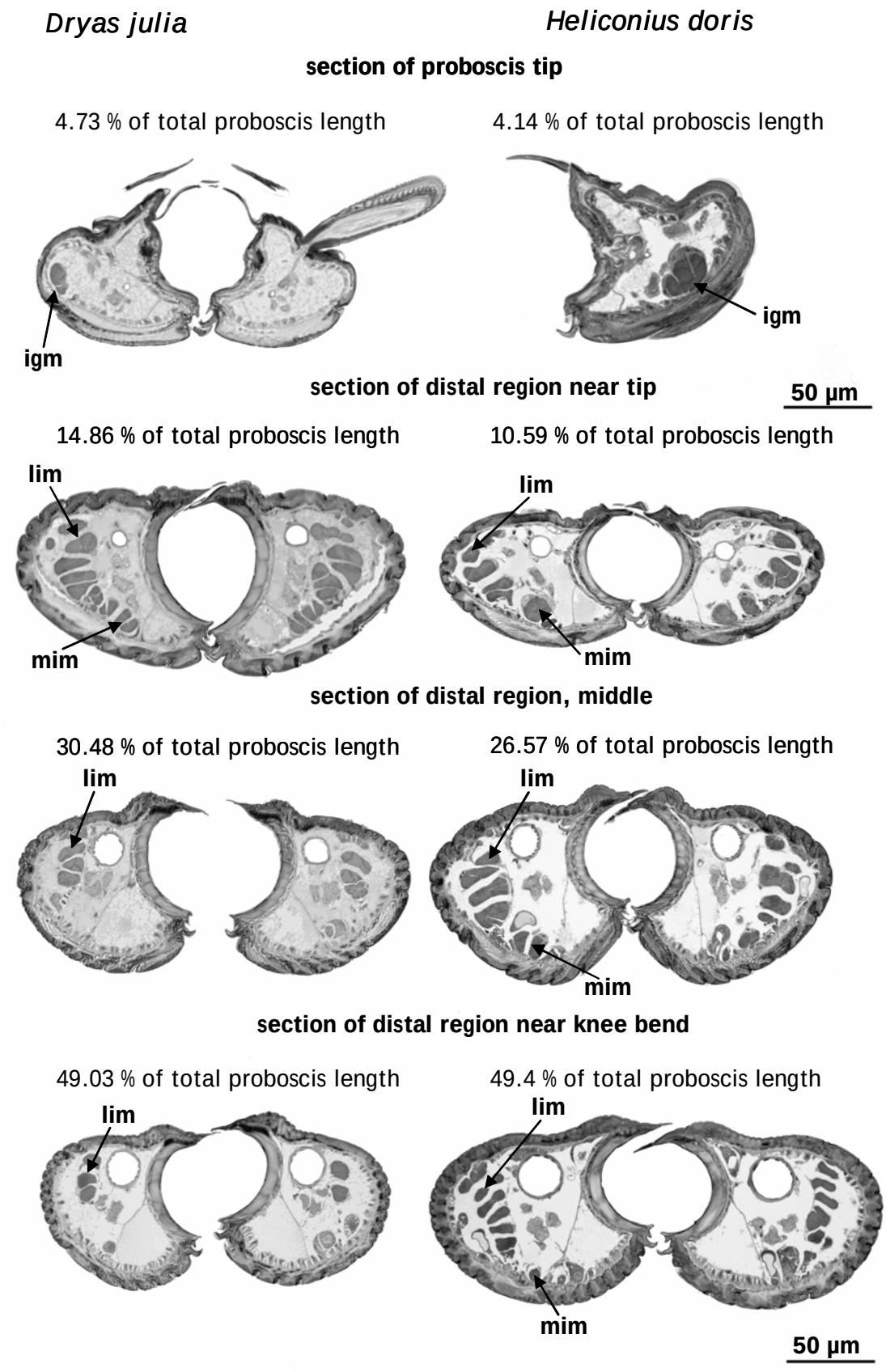

Figure 2. Semithin sections of various proboscis regions of *Dryas julia* as a representative of non-pollen feeding Heliconiini and *Heliconius doris* as a representative of pollen feeding Heliconiini. igm…intrinsic galeal musculature, lim…lateral intrinsic musculature, mim…median intrinsic musculature.

3. 2. *Number of muscle units*

In all regions where both muscle types are present, the number of lateral intrinsic muscles counted per galeal cross section exceeds these of median intrinsic muscles (Table 7).

Table 7. Number of muscle units counted per galeal cross section, with minimum and maximum values for the two feeding guilds in each proboscis region. igm…intrinsic galeal musculature, lim…lateral intrinsic galeal musculature, mim…median intrinsic galeal musculature, NPF…non-pollen feeding, PF…pollen feeding.

Proboscis region	Muscle series	Feeding guild	
		NPF	PF
tip	igm	1 - 3	1 - 3
distal, tip	lim	2 - 8	2 - 8
distal, tip	mim	1 - 6	1 - 5
distal, middle	lim	2 - 5	2 - 9
distal, middle	mim	0 - 3	0 - 4
distal, knee bend	lim	2 - 8	1 - 8
distal, knee bend	mim	0 - 3	0 - 2

The mean number of muscle units per galea for all examined individuals are given in table 8. The mean number of total muscle units per galea does not differ significantly among pollen feeding and non-pollen feeding Heliconiini in the proboscis tip ($n = 14$, $Z = - 0.834$, $p = 0.404$), in the distal region near the base of the tip ($n = 19$, $Z = - 1.143$, $p = 0.253$) and close to the transition to the knee bend ($n = 19$, $Z = - 1.539$, $p = 0.124$). However, in the middle of the distal region the mean number of total musculature per galea differs significantly between the two feeding guilds ($n = 19$, $Z = - 2.371$, $p = 0.018$). The difference is due to a lower mean number of median intrinsic muscle units per galea in non-pollen feeding species ($n = 19$, $Z = - 2.008$, $p = 0.045$, figure 3). In non-pollen feeding species the mean number of median intrinsic muscles per galeal cross section ranges between 26 and 36 % of the galeal length and contains 0 to 0.9 muscle units per galea. These muscles are more numerous in pollen feeding butterflies ranging from 0 to 3.1 muscle units per galeal cross section.

Table 8. Number of muscle units per galeal cross section in various proboscis regions, expressed as mean (n = 4 in each case) per galea, minimum and maximum values are given in parenthesis, approximated to one decimal place. lim…lateral intrinsic muscles, mim…median intrinsic muscles, NPF…non-pollen feeding, PF…pollen feeding, x…missing data.

		Tip	Distal, tip			Distal, middle			Distal, knee bend		
Individuals	**Feeding guild**	**igm**	**lim+mim**	**lim**	**mim**	**lim+mim**	**lim**	**mim**	**lim+mim**	**lim**	**mim**
Argynnini											
A. paphia	NPF	2.8 (2.0-3.0)	3.8 (3.0-4.0)	3.0 (3.0-3.0)	0.8 (0.0-1.0)	5.8 (5.0-6.0)	4.3 (4.0-5.0)	1.5 (1.0-2.0)	9.0 (9.0-9.0)	6.0 (6.0-6.0)	3.0 (3.0-3.0)
A. paphia	NPF	1.0 (1.0-1.0)	3.4 (3.0-4.0)	2.4 (2.0-3.0)	1.0 (1.0-1.0)	5.0 (4.5-5.5)	3.0 (2.5-3.5)	2.0 (2.0-2.0)	7.4 (6.5-8.0)	5.5 (4.5-6.5)	1.9 (1.5-2.0)
Heliconiini											
N. aoede	NPF	1.5 (1.0-2.0)	10.0 (9.0-11.0)	5.8 (5.0-6.5)	4.3 (4.0-4.5)	5.4 (4.5-6.0)	4.6 (4.0-5.0)	0.8 (0.5-1.0)	3.8 (3.0-4.0)	3.8 (3.0-4.0)	0.0
N. aoede	NPF	x	7.9 (7.5-8.5)	5.4 (5.0-5.5)	2.5 (2.0-3.0)	4.4 (3.5-5.5)	3.5 (3.0-4.0)	0.9 (0.5-1.5)	3.6 (3.0-4.0)	3.6 (3.0-4.0)	0.0
A. vanillae	NPF	2.0 (2.0-2.0)	3.1 (3.0-3.5)	2.9 (2.5-3.0)	0.3 (0.0-0.5)	2.9 (2.0-4.0)	2.9 (2.0-4.0)	0.0	3.4 (3.0-3.5)	3.4 (3.0-3.5)	0.0
A. vanillae	NPF	1.3 (1.0-2.0)	6.0 (5.0-7.0)	4.5 (4.0-5.0)	1.5 (1.0-2.0)	3.3 (3.0-4.0)	3.3 (3.0-4.0)	0.0	2.5 (2.0-3.0)	2.5 (2.0-3.0)	0.0
D. julia	NPF	1.0 (1.0-1.0)	10.3 (10.0-11.0)	5.9 (5.5-6.5)	4.4 (3.5-5.5)	4.1 (3.5-4.5)	3.5 (3.0-4.0)	0.6 (0.5-1.0)	2.5 (2.0-3.0)	2.5 (2.0-3.0)	0.0
D. julia	NPF	x	11.0 (10.0-12.5)	7.1 (6.5-8.0)	3.9 (3.0-4.5)	3.1 (2.5-3.5)	3.1 (2.5-3.5)	0.0	2.4 (2.0-2.5)	2.4 (2.0-2.5)	0.0
H. melpomene	PF	1.0 (1.0-1.0)	8.9 (8.5-9.5)	5.4 (5.0-5.5)	3.5 (3.0-4.0)	5.8 (5.0-6.5)	5.3 (4.5-6.0)	0.5 (0.5-0.5)	2.5 (2.0-3.0)	2.5 (2.0-3.0)	0.0
H. melpomene	PF	1.0 (1.0-1.0)	6.3 (6.0-7.0)	3.0 (3.0-3.0)	3.3 (3.0-4.0)	7.0 (6.0-8.0)	5.0 (4.0-6.0)	2.0 (2.0-2.0)	3.5 (3.0-4.0)	3.3 (3.0-4.0)	0.3 (0.0-1.0)

Table 8. Continued.

Individuals	Feeding guild	Tip	Distal, tip			Distal, middle			Distal, knee bend		
		igm	lim+mim	lim	mim	lim+mim	lim	mim	lim+mim	lim	mim
H. melpomene	PF	x	7.0 (7.0-7.0)	4.8 (4.5-5.5)	2.3 (1.5-2.5)		3.3 (2.5-4.0)	0.0	2.0 (1.5-2.5)	2.0 (1.5-2.5)	0.0
H. pardalinus	PF	1.0 (1.0-1.0)	6.3 (5.5-7.5)	5.0 (4.0-6.5)	1.3 (1.0-1.5)	6.4 (6.0-7.0)	5.3 (5.0-5.5)	1.1 (0.5-1.5)	4.1 (3.5-4.5)	4.1 (3.5-4.5)	0.0
H. pardalinus	PF	x	7.3 (7.0-8.0)	4.8 (4.0-5.0)	2.5 (2.0-3.0)	4.8 (3.0-6.0)	4.8 (3.0-6.0)	0.0	2.0 (2.0-2.0)	2.0 (2.0-2.0)	0.0
H. hecale	PF	3.0 (3.0-3.0)	10.8 (10.0-11.5)	6.4 (6.0-7.0)	4.4 (4.0-4.5)	5.9 (5.5-6.5)	4.1 (3.5-5.0)	1.8 (1.5-2.0)	3.3 (3.0-4.0)	2.9 (2.5-3.5)	0.4 (0.0-0.5)
H. hecale	PF	2.0 (1.0-3.0)	5.0 (4.5-5.5)	2.0 (2.0-2.0)	3.0 (2.5-3.5)	5.5 (5.0-6.5.)	3.8 (3.5-4.5)	1.8 (1.5-2.0)	4.4 (4.0-4.5)	4.4 (4.0-4.5)	0.0
H. doris	PF	1.3 (1.0-2.0)	6.3 (5.0-7.5)	3.9 (3.0-5.5)	2.4 (2.0-3.0)	8.6 (7.5-10.0)	5.5 (4.5-7.0)	3.1 (3.0-3.5)	7.8 (7.5-8.5)	6.8 (6.5-7.5)	1.0 (1.0-1.0)
H. doris	PF	1.5 (1.0-3.0)	8.0 (7.0-9.0)	5.3 (4.0-6.0)	2.8 (2.0-3.0)	7.8 (7.0-8.0)	6.8 (6.0-7.0)	1.0 (1.0-1.0)	5.3 (5.0-6.0)	5.3 (5.0-6.0)	0.0
H. sara	PF	1.0 (1.0-1.0)	5.0 (4.0-6.0)	3.0 (3.0-3.0)	2.0 (1.0-3.0)	4.4 (3.5-5.0)	2.9 (2.0-3.5)	1.5 (1.5-1.5)	3.8 (3.5-4.0)	3.8 (3.5-4.0)	0.0
H. sara	PF	1.0 (1.0-1.0)	4.6 (4.0-5.5)	2.8 (2.5-3.5)	1.9 (1.5-2.0)	3.6 (3.0-4.0)	3.6 (3.0-4.0)	0.0	4.6 (4.0-6.0)	4.6 (4.0-6.0)	0.0
H. charitonia	PF	1.0 (1.0-1.0)	3.3 (3.0-4.0)	2.0 (2.0-2.0)	1.3 (1.0-2.0)	4.8 (4.0-5.0)	3.5 (3.0-4.0)	1.3 (1.0-2.0)	4.5 (4.0-5.0)	4.0 (4.0-4.0)	0.5 (0.0-1.0)
H. charitonia	PF	x	4.5 (3.0-6.0)	2.8 (2.0-4.0)	1.8 (1.0-2.0)	4.3 (4.0-5.0)	2.5 (2.0-3.0)	1.8 (1.0-2.0)	4.3 (3.0-5.0)	2.5 (2.0-3.0)	1.8 (1.0-2.0)

3. 3. *Insertion of musculature at the proboscis tip*

As mentioned, the apex of the proboscis tip is the only region in the proboscis that does not contain muscles. This is because musculature does not reach the apex of the galea. Musculature inserts between 317 µm and 896 µm depending on the examined individual (Table 9). In this range, the galea contains the lowest number of muscle units. In the distal region near the base of the proboscis tip, the number of muscle units reaches its maximum, whereas muscle number decreases from the middle of the distal region towards the transition to the knee bend (Figure 3).

Musculature insertion differs among species and individuals. In the species *Argynnis paphia*, musculature inserts farther proximal than in Heliconiini. Comparing musculature insertion points expressed as percentage of overall proboscis length between non-pollen feeding and pollen feeding Heliconiini, it becomes apparent that musculature extends significantly further to the apical end of the tip in pollen feeding than in non-pollen feeding species ($n = 14$, $Z = -2.263$, $p = 0.024$; figure 4). However, the length of the proboscis tip does not differ significantly between these two groups ($n = 19$, $Z = -0.263$, $p = 0.792$).

Table 9. Insertion of musculature in the proboscis tip and length of tip, NPF...non-pollen feeding, PF...pollen feeding.

Individuals	Feeding guild	Insertion of musculature in the tip [µm]	Insertion of musculature in the tip [% of total galeal length]	Length of tip [µm]	Length of tip [% of total galeal length]
Argynnini					
A. paphia	NPF	594	4.16	1226	8.58
A. paphia	NPF	896	7.15	1161	9.26
Heliconiini					
N. aoede	NPF	597	4.33	1016	7.38
A. vanillae	NPF	719	3.89	936	5.06
A. vanillae	NPF	672	3.58	919	4.89
D. julia	NPF	668	4.73	1500	10.63
H. melpomene	PF	626	3.45	1113	6.13
H. melpomene	PF	598	3.16	940	4.97
H. pardalinus	PF	569	2.62	1339	6.16
H. hecale	PF	317	1.98	1129	7.04
H. hecale	PF	537	2.65	1371	6.76
H. doris	PF	699	3.9	1548	8.65
H. doris	PF	764	4.14	1710	9.28
H. sara	PF	494	3.37	968	6.61
H. sara	PF	499	3.51	984	6.92
H. charitonia	PF	335	2.23	710	4.73

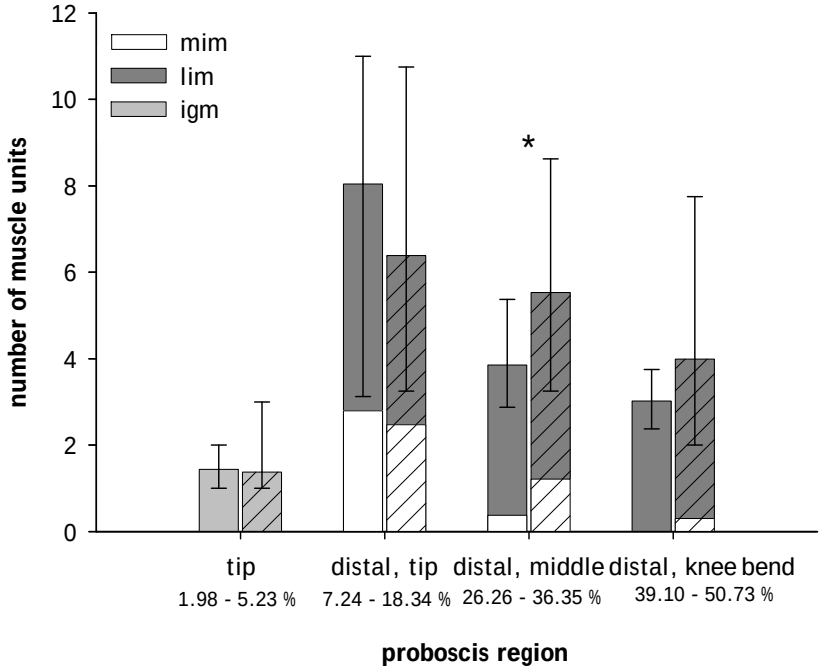

Figure 3. Number of muscle units, expressed as mean per galea in pollen feeding (hatched bars, proboscis tip: n = 10, distal region: n = 13) and non-pollen feeding species (empty bars, proboscis tip: n = 4, distal region: n = 6). Error bars show minimum and maximum of total muscle units (sum of lim and mim). Asterisk denotes presence of a significant difference in the total number of musculature and the number of median intrinsic muscles between pollen feeding and non-pollen feeding Heliconiini in the middle of the distal region (n = 19, Z = - 2.371, p = 0.018, n = 19, Z = - 2.008, p = 0.045, respectively). igm…intrinsic galeal muscles, lim…lateral intrinsic muscles, mim…median intrinsic muscles.

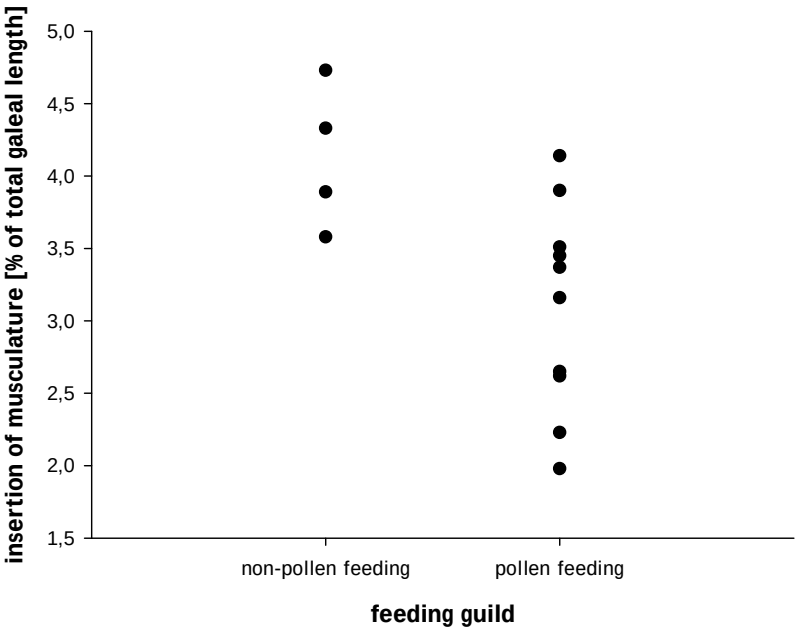

Figure 4. Insertion of musculature in pollen feeding and non-pollen feeding Heliconiini. Each symbol represents one individual. Intrinsic galeal muscles extend significantly further to the tip in pollen feeding species. Mann-Whitney-U-Test (n = 14, Z = - 2.263, p = 0.024).

4. Discussion

4. 1. *Anatomical aspects*

Butterflies belonging to the tribes Heliconiini and Argynnini have, in principle, a similar musculature arrangement in the proboscis, including series of lateral and median intrinsic galeal muscles in the distal region.

In the proboscis tip of all investigated species, muscles could not be assigned to two distinct series. Corresponding to the narrowing of the galea, the slender muscle strands take a more longitudinal course (KRENN & KRISTENSEN 2004) to fit in the small lumen of the galea in the proboscis tip. Longitudinal intrinsic proboscis musculature that is not divided into two series is also present in the proboscis of basal Myoglossata. This arrangement is considered as the plesiomorphic condition of the clade (KRENN & KRISTENSEN 2004).

Differences among the investigated individuals concern the occurrence of median intrinsic musculature in the distal region. In all examined species both muscle series occur at the base of the distal region near the transition to the proboscis tip between 7 and 18 % of the galeal length. In other parts of the distal region, median intrinsic musculature is present to varying degrees. *Argynnis paphia* as a member of the closely related tribe Argynnini resembles the groundplan anatomy for most Nymphalidae, which possess both muscle series throughout the distal region (KRENN & MÜHLBERGER 2002). Comparing non-pollen feeding and pollen feeding Heliconiini, there is a slight tendency to a minor expansion of median intrinsic musculature in non-pollen feeding Heliconiini, since in all non-pollen feeding species the distal region beyond the knee bend contains only lateral intrinsic musculature and in some cases this muscle series terminates before the middle of the distal region. However, this pattern can also be observed in some pollen feeding species. In contrast, some pollen feeding species exhibit the same muscle arrangement as the non-pollen feeding *Argynnis paphia* and have both muscle series throughout the distal region. Therefore it remains ambiguous whether pollen feeding species actually possess only lateral intrinsic musculature in the distal region and both muscle series in the proboscis tip, as has been previously concluded (KRENN & MÜHLBERGER 2002). To confirm the assumption that non-pollen feeding species

possess median intrinsic musculature with a minor expansion in the distal region, it is necessary to increase taxon sampling.

The total number of muscle units differs significantly between pollen feeding and non-pollen feeding Heliconiini in the middle of the distal region between 26 and 36 % of the galeal length. This is due to a significant difference in the number of median intrinsic musculature, but not in the number of lateral intrinsic musculature. The proboscis of pollen feeding Heliconiini contains a higher number of median intrinsic muscle units than the proboscis of non-pollen feeding Heliconiini in this region.

Likewise, the insertion of musculature in the proboscis tip differs among pollen feeding and non-pollen feeding Heliconiini. In non-pollen feeding Heliconiini and *Argynnis paphia*, musculature inserts more proximally to the transition to the distal region than in pollen feeding Heliconiini. Therefore, intrinsic galeal musculature extends farther to the apex of the proboscis in pollen feeding species than in non-pollen feeding species, although both groups do not differ in the length of the proboscis tip.

4. 2. *Evolution of intrinsic galeal musculature*

In the ancestral condition of the lepidopteran proboscis only extrinsic galeal musculature is present in the basal joint region which connects the proboscis base with the basal maxillary sclerite. The presence of intrinsic galeal muscles is regarded as a morphological novelty of the Myoglossata that evolved in context with long proboscides adapted to nectar feeding. This musculature is derived from the basal galeal musculature (KRENN & KRISTENSEN 2004) and consists of one or few longitudinal muscle strands in the lineages of Myoglossata except for Ditrysia (KRISTENSEN & NIELSEN 1981, KRISTENSEN 1998). Multiple intrinsic galeal muscles, of which both the origin and attachment sites are markedly distal from the basal joint region are a groundplan autapomorphy of the Ditrysia. Two distinct series of intrinsic galeal muscles occur in most Apoditrysia and in all Macrolepidoptera (KRENN & KRISTENSEN 2004, for review see STEKOLNIKOV & KORZEEV 2007).

KRENN & MÜHLBERGER (2002) found that the plesiomorphic character state of the muscle arrangements in the proboscis of Papilionoidea contains both lateral

and median intrinsic muscle series in the proximal region, the bend region and the distal region. This condition was found also in the outgroup taxa of Hesperiidae, Hedylidae and Geometridae, as well as in all Papilionidae, many Nymphalidae (all Limenitidinae, Nymphalinae, Morphinae, *Apatura* and *Archaeoprepona*) and one lycaenid species, *Hamearis lucina* (Riodininae). In light of the distribution of the median intrinsic muscles throughout the proboscis, three apomorphic character states can be distinguished: (1) the median intrinsic muscles are present in the proximal and bend regions, but absent in the distal region as in Pieridae, Satyrinae, Danainae and some Lycaenidae; (2) the median intrinsic muscles are present up to the bend region and again at the base of the tip region in Heliconiini; (3) all median intrinsic muscles are lost in some Lycaenidae (*Nymphidium*).

The results of this study are only partly consistent with the findings of MÜHLBERGER & KRENN (2002). They investigated two species of Heliconiini, *Dryas julia* and *Heliconius melpomene*, and failed to identify median intrinsic musculature in the range of 30-50 % of the galeal length. This muscle series is also present at the base of the proboscis tip between 10-20 % of the galeal length (MÜHLBERGER & KRENN 2002). *Argynnis paphia* as a member of the closely related tribe Argynnini resembles the groundplan anatomy of Papilionoidea with the presence of both muscle series throughout the distal region. This plesiomorphic condition was also found in some pollen feeding Heliconiini, for example *Heliconius charitonia*, *Heliconius hecale*, *Heliconius melpomene* and *Heliconius doris*. However, in these individuals the muscle series is weakly developed in the middle of the distal region and near the knee bend. According to these findings, the absence of median intrinsic musculature in the whole distal region is not a feature that applies to all members of Heliconiini. Therefore, this character state cannot be regarded as autapomorphy for Heliconiini.

4. 3. *Impact of galeal musculature on proboscis movement*

Several partly contradictory interpretations of the mechanism of proboscis movement have been worked out (BÄNZINGER 1971, EASTHAM & EASSA 1955, HEPBURN 1971, SCHMITT 1938) until the studies of KRENN (1990, 2000) and the

electrophysiological study of WANNENMACHER & WASSERTHAL (2003) elucidated the mechanism of coiling and uncoiling. Contraction of the intrinsic galeal musculature is responsible for the coiling movement of the proboscis (BÄNZINGER 1971, KRENN 1990, 2000, WANNENMACHER & WASSERTHAL 2003). The uncoiling of the proboscis is brought about by an increase of hemolymph pressure due to stipes pumping action (WANNENMACHER & WASSERTHAL 2003, KRENN 1990, 2000).

The functional consequences of the different musculature arrangements for recoiling the proboscis or proboscis movability remain unclear. Although it is clear that all types of intrinsic galeal muscles are used to coil the proboscis (BÄNZINGER 1971, KRENN 1990, 2000, WANNENMACHER & WASSERTHAL 2003), the reason why there are different muscle series in many species, is less obvious (KRENN 1990). KRENN (1990) demonstrated in a comparative anatomical study that Bänzinger's investigation of the mechanism responsible for proboscis movement was carried out in butterflies having various musculature arrangements, yet the results were the identical for all species. In other words, the arrangement of muscles has no bearing on the fundamental mechanism of coiling and uncoiling (KRENN 1990). The reason why there are two distinct muscle series in the proboscis of Macrolepidoptera at all, is still an open question because both muscle series obviously recoil the proboscis (BÄNZIGER 1971, KRENN 1990, 2000, SCHMITT 1938, WANNENMACHER & WASSERTHAL 2003). Therefore, the absence of median intrinsic musculature in a particular region of the proboscis would not impair the principle mechanism of proboscis coiling (KRENN & MÜHLBERGER 2002).

4. 4. *Relevance of galeal musculature for pollen collecting and processing*

The development of a more powerful mechanism of proboscis movement in Ditrysia reflects an adaptation to flowers with deep corollas. This is achieved by the series of oblique muscles permitting a dense packing of the intrinsic galeal muscles (KRENN & KRISTENSEN 2004). In this respect, it is impossible to give an explanation for the different musculature arrangements in the investigated individuals of Heliconiini.

However, the higher number of median intrinsic muscles in the middle of the distal region might help pollen feeding Heliconiini to partially coil the proboscis during the pollen processing behaviour. However, *Argynnis paphia*, which is a related species that does not feed on pollen, possesses a number of median intrinsic muscle units in this proboscis region similar to pollen feeding Heliconiini. Therefore, a higher number of median intrinsic muscles in the middle of the distal region is not a feature that applies exclusively to pollen feeding Heliconiini. Moreover, as mentioned before, the functional importance of median intrinsic musculature, in contrast to lateral intrinsic musculature, is not fully understood.

Also the functional significance of muscles that extend close to the apex of the proboscis in pollen feeding Heliconiini remains unclear. It was assumed that the sideways movements of the proboscis tip, that can also be observed in other Lepidoptera, do not involve intrinsic galeal musculature, but can be explained by compressions of only one stipes that causes asymmetrical pressure conditions inside the galea (KRENN 1990, WANNENMACHER & WASSERTHAL 2003). Possibly, muscles that extend far to the apical end of the proboscis tip may support an efficient and tight coiling of the apical parts of the proboscis during pollen processing. It might also ensure improved movability within the narrow corolla tubes during pollen collecting. The possibility that contraction of these muscles may cause delicate sideways movements cannot be ruled out.

Several previous studies have addressed the question, why *Heliconius* butterflies alone have developed the unique behaviour of pollen collecting and processing with the aid of their proboscides and, especially, which adaptations made this evolutionary step possible (EBERHARD ET AL. 2007, IN PRESS; KRENN 2008; KRENN & PENZ 1998, 2000) . None of these studies discovered anatomical or morphological traits that are unique for pollen feeding Heliconiini. However, details of morphology and behaviour significantly differ among pollen feeding and non-pollen feeding passion-vine butterflies, i. e., modifications in the proboscis length, the abundance and length of bristle-shaped sensilla on the proboscis, the length of the labial palpi (KRENN & PENZ 1998) and the volume of the salivary glands (EBERHARD ET AL., IN PRESS). The presence of proteases in the saliva of pollen feeding butterflies was also regarded as adaptation to pollen utilization (EBERHARD ET AL. 2007). Similarly,

behavioural modifications of the flower probing behaviour (KRENN & PENZ 2000, KRENN 2008) contributed to the adoption of the unique pollen feeding behaviour that is regarded as key innovation for the radiation of *Heliconius* butterflies (GILBERT 1991). Furthermore, the higher number of muscle units in the distal proboscis region and the musculature that nearly reaches the apical tip can be considered as exiguous modifications of existing features which probably evolved in context with pollen feeding. Both may account for improved flexibility of the proboscis and, in particular, of the tip region, as well as for a more powerful mechanism enabling proboscis movement. It is certainly possible that the examined characters of intrinsic galeal musculature constitute but a fraction of the subtle changes in the features of *Heliconius* butterflies that are needed to explain the evolution of a new feeding habit.

5. Conclusion and future work

Butterflies belonging to the tribes Heliconiini and Argynnini have, in principle, a similar musculature arrangement in the proboscis. Lateral intrinsic muscles are present throughout the distal region, whereas median intrinsic muscles are present to varying degrees. The absence of median intrinsic muscles in the whole distal region cannot be regarded as a derived feature for Heliconiini as claimed by KRENN & MÜHLBERGER (2002). However, there is a tendency to a minor expansion of median intrinsic muscles in non-pollen feeding Heliconiini. Pollen feeding species possess a higher number of median intrinsic muscle units in the distal region between 26 and 36 % of the galeal length. Furthermore, in pollen feeding Heliconiini musculature extends further to the apical end of the proboscis tip.

Whether these findings entail functional consequences for proboscis movability in pollen feeding Heliconiini must to be determined in future studies. Video analyses with special focus on the distal region and the proboscis tip of the studied species should be performed to detect differences in the mechanism of movements and movability. Electrophysiological studies with intracellular recordings to determine whether there is a functional difference between lateral and median intrinsic muscles are lacking, so far.

6. Abstract

Neotropical butterflies of the genus *Heliconius* possess the ability to actively collect pollen onto the outside of their proboscides and to ingest amino acids extracted from the pollen grains with a particular processing behaviour. During typical pollen processing behaviour the proboscis is partly coiled and uncoiled repeatedly. Both flower-visiting behaviour and morphology of mouthparts show adaptations to this unique form of nutrition. Since the contraction of the intrinsic galeal muscles is responsible for the coiling movement, the arrangement and number of the intrinsic galeal muscles was studied in detail. Previous anatomical studies of the proboscis musculature in Heliconiinae showed that median intrinsic musculature is present up to the bend region and occurs again at the base of the tip region whereas the lateral intrinsic muscles occur throughout the whole galeal length.

The unique muscle arrangement probably provides the proboscis with its extraordinary flexibility. It is hypothesized to be a functional adaptation which evolved in association with pollen feeding.

Semithin sections of the distal region and the proboscis tip reveal that species of non-pollen feeding Heliconiini tend towards a minor expansion of the median intrinsic musculature in the distal region than pollen feeding species. Furthermore, the proboscis of pollen feeding Heliconiini contains a higher number of muscle units of the median intrinsic musculature in the middle of the distal region. In pollen feeding Heliconiini, intrinsic galeal musculature extends further, almost to the apical end of the proboscis tip.

It is concluded that the higher number of muscle units in the distal proboscis region may help the proboscis to coil during the pollen processing behaviour. Intrinsic galeal muscles that extend far to the apical tip may be responsible for the flexibility of the proboscis tip and might therefore contribute to enhanced efficiency in flower handling and pollen collecting behaviour.

7. Zusammenfassung

Die neotropisch verbreiteten Schmetterlinge der Gattung *Heliconius* besitzen die Fähigkeit, mit ihrem Rüssel aktiv Pollen zu sammeln. Die Pollenpakete werden durch wiederholtes teilweises Ein- und Ausrollen des Rüssels bearbeitet, bis Aminosäuren freigesetzt werden und diese aufgenommen werden können. Sowohl das Verhalten während des Blütenbesuchs, als auch die Morphologie der Mundwerkzeuge zeigen Anpassungen an diese einzigartige Ernährungsweise. Da für die Einrollbewegung des Rüssels die Kontraktion der intrinsischen galealen Muskulatur verantwortlich ist, wird die Anordnung der intrinsischen galealen Muskulatur in dieser Studie untersucht. Vorangegangene anatomische Studien der Rüsselmuskulatur bei Heliconiinae zeigten, dass die medianen Muskeln bis zur Knieregion vorhanden sind und erst wieder an der Basis der Rüsselspitze auftreten, während die laterale Muskelserie über die gesamte Länge des Rüssels vorhanden ist. Die einzigartige Muskelanordnung im Rüssel, die zu einer besseren Beweglichkeit beitragen könnte, kann als funktionelle Anpassungen betrachtet werden, die im Zusammenhang mit Pollenfressen entstanden ist.

Semidünnschnitte der distalen Region und der Rüsselspitze zeigen, dass nicht pollenfressende Heliconiini in der distalen Region weniger mediane Muskeln besitzen als pollenfressende Heliconiini. Im Rüssel von nicht pollenfressenden Arten findet sich eine signifikant geringere Zahl an medianen Muskelsträngen in der Mitte der distalen Region. Bei pollenfressenden Heliconiini reicht die Muskulatur weiter in die Rüsselspitze als bei nicht pollenfressenden Arten.

Die größere Anzahl von Muskeln in einer bestimmen Region des Rüssels könnte beim Einrollen des Rüssels während der Pollenbearbeitung helfen. Muskeln, die weit in die Rüsselspitze hineinreichen, könnten für die größere Beweglichkeit der Spitze verantwortlich sein und so zu einer größeren Effikivität beim Pollensammeln beitragen.

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