



Fruit-feeding behavior of the butterfly *Consul fabius* (Charaxinae, Nymphalidae, Lepidoptera)

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Abstract: Fruit-feeding behavior of the butterfly *Consul fabius* (Charaxinae, Nymphalidae, Lepidoptera). Fruit-feeding is a common behavior in many tropical butterflies. *Consul fabius* (Charaxinae) was studied to analyse the two applied feeding techniques in detail, i.e., fruit-piercing and fruit-sweeping. Fruit-piercing was performed with a straight proboscis, accompanied by up and down pushing movements of the whole body; up to 90 % of the proboscis length was inserted into a soft fruit. During fruit-sweeping, the dorsal side of the proboscis drinking region was turned towards the fruit to ingest liquid from the surface. Characteristic sweeping movements of the proboscis occurred that scanned the fruit surface while the body stayed motionless. The autofluorescence of the proboscis cuticle indicated increased sclerotization towards the tip. Strong sclerotization in the cuticle occurred in the galea linking structures and the sensilla styloconcia near the tip. These sensilla extend from soft cuticle of the galea, indicating a flexible socket of each sensillum. This arrangement might help to mash fruit tissue during piercing. In some individuals, the two galeae were shifted antiparallel so that an apical opening into the food canal was formed. The fruit-piercing behavior and the typical proboscis morphology of Charaxinae are autapomorphic features of this clade, whereas sweeping fruit-feeding behavior evolved several times independently in various lineages of the nymphalid butterflies.

Keywords: fruit-piercing, feeding behaviour, proboscis, butterfly, Papilionoidea, Lepidoptera

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Introduction

Adult butterflies (Papilionoidea) exclusively feed on fluids that are obtained from a variety of nutrient sources using their coilable proboscis (SCOBLE 1992, KRENN 2010). Normally butterflies use floral nectar and supplement this sugary diet with dissolved mineral substances. However, some species exclusively rely on juices of squashed fruits, honey dew, fresh dung or tree sap as a main food source (DEVRIES 1987, SCOBLE 1992, KRENN 2010). Similar to all adult glossatan Lepidoptera the mouthparts of Papilionoidea are characteristically modified into a siphoning proboscis which is simple in composition and has rather uniform morphology throughout the megadiverse taxon (KRISTENSEN 1998). The labrum is small, the mandibles are reduced, and the vestigial labium forms the ventral head bearing a pair of large labial palps. The proboscis of Lepidoptera is composed of the

two elongated and interlocked galeae of the maxillae. The basal maxillae form a pair of hemolymph pumping structures that enable uncoiling of the proboscis from the spirally coiled resting position (EASTHAM & EASSA 1955, BÄNZIGER 1971, KRENN 1990; reviewed in KRENN 2010). Encompassing the food tube, the galeae are linked with each other on the dorsal and ventral sides by cuticle processes (called legulae) of the galeal wall (DAVIS 1986, KRENN & KRISTENSEN 2000). These cuticle structures firmly interlock the two galeae on the ventral side by a series of toothed hooks (EASTHAM & EASSA 1955). The dorsal legulae extend from the dorso-medial galeal wall horizontally to the opposite galea overlapping those of the opposite galea; they form slits into the food canal near the proboscis tip (KRENN & KRISTENSEN 2000). This apical part of the proboscis is called “tip region” or “drinking region” as it allows the uptake of liquid into the food canal (KRENN 1990, KRENN & MÜHLBERGER 2002, MONAENKOVA et al. 2012, LEHNERT et al. 2013, 2016, LEE et al. 2014).

In most butterflies the uncoiled proboscis usually assumes a position showing a distinct bend after one third of the length during nectar feeding. Forward and backward movements of the distal proboscis are combined with up-and-down motions of the entire proboscis during flower-probing (EASTHAM & EASSA 1955, KRENN 1990, 2008). A hydraulic mechanism, the elastic properties of the galeal cuticle and galeal musculature cause the proboscis movements (BÄNZIGER 1971, KRENN 1990, 2000, WANNENMACHER & WASSERTHAL 2003; reviewed in KRENN 2010).

The proboscis movements are controlled by three main types of sensilla in butterflies (PAULUS & KRENN 1996, KRENN 1998, PETR & STEWART 2004, FAUCHEUX 2013). The bristle-shaped sensilla trichodea/chaetica contain mechanoreceptors. Their aporous setae are usually longest on the ventral side of the proximal region and shortest near the tip of the proboscis (KRENN 1998). Sensilla basiconica exist on the external surface of the proboscis, but also inside the food canal. These short, uniporous sensilla are chemoreceptive and enable taste sensation (KRENN 1998, FAUCHEUX 2013). Sensilla styloconica have a variously shaped stylus and short sensory cone located at the tip (KRENN 1998, KRENN et al. 2001, PETR & STEWART 2004). Sensilla styloconica are restricted to the drinking region of the proboscis. Concluded from ultrastructural evidence, sensilla styloconica are bimodal taste/mechanoreceptive sensilla with high sensitivity to various sugars (SALAMA et al. 1984, BLANEY & SIMMONDS 1988, KRENN 1998, KVELLO et al. 2006). Depending on the feeding guild, there is a lot of variation in length and sensilla arrangement especially in fruit-feeding butterflies (KRENN et al. 2001, LEHNERT et al. 2016).

The feeding behavior, the proboscis morphology and the micromorphology of the sensilla in particular have been discussed to be adaptations to fluid uptake from rotting fruits and other non-floral fluid sources in some taxa of the Nymphalidae (KRENN et al. 2001, MOLLEMAN et al. 2005a, LEHNERT et al. 2016). MOLLEMAN et al. (2005a) distinguished two principle feeding techniques in frugivorous butterflies, i.e. fruit-sweeping and fruit-piercing behavior. During fruit-sweeping, the proboscis ingests juice from the moist surface of a fruit. Fruit-piercing behavior, in contrast to fruit-sweeping, enables uptake of juice from inside a fruit. In Lepidoptera, piercing behavior is predominantly associated with fruit- and blood-feeding of highly specialized Noctuoidea, and was extensively studied

under various aspects in these moths (e.g., BÄNZIGER 1970, 1980, 2007, ZASPEL et al. 2007, 2011). In the piercing-sucking Noctuoidea, antiparallel movements of the two galeae have been observed during penetration of the proboscis and special cuticle structures as well as the sensilla styloconica are modified to alternately help anchor the galeae during piercing the fruits or skin (BÄNZIGER 1970, 1980, BÜTTIKER et al. 1996, ZASPEL et al. 2011). Likewise, butterfly species of the nymphalid subfamily Charaxinae were observed to pierce fruits (NORRIS 1936). In these fruit-piercing butterflies the proboscis lack such specialized piercing structures at the tip (MOLLEMAN et al. 2005a). Therefore it is hypothesized that a different piercing technique is applied in butterflies than in fruit-piercing moths. The present study analysed the fruit-piercing feeding behavior of a charaxinae butterfly in detail. The cuticle structures of the proboscis are studied using confocal laser scanning microscopy to gain information of their mechanical properties and possible functions in context with the fruit-piercing technique of butterflies.

Material and Methods

Consul fabius (CRAMER, 1776)

Twenty-five pupae of *Consul fabius* (Charaxinae, Nymphalidae) were purchased from London Pupae Supplies Ltd. After emerging, the adult butterflies were transferred into an experimental chamber (approximately 2 × 2 × 2 m) inside a greenhouse of the Faculty of Life Sciences at the University of Vienna. Temperature was between 27.8°C and 30.7°C with a relative humidity between 58% and 86%.

Analysis of the feeding behavior

Videography of feeding behavior started three days after eclosion. The butterflies were not fed prior to experiments. First, they were offered inflorescences of *Lantana camara* (Verbenaceae) as these flowers are known to be attractive food sources to many butterflies (WEISS 1991, PENZ & KRENN 2000). On the next day pieces of overripe bananas (25% sucrose) or mangos (15% sucrose) were offered. The percentage of sucrose in solution was measured with a hand-held refractometer (Ataga). Feeding behavior was recorded with a digital video camera (Sony HDD, HDR XR550VEB). The video footage (in total 356.5 min showing feeding behavior) was analyzed using the software Magix Video Deluxe 2016 Plus (version 15.0.0.107, Magix Software GmbH). Single frames were used to describe various positions of the proboscis during feeding. The butterflies could feed ad libitum and, in most cases, they could be filmed until they left the food source. *Consul fabius* seemed to be unaffected by the video recordings since there was no escape behavior as soon as feeding had started.

The butterflies were weighed with an electronic balance (Sartorius Elektronische Analysewaage EG; Sartorius AG, Goettingen, Germany) before and after feeding in order to measure the intake of fruit juice.

Imaging and proboscis morphology

Five butterflies were anesthetized with CO₂ and frozen in a deep freezer at -25°C for later studies in the light microscope, confocal laser scanning microscope (CLSM) and scanning electron microscope (SEM).

The head and whole mounts of the proboscis were viewed and photographed using a stereomicroscope (Nikon SMZ25). For these methods both deep-frozen butterflies and FAA fixed specimens (4 parts formaline, 1 part acetic acid, 10 parts alcohol for 3 to 7 days) were used.

Confocal laser scanning microscopy was used to analyze the cuticle composition of the proboscis by detection of its autofluorescence. The coiled proboscises were cut off the defrosted bodies, transferred into a droplet of glycerine located on depression microscope slides and covered with a cover slip. The specimens were visualized with the confocal laser scanning microscope Leica TCS SP5 II (Leica Microsystems, Wetzlar, Germany) equipped with three diode lasers at wavelengths of 488 nm, 561 nm and 633 nm as well as a 405 nm argon laser source. The latter was used to visualize the autofluorescence emitted by resilin. A bandpass emission filter was chosen for the 405 nm argon laser, for the other lasers longpass emission filters were used. The different wavelengths were excited and detected sequentially. Image stacks were recorded with 0.3–0.45 μm step size along the z-axis. Colors were chosen following the standards of MICHELS & GORB (2012), i.e., blue: excitation at 405 nm and emission 410–480 nm, green: excitation at 488 nm and emission 500–795 nm, red: excitation at 561 nm and emission 570–795 nm as well as excitation at 633 nm and emission at 640–795 nm. This technique has successfully been used to study the material properties of arthropod cuticle (MICHELS 2007, MICHELS & GORB 2012, PEISKER et al. 2013, MICHELS et al. 2015, WILLKOMMEN et al. 2015, RAJABI et al. 2016). Image stacks were captured as maximum intensity projections created by the CLSM software LAS AF.

The specimens analyzed with CLSM were subsequently used in scanning electron microscopy (SEM). The glycerine was removed from the proboscides by washing them three times in 30% ethanol for 20 minutes. After that, specimens were dehydrated in an ascending ethanol series, submerged in a 1:1 hexamethyldisilazane (HMDS)-alcohol solution for 35 minutes and in 100% HMDS for 40 minutes prior to air drying. Samples were mounted on SEM viewing stubs using graphite adhesive tape. They were sputter-coated with gold for 180 seconds and examined in a Philips XL30 ESEM scanning electron microscope at a voltage of 15kV.

Results

Fruit-feeding behavior

All butterflies accepted slices of overripe mango and banana as food sources while flowers of *L. camara* were not probed by *C. fabius* in the feeding trials. Within 1–3 seconds after landing on the fruit, butterflies uncoiled their proboscides and started to probe a fruit with the tip. All six individuals performed piercing behavior, but only four butterflies also used the sweeping feeding behavior (Fig. 1). Minimum total feeding time was 7 minutes; the maximum observed feeding time was 210 minutes in one butterfly. The sweeping behavior was performed only on the wet surface of mango, whereas piercing behavior occurred in feeding trials using peeled mango and banana as experimental food source.

During fruit-sweeping behavior, fluid was ingested only from the moist surface of a mango fruit. The extended proboscis was bent in a quarter of a circle so that the dorsal

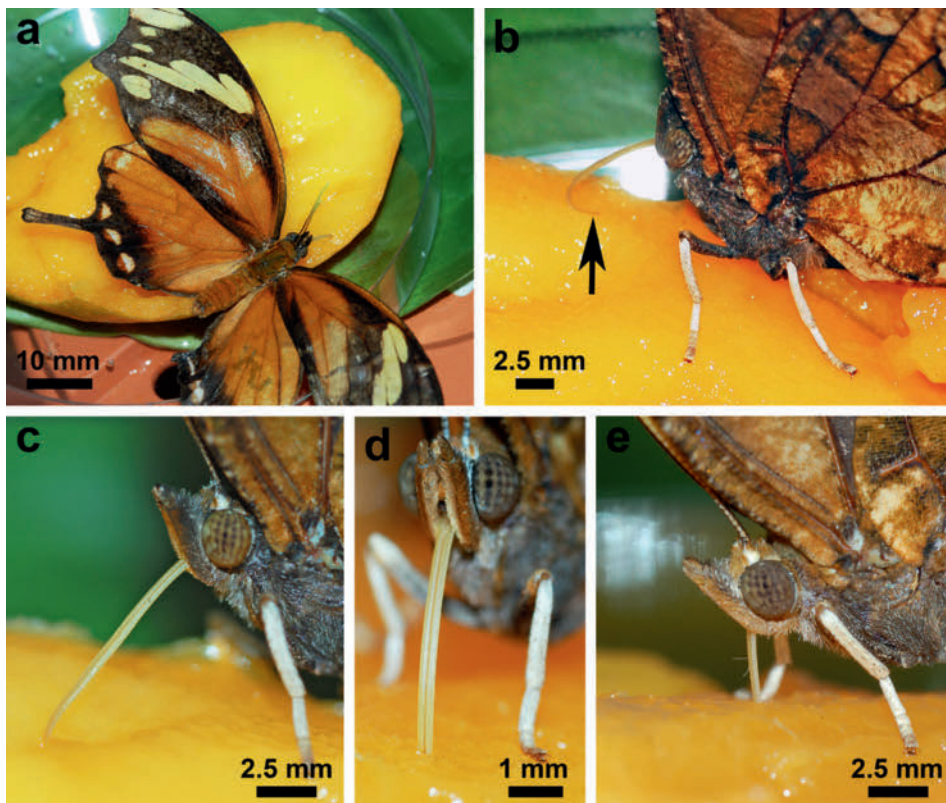


Figure 1. Proboscis positions during fruit-feeding behavior of *Consal fabius* (Charaxinae, Nymphalidae). (a, b) Sweeping fluid-feeding technique: The dorsal side of the proboscis tip (arrow) is turned towards the fruit and liquid enters the food canal through drinking slits on the dorsal side. (c, d, e) Fruit-piercing technique. (c) The tip is placed on the surface of a soft fruit. (d) Tip pierce into the fruit tissue. (e) Proboscis is penetrated deeply into fruit by pushing downward with the whole body.

side of the proboscis' tip touched the fruit (Fig. 1a, b). The ingestion of liquid could be observed through the drinking slits. During probing movements, the proboscis tip was lifted and then placed elsewhere or it was moved back and forth longitudinally to the body axis or the tip was bent sideways. These sweeping movements enabled the otherwise motionless butterfly to scan the fluid source and drink from an area between the tarsi of the middle legs to an area under the tips of the antennae. The time in which the proboscis was motionless lasted between 2–40 seconds, whereas the maximum time of the continuous sweeping movements of proboscis but motionless legs was 20.5 minutes.

All studied individuals of *C. fabius* performed fruit-piercing. The fruit-piercing behavior was characterized by penetration of the proboscis tip into the fruit tissue (Fig. 1c–e). Before piercing, experimental specimens showed long series of quick dabbing movements in which the butterfly probed the fruit with the proboscis tip. During this searching behavior, the drinking region was slightly flexed into various directions on the fruit. As soon as an appropriate spot was found, the proboscis was stuck into the fruit. The proboscis was held

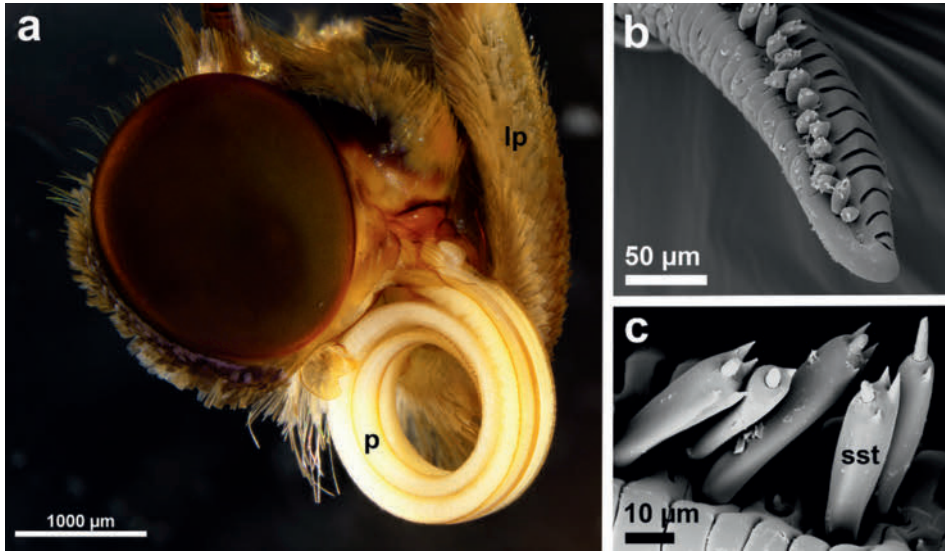


Figure 2. Mouthparts of *Consul fabius* (Charaxinae, Nymphalidae). (a) Head with coiled proboscis in resting position; proboscis cuticle is light hued and coiled in approximately two coils; the right labial palpus removed. (b) Proboscis tip equipped with short sensilla styloconica in two rows; dorsal legulae form slits between each other. (c) Sensilla styloconica with flexible base.

nearly straight without the distinct bend during the piercing process (Fig. 1 c, d). When feeding on banana, butterflies just inserted the drinking region of the proboscis into the fruit that is approximately the most distal 10 % of total proboscis length. A single piercing action was usually interrupted by new dabbling actions or a change in piercing depth by continuous up and down pivoting of the whole body. When feeding on mangos, the depth of piercing greatly varied in the different piercing events. In some individuals up to 90 % of proboscis' total length penetrated the fruit by pushing the proboscis deep into the tissue (Fig. 1e). Piercing was performed in different angles of the proboscis against the substrate varying from a straight vertical (Fig. 1e) or oblique (Fig. 1c) to nearly horizontal position of the proboscis. Up and down movements of the whole proboscis followed and were caused by movements of the whole body. In the observed individuals of *C. fabius*, 95–100 % of total feeding time was used for piercing behavior combined with searching, while only 0–5 % was used for sweeping. However, one individual spent approximately 70 % of its feeding time with sweeping combined with searching and pierced the fruit during 30 % of the total feeding time. This was performed on a mango in the second longest feeding event observed lasting for approximately 150 minutes.

The body weight of *C. fabius* butterflies ranged between 172.6 mg and 272.8 mg before food uptake. The weight increase after fruit-feeding ranged between 4 mg and 24.3 mg that was equivalent to 2.3–14.1 % of body weight.

Proboscis structures and function during fruit-piercing behavior

The smooth proboscis of *C. fabius* has a light yellowish cuticle and it forms a spiral of 2–2.5 coils in the resting position (Fig. 2a). The total length of the proboscis ranges

from 9.55 mm to 10.13 mm ($n = 5$) with an apical drinking region spanning approximately 10% of the total length where the dorsal legulae form drinking slits (Fig. 2b). In some individuals the interlocked galeae were held in a position where one galea protruded the other forming an opening to the food canal at the apex through which ingestion of liquid directly into the food canal could be possible inside the fruit (Fig. 3).

The sensory equipment of the proboscis tip consisted of 38–41 sensilla styloconica found on each galea in dorsolateral position in distal-most 0.7 mm of the proboscis. Restricted to the drinking region of the proboscis, sensilla styloconica were arranged in 1–2 rows next to the dorsal drinking slits (Fig. 2b, c). These sensilla were composed of a short uniporous sensory cone located on a $45 \mu\text{m}$ ($\pm 8.6 \mu\text{m}$; $n = 5$) long stylus with 1–4 small apical spines (Fig. 2c).

Analysis of the proboscis using CLSM indicated a material gradient of the cuticle from the proximal to distal proboscis in *C. fabius* (Fig. 3). Stimulated by the 405 nm laser light, blue autofluorescence was dominant in the proximal and middle regions of the proboscis that indicated weakly sclerotized chitinous material. The nearer to the tip, increased cuticle sclerotization could be recognized by green autofluorescence. The legulae and sensilla styloconica had red autofluorescence indicating strongest sclerotization of the cuticle of the proboscis wall. However, the cuticle areas from where the sensilla styloconica extend from the galeae showed blue autofluorescence indicating soft cuticle (Fig. 3).

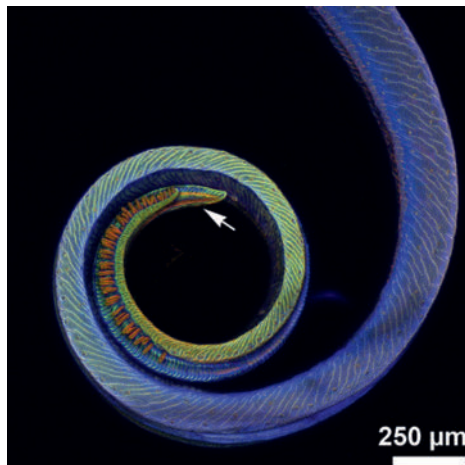


Figure 3. Proboscis of *Consul fabius* (Charaxinae, Nymphalidae) in lateral view using confocal laser scanning microscopy (CLSM maximum intensity projections). Tips of the galeae are dislocated against each other opening the food canal at the apex of the proboscis (arrow). The color gradient of the autofluorescence of the proboscis cuticle from proximal to distal indicating weakly-sclerotized chitinous material proximally (blue autofluorescence) and increased sclerotization distally (yellow/green autofluorescence); the legulae as well as the sensilla styloconica near the tip consist of stronger sclerotized cuticle (red autofluorescence); the sensilla styloconica extend from weakly-sclerotized cuticle (blue autofluorescence).

Discussion

Fruit-sweeping versus fruit-piercing feeding behavior

The present study examined the fruit-piercing behavior of a Neotropical butterfly in detail. In previously studied fruit-feeding African nymphalid butterflies fruit-piercing and fruit-sweeping feeding techniques have been distinguished (MOLLEMAN et al. 2005a). Various nymphalid butterflies from different lineages use sweeping movements of the proboscis to take up fluids from wet surfaces of a rotting fruit or other wet organic substances. During this fruit-sweeping behavior the dorsal side of the distal proboscis is characteristically bent towards the moist surface whereby fluid accumulates around the tip sensilla and the drinking slits. The proboscis tip region scans the ground for an

appropriate feeding spot in a stereotypical pattern of movements in obligatory non-flower visiting African and Neotropical Nymphalidae (KNOPP & KRENN 2003, MOLLEMAN et al. 2005a, KRENN 2008); a behavior that was likewise found in *C. fabius* (Charaxinae). In addition, *C. fabius* butterflies are able to deeply push the proboscis into the fruit by holding the proboscis straight and applying pressure. In contrast to sweeping behavior that is performed with a motionless body, the fruit-piercing process is characterized by up and down pushing movements of the whole body.

Function of proboscis structures during fruit-piercing

During the initial search behavior of *Consul*, the flexible tip bends in all directions. Such behavior was observed in many fruit-feeding Nymphalinae and Satyrinae (MOLLEMAN et al. 2005a, KRENN 2008) or in *Morpho peleides* (Morphini, Satyrinae) in which the movement pattern has been described in detail (KNOPP & KRENN 2003). Many fruit-feeding Nymphalidae have dense rows of elongated sensilla styloconica that together form a brush-shaped proboscis tip (KRENN et al. 2001, KNOPP & KRENN 2003, MOLLEMAN et al. 2005a, LEHNERT et al. 2016). Particularly the numerous and long sensilla styloconica characterize the proboscis tip region of obligatory non-flower visiting butterflies (KRENN et al. 2001). These structures facilitate fluid uptake by forming a small pool of fluid around the hydrophilic cuticle in the drinking region (LEE et al. 2014). It was assumed that the sensilla styloconica act like a nano-sponge which help to accumulate fluid droplets during fluid sweeping next to the drinking slits since their cuticle was found to be wettable (MONAENKOVA et al. 2012, LEHNERT et al. 2013, 2016, LEE et al. 2014). The numerous sensilla styloconica form a large hydrophilic surface, but their length prevents the tip from piercing fruit. However, only few and rather short sensilla styloconica occur in *C. fabius* and other species of the Charaxinae (KRENN et al. 2001, MOLLEMAN et al. 2005a). Autofluorescence of the proboscis of *C. fabius* indicated that the cuticle of the sensilla styloconica is strongly sclerotized whereas each sensillum extends from a spot of soft cuticle that might allow deflection of the sensilla styloconica against the galea. It is hypothesized that the rows of sensilla styloconica could mash up the fruit inside and help to liquefy the tissue for ingestion in addition to chemo- and mechano-sensitive functions (KRENN 1998). Furthermore, it is supposed that the antiparallel position of the galeae, in which one of the two galeae overlap the other, form an opening to the food canal. This enables easier uptake of semiliquid substances into the food canal and could be especially useful when feeding on highly viscous fruit juice (LEHNERT et al. 2016). In nectar-feeding butterflies similar “galeal sliding” has been observed probably to overcome the biophysical problem of the narrowing of the food canal toward the tip (TSAI et al. 2014).

Comparison to fruit-feeding moths

Fruit-piercing proboscises were also found in various representatives of the Calpinae (Erebidae, Noctuoidea), as for example, in *Calyptra* (BÄNZIGER 1970) and *Othreis* (SRIVASTAVA & BOGAWAT 1969). As these moths often pierce hard-skinned fruits or mammal skin for blood-feeding, the proboscis tip is pointed and the cuticle is supposed to be hard and stiff while in Charaxinae, which normally feed on soft fruits the proboscis apex is rather broad and blunt (KRENN et al. 2001, MOLLEMAN et al. 2005a).

The piercing behavior of fruit- and skin-piercing Calpinae differs fundamentally from that of fruit-piercing Charaxinae. In *Calyptra*, the proboscis starts with performing vibrations while at the same time the middle of the proboscis bends out laterally while the base and tip stay stable and oscillations of the head are visible. After the skin of a fruit has been pierced, anti-parallel movements of the two galeae perform deeper penetration. Specialized erectile sensilla styloconica as well as modified cuticle tearing hooks and barbs have been described in the distal proboscis that anchor the galea during piercing and lacerate the fruit (BÄNZIGER 1970, ZASPEL et al. 2011). In *Eudocima materna* a second technique was observed in addition: The distal part of the proboscis with the tip region is placed transversely on the fruit surface, and forward and backward movements of the head enable rubbing the fruit with spiny structures. This behavior has a similar effect as a saw opening wounds through that the fruit is opened (SRIVASTAVA & BOGAWAT 1969).

The CLSM analysis of the autofluorescences of the proboscis cuticle indicated increasing sclerotization of the proboscis wall toward the distal region and the tip. HEPBURN (1971) discussed the presence of a regular arrangement of soft and hard cuticle rings with alternating rows of ridges and troughs. In *C. fabius* the proboscis is rather smooth and no ridges were detectable. Several studies on the material composition of arthropod cuticle using CLSM (MICHELS 2007, MICHELS & GORB 2012, PEISKER et al. 2013, MICHELS et al. 2015, WILLKOMMEN et al. 2015, RAJABI et al. 2016, APPEL et al. 2015) showed that blue autofluorescence indicates weakly sclerotized cuticle with high portions of the rubber-like protein resilin using excitation standards of MICHELS & GORB (2011). As same CLSM standards and settings were used in the present study of *C. fabius*, it is concluded that blue autofluorescence of the proboscis indicates high portions of soft and elastic cuticle. Already HEPBURN (1971) underscored that resilin gives a strong blue emission under ultraviolet light with a maximum absorption at 420 nm. The rubber-like protein occurs in those parts of arthropods' cuticle where a high grade of elasticity is required, often in contact regions under stress (RAJABI et al. 2016, APPEL et al. 2015). As the proboscis of a fruit-piercing butterfly is exposed to stress and compression all over the surface, the presence of elastic and soft cuticle might be advantageous while sclerotized cuticle occurs in the interlocking structures in addition to the sensilla styloconica.

Fruit-feeding guild in Nymphalidae

Fruit-feeding behavior takes longer time than nectar-feeding from flowers (KRENN 2008). Usually non-flower visiting butterflies close their wings during feeding and most members of this feeding guild show camouflage wing patterns on the underside (KOEPECKE 1980). Likewise, *C. fabius* usually closed the wings during fruit-feeding showing their underside pattern that imitates dead leaves (DEVRIES 1987). Decaying fruits often contain high portion of ethanol, which was supposed to reduce escape behavior due to intoxication (YOUNG 1979). In addition, a butterfly that has stuck the proboscis deeply into a fruit, as we showed in *C. fabius*, is not able to take off instantaneously. This implies that the predation risk is higher while fruit-piercing and camouflage wing colors could be essential for survival.

Despite the long feeding duration the amount of ingested fluid was rather small in *Consul fabius* compared to *Morpho peleides* that could imbibe up to 50 % of its body weight in

feeding trials from wet plotting paper (KNOPP & KRENN 2003). African piercing Charaxinae were found to be more effective in feeding from various forest fruits having higher intake rates than fruit-sweeping butterflies which were superior in ingesting fluid from wet surfaces (MOLLEMAN et al. 2005b). It could be demonstrated in African Charaxinae that nutrients from the fruits increased fertility and positively affected life-span and reproduction of these butterflies in natural habitats (MOLLEMAN et al. 2008, 2009).

Evolution of fruit-piercing behavior in Nymphalidae

The Papilionoidea originated in the Cretaceous about 119 million years ago and most extant lineages diverged in the Paleogene 65 million years ago (ESPELAND et al. 2018). The majority of species in all lineages are flower-visitors which primarily feed on nectar (KRENN 2010). There is no doubt that nectar-feeding is the plesiomorphic feeding behavior for adult butterflies. Since many representatives among the Apaturinae, Limenitidinae, Nymphalinae, Biblidinae and Satyrinae are obligatory non-flower visitors it is concluded that multiple shifts from flower-feeding to alternative food sources took place. Convergent evolution led to sweeping feeding behavior from openly accessible non-floral liquid nutrients accompanied by a derived proboscis morphology characterized by a brush-shaped tip (KRENN et al. 2001, KNOPP & KRENN 2003). DEVRIES (1987) noticed that at least some representatives of the Neotropical butterfly genus *Opsiphanes* (Brassolini, Satyrinae) also possess the ability to pierce the skin of soft fruits. Although detailed behavioral studies are missing it is concluded that fruit-piercing behavior also evolved twice independently in Neotropical Nymphalidae.

Within the Charaxinae, a characteristic piercing proboscis and similar piercing behavior occurs in all so far studied species (KRENN et al. 2001; MOLLEMAN et al. 2005a). Since the examined species represent various taxa from different lineages of the Charaxinae (according to the phylogeny of ORTIZ-ACEVEDO & WILLMOTT 2013), it is concluded that the derived fruit-piercing behavior along with the apomorphic character state of the proboscis morphology is an autapomorphy of this taxon of butterflies that probably evolved more than 20 million years ago (ESPELAND et al. 2018).

Zusammenfassung

Viele tropische Tagfalter ernähren sich von Früchten und suchen keine Blüten zur Nahrungsaufnahme auf. Bei einem neotropischen Vertreter der Charaxinae, *Consul fabius*, konnten zwei verschiedene Techniken dieser Nahrungsaufnahme unterschieden werden: Anstechen von Früchten oder wischend-tupfende Rüsselbewegungen auf der Fruchtoberfläche. Das Stechverhalten wird mit gerade gestrecktem Rüssel ausgeführt und von Auf- und Abbewegungen des ganzen Körpers begleitet; bis zu 90 % der Rüssellänge wird in weiche Früchte eingestochen. Dagegen wird bei Flüssigkeitsaufnahme von der Fruchtoberfläche der distale Rüssel so gebogen, dass die dorsale Rüsselseite nach unten weist und Flüssigkeit eingesaugt werden kann. Charakteristische tupfend-wischende Bewegungen erfolgen nur mit dem Rüssel, der Körper aber bleibt bewegungslos. Die Untersuchung der Autofluoreszenz des Rüssels weist darauf hin, dass die Kutikula des Rüssels zur Spitze hin härter wird. Besonders starke Sklerotisierung war im Bereich der Galeaverbindungen und auf den Sensillen der Rüsselspitze zu erkennen. Jedoch

entspringen diese Sensillen Bereichen mit schwach sklerotisierter Kutikula. Die kurzen aber harten Sensillen helfen möglicherweise beim Zerreiben des Fruchtfleisches. Bei einigen Individuen wurde antiparallel Verschiebung der Galeae beobachtet, die eine apikale Öffnung der Rüsselspitze ergibt. Das Früchtestechverhalten und die charakteristische Rüsselmorphologie stellen eine Autapomorphie der Charaxinae dar, wogegen wischend-tupfende Flüssigkeitsaufnahme von Oberflächen mehrfach unabhängig in verschiedenen Gruppen der Nymphalidae entstanden ist.

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