**A biomechanical analysis of prognathous and orthognathous insect head capsules: Evidence for a many to one mapping of ridge strain to head strain.**

**Supplementary methods and discussion**

**Supplementary methods**

Two of the investigated lineages – mayflies and stoneflies – perform most of their food uptake during the aquatic larval stage (mouthparts are even vestigial in adult mayflies and many stoneflies) and have head widths of only 2-5mm. Therefore it is not possible to reliably measure basic biomechanical parameters such as bite force. Instead, our study relies on the well-established technique of multibody dynamics analysis (MDA) to obtain the boundary conditions for subsequent finite element analysis (FEA). Given precise 3D geometries, muscle attachment areas and information about the general movement of the mouthparts, MDA has been used to simulate mouthpart movement and predict bite forces and muscle forces in vertebrates (Gröning *et al.*, 2012, 2013) and insects (Blanke *et al.*, 2017b). FEA with different material property combinations for the dorsal tentorial arms (DTAs) and eyes then allows an estimation of the strain in the head capsule during biting, and thus the influence of DTA and eye material on head mechanics to be investigated.

*Multibody dynamics analysis (MDA)*

Using well established and validated routines (Curtis *et al.*, 2010, 2013; Gröning *et al.*, 2013; Watson *et al.*, 2014), 3D surface models of the head capsule and mandibles, with joint- and muscle coordinates were imported into the ADAMS software (MSC Software Corp. USA) and spherical joints specified between the head capsule and the mandibles. Each muscle was modelled as a series of strands to allow the *in vivo* pennation angles to be represented. The Musculus hypopharyngomandibularis was not considered in these analyses since it is a small muscle connecting two movable parts (mandible and a hypopharyngeal sclerite). Therefore, the influence of this muscle cannot be modelled with certainty.

The maximum intrinsic force capability of each muscle was calculated by multiplying the measured physiological cross sectional area with the muscle stress value obtained in earlier studies (33 N/cm² (David *et al.*, 2016) as used in other studies estimating bite force magnitudes (Wheater & Evans, 1989; Meijden *et al.*, 2012; Goyens *et al.*, 2014; Weihmann *et al.*, 2015). The muscle strands were then activated using a dynamic geometric optimization method (Curtis *et al.*, 2010), which calculates the force within each strand based upon its orientation at each time-step, in order to cause the mandible to follow a specific motion. We simulated an initial jaw opening phase to enable a food particle to be placed at the mid-point between the mandibles. During the subsequent closing phase, the mandibles contacted the food particle and generated a bite force and balancing joint reaction (JRF) and muscle forces until the maximum bite force is achieved (Fig. 1). These JRFs and muscle forces were then exported as boundary conditions for the finite element solver VOX-FE2. Please refer to the main text for further details.

*Measurement of material parameters*

The Young’s modulus of hydrated cuticle of the heads and eyes was measured for three adult dragonflies (*Lestes virens*, *Anax imperator*, *Cordulegaster bidentata*; One sample each) using nano-indentation and protocols already reported in previous studies (Klocke & Schmitz, 2011, 2012; Blanke *et al.*, 2017a). All measurements were taken with at least 4µm distance between adjacent edges of the indents. We measured two locations on the head for each species, the medial part of the epistomal ridge/clypeus and the connection of the anterior tentorial arms (ATA) to the head capsule since these locations are nearby the connection of the mandibles to the head or nearby a presumably highly loaded ridge. For the eye, it was only possible to measure two species (*Lestes* and *Anax*) due to loss of material during the embedding procedure. All measurements were taken orthogonal to the external surface of each tested location and for each location 8-13 measurements were made resulting in the following moduli for each species (all values in GPa, first one = epistomal ridge/clypeus, second one = ATA, third = eye):

Lestes: 8.16±1.4 / 7,00±1.8 / 4.80±1,0

Anax: 7.42±0.9 / 5.29±0.7 / 3.34±0.7

Cordulegaster: 8.69±2.4 / 6.58±1.2 / --

We took the mean of the measured head locations of all species as the *ex vivo* modulus for the head capsule (7.3 GPa) and the mean of the measured eye locations (4 GPa) as the *ex vivo* value for the eye. Material parameters for the ligamentous tissue were obtained from the literature (Zajac, 1989; Maganaris *et al.*, 1998). Due to the high variability of cuticle material parameters reported here and in other studies (Vincent & Wegst, 2004), the measured values should be taken as representative only for our sample rather than measurements which can readily be extended to other insect structures.

**Supplementary results**

The chosen species are representatives of the currently hypothesized most basal, recent, winged insect orders (Misof *et al.*, 2014) and show varying mandibular muscle configurations and endoskeletal configurations. All investigated species possess internally hollow biting-chewing mandibles with a proximal orifice and several incisivi which are used for initial processing of the food material. A varying set of muscle bundles inserts through the orifice within the mandible or at the ridge of the orifice, with five in *Siphlonurus* and *Lestes* and two in *Perla* and *Forficula*. All species additionally possess a large mandibular adductor muscle (M. craniomandibularis internus (von Kéler, 1963)) originating in the frontal and occipital area of the head and inserting via a cuticular structure (frequently referred to as “tendon”) at the proximal base of the mandibular orifice.

**Supplementary discussion**

Mayflies are considered to possess a range of plesiomorphic features such as a comparatively high number of mandibular muscles and an anterior mandibular articulation complex that shows (homologous) similarities with the sistergroup of winged insects, the wingless silverfish (Staniczek, 2000). Additionally, ridges such as the epistomal and the circumantennal ridge are rather areas of bent cuticle only slightly thicker than the surrounding material*.* Indeed, the mayfly studied here exhibits the aforementioned plesiomorphic morphology with respect to ridge configuration and mandible muscle set, and it also shows the lowest bite force of all studied specimens, although for example the earwig is comparable in head size. Despite largely similar mandibular advantages in all studied species, the low force transmission coefficient (22.5%, Table 2) and the unfavourable insertion of the main mandibular adductor (67-73°) in *Siphlonurus* means that this species, with its particular ridge configuration and head geometry, is apparently unable to transfer the muscle forces effectively to the food item, whereas the earwig is able to transmit 43% of the muscle force to its food. Furthermore, the absence of head ridges prevents higher force transmissions since this would inevitably lead to higher loading of the head, in particular in regions such as the eyes.

Although sampling of four species does not permit statistical testing, the measured head sizes and predicted bite forces are in line with earlier studies which revealed that absolute bite forces are not a strict function of head size (David *et al.*, 2016). For example, the dragonfly, despite having a larger head size, has only half the bite force of the smaller earwig (Table 3). Instead, it seems that factors such as head geometry, cuticle thickness at muscle origins, muscle set and probably muscle volumes (which are indirectly affected by head shape and proportions of other organs) largely determine absolute bite forces while the mandibular advantage for the distal mandibular incisivi in the species studied is largely the same (~0.4-0.5). The differing MA between left and right mandible in *Siphlonurus* can be explained by the higher asymmetry of the mandibles compared to the other species. Asymmetric mandibles are a common phenomenon in insects (Beutel *et al.*, 2010; Wipfler *et al.*, 2011, 2012) and the grade of asymmetry is suggested to be a function of food uptake requirements (Inoda *et al.*, 2003).

Loading in the region of the eyes was always very low as would be expected for such a structure with fragile sensory tissue (Fig. 2). Indeed, simulation of different material properties additionally confirms that the eyes have an overall low influence (<1%) on strain distributions across the head (Fig. S1) in species with a prominent circumoccular ridge. Strain magnitudes under less stiff eye material properties were distinctly higher in the mayfly (Fig. S1) which lacks a circumoccular ridge.

**The influence of the dorsal tentorial arms on head mechanics**

Material property variation in the DTA-head connection revealed its decisive influence on loading of other head structures such as ridges and endoskeletal elements. The stonefly and mayfly simulations with a stiffer DTA-head connection lead to higher loading in the eye and subgenal region. The ligamentous DTA-head connection thus helps to reduce strain imposed on the eyes during biting. By comparison, the simulation of a ligamentous DTA-head connection in *Lestes* and *Forficula* did not have a negative impact on loading of the eye region. We hypothesize that this is due to the presence of a well-developed circumoccular ridge, which helps to reduce strain imposed on the eye. In the mayfly *Siphlonurus* such a prominent circumoccular ridge is absent.

Supplementary figure

**Fig. S1 Cumulative histograms of the distribution of first and third principal strains (ɛ1 & ɛ3) to illustrate the influence of varying material properties of the dorsal tentorial arms and eyes on overall head strain.** Note that frequencies are relative to each other to account for the different element numbers of each structure. Small insets show strain distribution for structures with higher strain. (a) *Siphlonurus lacustris* (Ephemeroptera); (b) *Lestes virens* (Odonata); (c) *Perla marginata* (Plecoptera); (d) *Forficula auricularia* (Dermaptera). Abbreviations: DTA, dorsal tentorial arm; HT, “stiff” dorsal tentorium (7300 MPa); ST, “soft” dorsal tentorium (350MPa); SE, “limp” eyes and dorsal tentorium (350 MPa). Bold font indicates *in vivo* material combinations with respect to the eye and DTA connection.

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