**Abstract.** Bilateral symmetry is widespread across animals, yet, among bilaterians, many cases of conspicuous asymmetries evolved. This means that bilaterally homologous structures on the left and right side display divergent phenotypes. Evolution of such divergent phenotypes between otherwise similarly shaped structures can be thought to be favoured by modularity, but this has rarely been studied in the context of left-right differences. Here, we provide an empirical example, using geometric morphometrics, to assess patterns of asymmetry and covariation patterns between landmark partitions, in a grasshopper withconspicuously asymmetric mandibles. Our morphometric data confirms the presence of conspicuous directional asymmetry in the mandibles, and surrounding structures. Covariance patterns and tests hint at the strong integration between mandibles despite their divergent morphologies, and variational modularity with the head capsule While mandibles appear to have been selected to achieve a key and lock morphology by having different shapes, the developmental modularity required to achieve this appears to be overwritten by developmental and/or functional integration, allowing the precise matching required for feeding. The consequent conflicting covariation patterns are reminiscent of the palimpsest model.

**Keywords.** Bite force, Covariance ratio, EMMLi, geometric morphometrics, 3D landmarks, Orthoptera.

**Introduction**

Bilateral symmetry is one of the most widespread morphological characters in animals. Yet, even animals with a well-defined bilaterally symmetrical body plan (e.g. most bilaterians) are *not strictly* symmetrical, since many structures such as internal organs are asymmetrical or even unilateral (Babcock, 2005; Palmer, 1996; Van Valen, 1962). The break-up of symmetry can take many other forms, sometimes less evident, and with different biological implications (Klingenberg, 2022; Palmer, 1994; Van Valen, 1962). Shape differences between left and right sides can be *subtle*, requiring quantitative measurements to detect them. Among such subtle asymmetry types, fluctuating asymmetry (FA) arguably is the best studied, mostly as an assumed proxy for developmental instability, relating to intrinsic or extrinsic stresses on organisms (Benítez et al., 2020; Graham et al., 1994; Klingenberg, 2022; Møller, 1990; Palmer, 1994; Savriama et al., 2016; Van Valen, 1962). When studying fluctuating asymmetry, it is assumed that symmetry is the norm and the target phenotype to achieve (Hansen et al., 2006), and that “stressors”, such as random mutations or environmental conditions, lead to small left-right differences, the distribution of which should be normal and centered on zero (i.e. on perfect symmetry) at the population level (Palmer, 1994). Subtle asymmetry types also include directional asymmetry (DA) and antisymmetry, in which a symmetrical phenotype is *not* the norm, with the left-right differences being of consistent direction in the case of directional asymmetry, leading to a normal distribution *not* centered on zero, or of random direction in antisymmetry, leading to a platykurtic or bimodal distribution of differences. Directional asymmetry and antisymmetry are also found, and are characteristic, for *conspicuous asymmetries*, which are cases of asymmetry readily visible upon observation (Babcock, 2005; Palmer, 2004).

Because directional asymmetry and antisymmetry entail systematic differences between sides, they are generally considered to be inherited, and sometimes to be adaptive (Palmer, 2004). The latter point, however, is dependent on whether subtle or conspicuous asymmetries are studied. For example, *subtle* directional asymmetries in insect wings have been repeatedly demonstrated, but may be of little adaptive significance, due to limited functional consequences (Klingenberg et al., 1998; Pélabon & Hansen, 2008; Pither & Taylor, 2000). On the other hand, *conspicuous* directional asymmetry in the skull of toothed whales has been argued to be related to feeding or biosonar function (Churchill et al., 2019; del Castillo et al., 2016; Huggenberger et al., 2017; Laeta et al., 2023; Lanzetti et al., 2022; Macleod et al., 2007); extreme conspicuous directional asymmetry in the skulls of flatfishes relates to their benthic ecology (Evans et al., 2021); conspicuous antisymmetry in many Crustacean appendages has been related to divergent left and right functions (Govind, 1989; Govind & Blundon, 1985; Levinton, 2016; Pratt & Mclain, 2002); and in humans and mice, the mirroring of internal organs, or *situs inversus*, is mostly problematic when only some of the organs are concerned, while complete *situs inversus* entails no major health defects (Palmer, 2004).

If one considers that there is not just one left-right (or right-left) body axis, but instead two opposite medio-lateral axes (Meinhardt, 2001; Palmer, 2004), the emergence of conspicuously different left and right phenotypes can be interpreted as the relatively independent expression of two different developmental programs, which can be initiated genetically (e.g. usual *situs solitus*), randomly (e.g. *situs inversus* in *iv* mutant mice), or environmentally (e.g. lobster claws)(Brown & Wolpert, 1990; Govind, 1989; Palmer, 2016). It should however be kept in mind that even in extreme cases of antisymmetry (e.g. in fiddler crabs), the development of both sides has been shown to be positively correlated, at least in terms of resource investment (Levinton, 2016). With this view in mind, in cases of conspicuous directional asymmetry or antisymmetry both medio-lateral axes may be akin to *quasi-autonomous components* (Wagner et al., 2007; Zelditch & Goswami, 2021), producing a special case of modularity in which left and right sides of a bilaterally homologous structure represent developmental modules, anatomically separated and expressing different ontogenetic pathways. In addition to being developmentally modular, each side may also sometimes represent a quasi-autonomous functional module, in the biomechanical sense, illustrated perhaps most obviously in male fiddler crabs which use their minor claw to feed and their major claw for fights and displays, certainly leading to very different selective pressures on the opposite sides of the animal (Pratt & Mclain, 2002). Such functional and/or developmental left-right modularity may be key in allowing bilaterally homologous structures to evolve different morphologies, and in some cases different functions (Clune et al., 2013; Wagner et al., 2007). Although the modularity of crab claws has, to our knowledge, not directly been assessed, studies on the skull of toothed whales do suggest a link between the presence of directional asymmetry and a modification of the modularity pattern of the skull (Churchill et al., 2019; del Castillo et al., 2016, 2017). The situation in this latter case is slightly more entangled than in the crab claws example, because both sides of the skull, which may develop differently, may at the same time be involved in the same function, here feeding or sound production. On the other hand, the general shape of the skull should achieve some degree of symmetry to retain hydrodynamic properties, which would require that some skull modules develop symmetrically, while others develop asymmetrically.

Another case of conspicuous asymmetry are the mandibles of many insects, and among them Orthopterans, i.e. grasshoppers, crickets and relatives (Ball, 1992; Chapman, 1964; Clissold, 2007). Mandibles are used to induce shear and crush food, and their asymmetric shapes allow the distal parts (incisivi) to cross and act as double blades, and the proximal parts (molars) to occlude, forming a key-and-lock morphology (Fig. 1), functionally akin to upper and lower molars in mammals (Clissold, 2007). The left and right mandibles each rotate around an axis formed by two articulation points with the head capsule. They can move independently from each other, meaning they are by definition anatomical modules, and, in Orthopterans, they are each actuated by one adductor and one abductor muscle which are attached to the inside of the head capsule cuticle (Clissold, 2007). Contrary to the bones of the Cetacean skull, the Orthopteran mandibles remain to a large extent physically autonomous from each other, while, contrary to the fiddler crab claws, both mandibles must work together to achieve efficient feeding. What we refer to as a key-and-lock morphology means that the left and right mandibles must be interlocking. As such, it is clear that a bilaterally symmetrical shapes would not function, since the various ridges and cusps would simply hit each other, not allowing any extent of occlusion. Therefore, contrary to the assumption made for fluctuating asymmetry studies, here perfect symmetry cannot be considered the functional and selective optimum. On the other hand, extremely asymmetric mandibles, for instance one very long and one very short, or each with completely unrelated shapes (one could think of it as a random key and a random lock), would also not function properly to shear and crush, since they would not properly come into contact. Therefore, it must be assumed that each mandible has its own optimal shape, whose functional optimality is *in fine* dependent on the shape of the opposite mandible. Consequently, the achievement of optimal feeding function relies on an optimal degree of asymmetry between both mandibles (i.e. a key fitting the lock as well as possible). The determinism of which shape corresponds to which side (in other words which is the key and which is the lock) appears to be genetic, supported by the fact that the left mandible is always larger and overlaps the right mandible (Chapman, 1964; Clissold, 2007). While the different shapes of opposite sides would require divergent developmental pathways, the coordination of those shapes to achieve interlocking certainly must entail precise integration of the mandibular (and head) system as a whole. It can therefore be hypothesized that the mandibular morphological system is under hierarchical genetic control, with one or several shared factors determining the “handedness” of mandibles, unique factors affecting each mandible to produce either a left or a right morphology, and one or several integration factors producing left-right covariation to achieve interlocking morphologies (Hallgrímsson et al., 2009).

The Orthopteran head (including mandibles) therefore constitutes an interesting study case of an integrated structure, or *tagma* (Minelli et al., 2013), combining symmetric and asymmetric structures: Left and right mandibles are physically independent, but work together in a key-and-lock principle, while the rest of the head capsule should be under selective pressure for symmetry, given it holds sensory structures such as the eyes and antennae. On the other hand, asymmetric mechanical processes from the mandibles may also produce plastic asymmetry in the head. In addition to a common function, left and right mandibles share a common developmental origin (Posnien & Bucher, 2010), and must be tightly integrated to produce interlocking morphologies.

We propose that (i) to achieve conspicuous asymmetry in the mandibular system, two modular medio-lateral developmental pathways must co-exists. At the same time, because head symmetry should be generally maintained, we may expect that (ii) the head capsule should constitute a separate developmental module from the mandibles, in which developmental factors are entirely shared by the left and right half.. (iii) Since the left and right asymmetric components of the mandibular system must work together to achieve a common function, developmental and functional constraints may also counteract the right-left developmental modularity suggested in point (i), thereby producing integration between the left and right modules, to achieve proper feeding function. An additional possibility is that (iv) asymmetries may “spill-over” to neighboring areas, for example here through asymmetric mechanical loads on the head capsule (Levinton, 2016; Pratt & Mclain, 2002; Tiwari et al., 2017), which would increase covariation between the conspicuously asymmetric structures (mandibles) and their anatomical attachment and articulation structure (the head capsule).

To test these hypotheses, we focus here on the Orthopteran head and mandibles shape, which we quantify using geometric morphometrics. We measure morphological (co)variation in adults at the population level, and decompose it into various types of asymmetries. Taking advantage of the multivariate nature of geometric morphometric data, we study *variational* modularity between anatomical parts of the mandible-head system and use covariation patterns within and between parts as evidence for *developmental* modularity and integration (Klingenberg et al., 2001; Wagner et al., 2007; Zelditch & Goswami, 2021).

We expect that mandibles should show strong directional asymmetry, as already shown qualitatively. We also expect that their asymmetrical mechanical actions may induce asymmetry in the neighboring regions, but less so in more anatomically distant regions of the head, especially around sensory structures for which symmetry is functionally advantageous (e.g. compound eyes). Second, we expect that, according to point (i), the left and right mandibles may constitute variational modules, with large covariation within each mandible and lower-than-expected covariation between them, allowing their divergent morphologies to emerge. We also expect that mandibles will show low levels of covariation with regard to the head capsule structures, in which symmetry should be maintained (point (ii)). Additionally, because the mandibles should enable proper shearing and occlusion, it may be expected as suggested by point (iii), that their development is tightly integrated, leading to strong covariation between them. If this is the case, it may also be expected that the level of asymmetry is under tight selective control, showing strong adaptive accuracy (Hansen et al., 2006; Pélabon & Hansen, 2008), with individuals deviating from the optimal level of asymmetry having worse feeding performance, as measured here though *in vivo* bite force.

**Materials and Methods**

*Specimens and measurements*. Forty-nine live adult specimens of *Schistocerca gregaria* (Forskål, 1775) were purchased from Fressnapf© (Krefeld, Germany). The animals were brought back to the lab, were fed, and their maximal bite forces were measured using the setup developed by (Rühr & Blanke, 2022) on the same day or the next. Bite forces were measured by two different users (SG and SS), by holding the animals between thumb and index, and allowing them to bite voluntarily on the bite plates. Continuous sequences of bites were recorded, and the highest measured bite force (i.e. maximal voluntary bite force) was extracted and used in further analyses. Maximum voluntary bite forces have been shown in other insects to match physiologically maximum bite forces (Püffel et al., 2023).

*Fixation and microCT scanning*. Specimens were then fixed in Bouin solution for ~72 hours, their heads were cut off the body, and rinsed repeatedly in 70% ethanol. Following this, the heads were dehydrated in a series of increasingly concentrated ethanol solutions, going from 70% to 100% by steps of 10%, with 1 hour at each step. After this process, the heads were critical-point dried (Tousimis Autosamdri 931.GL) before microCT using a Bruker SkyScan 1272 (voltage = 50 kV, current = 200 µA, Image pixel size = 6.0 µm or 7.5 µm) and reconstructed using NRecon. All heads had fully closed mandibles when scanned, to ensure anatomical comparability between specimens while landmarking.

*Landmarking*. 3D reconstructed heads were imported and rendered in the software MorphoDig (Lebrun, 2018). In total, 38 homologous landmarks were placed across the head. However, three landmarks had to be excluded because they could not be placed accurately in all individuals, leaving a total of 35 landmarks (Fig. 1). All landmarks were digitized by the same user (SG) and replicated once, to allow discrimination between the various components of inter- and intra-individual shape variation (i.e. asymmetry, see below).

*Shape variation decomposition and analysis of asymmetry*. Entire landmark configurations were separated between mandible landmarks and head capsule landmarks, meaning that in further analyses, the mandible configurations array was analyzed using matching symmetry, while the head capsule configurations array was analyzed using object symmetry. This means simply that in the case of the mandibles, left mandibles were mirrored along the medio-lateral axis to match the right mandibles, while for the head capsule, each complete configuration was mirrored, and the order of the landmarks modified accordingly in the mirrored configurations, so that the difference between a configuration and its mirror constitutes a measure a asymmetry for the whole head capsule. Configurations and their replicates were aligned by partial Generalized Procrustes Analysis and orthogonally projected onto tangent Euclidean space, using functions ‘pgpa’ and ‘orp’ from (Claude, 2008). Two different approaches were used in the decomposition of asymmetric variation. First, we implemented the approach from (Neubauer et al., 2020), with custom code, to obtain estimates of individual fluctuating asymmetry (iFA) and individual directional asymmetry (iDA) at the individual level, and separately for mandibles and head capsule. This relies on the use of non-centered PCA on the matrix of differences between the configuration of a given individual and its reflection across the sagittal plane (in the case of object symmetry, i.e. for the head capsule), or alternatively the matrix of difference between right and mirrored left object (in the case of matching symmetry, i.e. for the mandibles). In the resulting PCA, the center of the space has a biological meaning, since it represents perfect symmetry (i.e. no difference between a configuration and its reflection or between the configuration of one side and the mirrored configuration of the other side). If the population average coordinate along one of the PC axes is significantly different from 0, it can be concluded that this axis represents DA variation. In the case of conspicuous directional asymmetry, this axis can be expected to be the first PC. Individual positions (averaged across replicates) along this axis can therefore be used as a proxy for iDA in shape differences represented by this axis. When the population average along an axis is not different from 0 (and the distribution is not bimodal, which would suggest antisymmetry), the individual positions can serve as a proxy for iFA. In addition, individual total asymmetry (iTA) was computed as the distance between a head capsule configuration and its reflection, or as the distance between mirrored left and right mandible configuration, averaged across replicates for the same individual. For mandibles only, the size iTA was also computed as the difference in centroid size between left and right mandible configurations averaged across replicates.

The second decomposition approach estimates DA and FA (as well as inter-individual variation) as population-level values, and relies on ANOVA (Palmer, 1994), as implemented in the geomorph R package (function bilat.symmetry) (Adams & Otárola-Castillo, 2013). The ANOVA has two explanatory factors, individual (representing inter-individual variance), side (or mirroring, representing DA), with their interaction representing FA. Finally, using replicates allows one to take into account the landmarking error, and to test for significance of DA and FA.

Asymmetry patterns were visually represented by 3D deformed meshes. This was achieved by importing arbitrarily selected mesh templates for the head capsule, the left mandible and the right mandible (all belonging to the same individual), with their corresponding landmark configurations, and warping them to various reference configurations, using functions ‘read.ply’ and ‘warpRefMesh’ from ‘geomorph’. A mandible configuration difference matrix was computed by subtracting the mean configuration of right mandibles from the mean configuration of mirrored left mandibles. This difference matrix, multiplied by 2, was *added* to the mean configuration of mirrored left mandibles to produce an exaggerated left mandible configuration. The same matrix was *subtracted* from the mean configuration of right mandibles to produce an exaggerated right mandible configuration. The right mandible and left mandible template meshes were then warped to both the average left and right configurations, and to the exaggerated configurations, to produce a deformation gradient of left-right mandible asymmetric differences. Note that because we are using average left and right configurations, the FA component is lost (average FA is expected to be 0), and the asymmetric differences represented should be restricted to DA patterns. A similar approach was used for the head capsule, using the average configuration and mirrored average configuration with reordered landmarks to produce the difference matrix, which was then added or subtracted from a purely symmetrical average configuration (i.e. mean shape of average and mirror average configurations). The template head capsule mesh was then warp to the symmetrical configuration, to the average and mirrored average configurations, and to the exaggerated average and mirrored average configurations.

*Covariation patterns analyses*. Replicated landmark configurations for each individual were averaged, and the resulting shapes were used for all following analyses. As mentioned in the previous section, mandibles and head capsule configurations are superimposed separately. Because mandibles are anatomically separate with respect to each other and to the head capsule, we postulated that the head-mandible morphological system is formed by at least three modules: the head capsule, the right mandible and the left mandible. In addition, we also considered the possibility of the existence of two modules in the head capsule: one formed by sensory structures on the dorsal half of the head, while the ventral half, including the clypeus and labrum, would be functionally related to the mandibles. We then tested for variational modularity, i.e. whether covariation is stronger within modules than between modules regions (Zelditch & Goswami, 2021), separately for the head and for the mandibles, using the ‘modularity.test’ function from the ’geomorph’ package. In addition, pairwise correlations between each of the four proposed modules were tested via two-block partial least squares analyses, as implemented in the ‘integration.test’ function of ‘geomorph’. Finally, to achieve a finer understanding of covariation patterns across the head-mandible system, we computed covariance matrices of Procrustes coordinates, as well as 3D landmark correlation matrix, using congruence coefficients, as implemented in the ‘dotcorr’ function of package ‘paleomorph’. We then proceeded to produce visual representations of these matrices as heatmaps, with variables ordered according to the module they belong to. This allowed us to map qualitatively which modules demonstrate higher within- and between-module-covariation, but also coordinate-by-coordinate and landmark-by-landmark covariation patterns.

*Variation and correlations between bite force and asymmetry.* To test whether the degree of asymmetry in the head and mandibles was functionally driven, we assessed the correlation between individual *in vivo* maximum voluntary bite force (BF), and the various indices of individual asymmetry (iTA, iFA, iDA). One hypothesis was that the functional key-and-lock principle for good occlusion between mandibles would lead to an optimum asymmetry value maximizing bite forces, and therefore to a quadratic relationship between BF and iTA or iDA. On the other hand, FA is generally considered to worsen fitness, therefore possibly leading to a negative relationship between iFA and BF. Finally, to test whether these traits are themselves under selection, we computed their respective coefficients of phenotypic variation (CVp), which can be viewed as a measure of adaptive accuracy (Hansen et al., 2006; Pélabon & Hansen, 2008). Finally, we tested the relationship between bite force and left and right mandible shape using two-block partial least squares.

All statistical analyses were carried out in the R programming environment version 4.1.3 (R Core Team, 2022).

**Results**

*Head and mandible shape asymmetry*. As expected, mandible shape at the population level was strongly directionally asymmetric (Table 1, Fig. 3). This directional asymmetry (DA) is however located mostly at the incisivi (landmarks 18 to 23) which are the most conspicuously asymmetric structures, as well as the insertion area of the mandible closer muscle (landmarks 28-29, 32-33; Fig. 3). DA can also be noticed in head capsule structures which are located close to the mandibles, such as the clypeus-labrum region (landmarks 1-3 and 12), and the tentorial bridge (landmarks 14-15). The major directionally asymmetric patterns are the tilting of the clypeus-labrum region towards the right side, and the dorso-ventral and antero-posterior displacement of mandible incisivi. Fluctuating asymmetry (FA) is also significant, in the mandibles and in the head caspule, although its magnitude is much less than DA. FA is more spread out across the head than DA, however, the incisivi also show a higher FA variation component (Supp. Fig. 1).

When using Neubauer et al.'s (2020) approach, the asymmetry PCA of the head and mandible both show that the major part of the asymmetric variation is directional, with PC1 accounting for 62% of asymmetric variation for the head capsule, and up to 88% for the mandibles (Fig. 4). The second axis, which represents for the head and mandibles respectively about 10% and 3% of asymmetric variance is centered around 0 (respectively for the head and mandibles, one sample t-test, mean = 0.0002 and -0.0003, t = 0.101 and -0.160, df = 48, both P > 0.85), and normally distributed for the mandibles, for the head when removing two extreme data points (Shapiro-Wilk normality test, W = 0.9858 and 0.9704, both P > 0.2), suggesting it represents a FA component. Further axes of asymmetric variance were not explored.

*Covariation patterns*. Covariance ratio tests, as implemented in function ‘modularity.test’, were significant for a partition between left and right mandible (CR = 0.8739, P = 0.001), as well as between ventral and dorsal halves of the head (CR = 0.8900, P = 0.006). Both CR values are rather close to 1, suggesting that in both cases the covariance between the putative modules is only slightly lower than that within the modules. Pairwise two-blocks partial least squares analyses between the four putative modules show significant correlation between both head halves and between left and right mandibles, as well as between the ventral head module and left mandible (Fig. 2C). Covariance and correlation heatmaps display additional details: overall covariances and correlations are higher within and between mandibles than across the head, as illustrated by the warmer colors observed for mandibles in Fig 2B and C. In the head, the dorsal half, supporting the major sensory structures appear to have greater covariances and correlations compared to the ventral half of the head. Finally, it can be noted that the covariances are on average of the same magnitude between the left and right mandibles as within the left mandible. This may be at least in part driven by the high covariance/correlation between the homologous points on each side, however, strong covariances and correlations are also observed between all combinations of the points representing the muscle insertions both within and between mandibles.

*Variation and correlation in bite force, shape and asymmetry*. No shape component is correlated to *in vivo* bite force, whether individual mandibles, head capsule, or their symmetrical or asymmetrical components are tested (r-PLS = [0.45; 0.54], all P > 0.1). *In vivo* bite force is also correlated neither to iDA, nor to iFA, nor to iTA (including size iTA) in mandibles or in the head (Pearson’s correlation, all |r| < 0.25, all P > 0.1). Quadratic and linear model fits to the data were all non-significant (all R² < 0.1, all P > 0.2, Fig. XXX). This result held whether we used iDA, iFA, iTA, and for both the mandibles and for the head capsule. The coefficients of phenotypic variation (CVp) were rather low for mandible shape iTA (CVp = 0.1027), mandible size iTA (CVp = 0.1549), and head shape iTA (CVp = 0.1652). Similar values were found for shape iDA for the mandibles (CVp = 0.1044), the head (CVp = 0.1909). On the other hand, iFA values were very high for both the mandibles (CVp = 1.2784) and head (CVp = 1.3015). Bite force had an intermediate coefficient of phenotypic variation (CVp = 0.3147). ( For reference we also computed CVp for head centroid size, which had a much lower value of CVp = 0.0506, and head length with CVp = 0.0565.

**Discussion**

In this study, we showed large and significant directional asymmetry (DA) in the head of the grasshopper *Schistocerca gregaria* (Table 1, Fig. 4). Most of this directional asymmetry concentrates at the mandibles, as expected since they are conspicuously asymmetric structures, but DA was also found in surrounding head structures (Fig. 3). Fluctuating asymmetry (FA) was significant, although accounting for much less variation than DA. Despite being more spread out than DA, the largest FA was also found in the mandibles. In addition, we showed that the level of individual head asymmetry did not appear to influence individual biting performance (Fig. 7). Despite the asymmetry, strong covariation was found between left and right mandibles, while the head capsule showed weaker within-covariation overall.

*Conspicuous asymmetry, modularity and the palimpsest model.*

Despite being recognized as a *tagma*, and therefore being an integrated anatomical unit (Minelli et al., 2013), our results show evidence that the insect head is also a modular system. This result fits with the fact that different parts of the head derive developmentally from various extremely specialized segments (Posnien & Bucher, 2010), which are then used for different functions. Variation and covariation patterns support the idea that the mandibles form a functional module for feeding, with the left and right mandibles showing strong covariation, despite each having divergent shapes. Within the feeding module, we also propose that, given our data, the conspicuously asymmetric mandible shapes are best explained by developmental modularity between left and right sides. Indeed, both statistical and qualitative approaches used to test and compare modularity partitions of the head-mandible system lend some support to the fact that the left and right mandibles each constitute a variational module, with strong within-mandible covariation (hypothesis (i) of the Introduction). The CR modularity analyses showed significant variational modularity between left and right mandibles (Table 2), meaning the covariances between mandibles are significantly smaller than covariances within mandibles. What should be noted is that the CR test uses a null hypothesis of a CR ratio of 1, representing the expectation for sets of variables partitioned randomly (Adams, 2016).

Our results show a CR ratio between mandibles significantly different but fairly close to 1 (0.87), despite high covariance within each mandible. This highlights the fact that it is possible for a developmental system to have at the same time several well-defined modules, which can be strongly integrated with each other, producing between modules covariation, leading to CR values close (or possibly equal?) to one. Such type of developmental system may be problematic for tests of modularity relying on covariation patterns, because they generally focus on the end result of several genetic, ontogenetic and plastic processes producing layers of possibly non-matching covariation patterns. This has been coined the palimpsest model by (Hallgrímsson et al., 2009), referring to recycled scrolls on which several layers of written text were overlayed through time, obscuring each other. In our case for example, the common feeding function of left and right mandibles may have increased covariation (e.g. due to plastic shape changes, wear) between them, obscuring to some extent the original developmental modularity of the system. With this in mind, we propose that the significant variational modularity observed here between mandibles reflects developmental modularity, but may actually be underestimated due to the common function of mandibles leading to increased non-developmental covariation. Another non-exclusive possibility is that the strong covariation between mandibles is in fact driven by developmental integration through common factors overarching the factors unique to each mandible, necessary to achieve their directionally asymmetric shapes (Hallgrímsson et al., 2009; Meinhardt, 2001; Palmer, 2004). Fluctuating asymmetry may also be one factor blurring patterns of covariation. Indeed, our results show relatively large magnitude of FA located at the mandible incisivi (Supp. Fig. 1). Because FA is of random direction, it may reduce the correlation between left and right mandible shapes, which is of determinate direction. However, the amount of variation explained by FA is very limited in comparison to DA, which would suggest that the influence of FA on global covariation patterns is small, compared to developmentally or functionally driven covariation.

The impact of functional selection and/or plasticity for interlocking left and right mandible shapes is apparent in the strong within-mandible covariation, limiting deviations from the left and right respective target phenotypes, or at least constraining potential shape changes within each mandible to be correlated, as suggested by the “fly in a tube model” of phenotypic evolution (Felice et al., 2018). Constraints on each mandible must be strong enough to achieve their respective phenotype, while also leading to a tightly integrated combined phenotype. This is corroborated by the relatively small CVP of iTA and iDA, which fall in the range of values for characters under selection (Hansen et al., 2006; Pélabon & Hansen, 2008).

Variational modularity between the mandibles and the rest of the head might have been expected, considering that the mandibles constitute both a developmentally (Posnien & Bucher, 2010) and functionally distinct module. The observed pattern of weak constitutes evidence corroborating that modularity between the head and the mandibles. Such disconnection may be critical to allow conspicuous asymmetry to appear in the mandibles while maintaining the head's global symmetry. Indeed, if the factors defining the left-right conspicuous asymmetry of mandibles were equally expressed in the head capsule, one would expect equally conspicuous head asymmetry. Here, we instead observe significant subtle (rather than conspicuous) DA in the head capsule. This could potentially be a general rule in cases of conspicuous asymmetry arising in Bilaterians, where modularity may be an evolutionary way to relax locally the constraints of symmetry, akin to the more general idea that modularity may "favor evolvability by allowing one module to change without interfering with the rest of the organism" (Hansen, 2003). For the skull of toothed whales, it was suggested that it is asymmetry which drives modularity (Churchill et al., 2019). We instead propose that modularity is one prerequisite for conspicuous asymmetry to start evolving. This fits in our opinion better with the idea that modularity allows traits to evolve independently (Hansen, 2003; Zelditch & Goswami, 2021). It might also be the case that there are strong evolutionary positive feedbacks between modularity and asymmetry: for example, once conspicuously asymmetric structures appear, they may be used for divergent functions, which would entail disruptive selection, through integration *within* left and right structures and parcellation *between* them (Wagner & Altenberg, 1996).

Additionally, the link between conspicuous asymmetry and modularity is probably dependent on the type of anatomical and functional relationships between left and right structures, and their symmetrical surroundings. In the case of the grasshopper, mandibles are working together to achieve a single function, which certainly constrains the degree of covariation between left and right sides. In other cases, such as claws of lobsters or fiddler crabs, left and right functions are divergent, and one might therefore expect even stronger left-right autonomy, i.e. weaker integration, although there is data indicating significant linkage in dimorphic crab claws (Levinton, 2016). It should however be noted that such potential left-right autonomy does not necessarily entail differences in the level of within-side integration, and indeed no difference in integration was found in aeglid fighting and non-fighting claws (Nogueira et al., 2022). On the other hand, in cases where asymmetric structures are embedded within symmetric structures, such as the Cetacean skull, one may expect that the degree of modularity between them is reduced, compared to what we observe in our study. One may also expect that in typically symmetrical species, e.g. insects with symmetrical mandibles, Mysticete whales which have symmetrical skulls, or crustaceans with symmetric claws, selection for parcellation may not have acted, preserving pleiotropic effect and covariation patterns, both between left and right sides, and between the studied structure and its anatomical surroundings, to achieve a symmetrical *bauplan*. This idea is indirectly supported by results from Churchill et al. (2019), who found a larger number of modules in Odontocete whales skulls, compared to classical modularity patterns found in other mammals.

*Asymmetry "spill-over" between mandibles and head capsule*.

The significant variational modularity observed between the ventral and dorsal halves of the head may seem at first glance surprising, considering the head capsule is a rather continuous cuticular ensemble, in which junctions between segments are not visible anymore. The fact that DA is observed in the ventral half, and less so in the dorsal half (hypothesis (iv) in the introduction, Fig. 3) may give us a hint. Indeed, asymmetrical mechanical loads from feeding may explain why structures in the ventral half of the head show large DA compared to the dorsal half. This functional linkage, related to the spatial proximity and anatomical articulation of mandibles of the ventral half of the head, could participate in the higher covariation between the ventral half of the head and the left mandible, compared with the correlation between the ventral and dorsal half of the head (Fig. 6D). It should also be mentioned that the very strong and asymmetric closer muscles of the mandibles in fact originate from the internal side of the dorsal part of the head (Weihmann & Wipfler, 2019). The limited DA observed in this dorsal region may therefore be explained by asymmetric muscle actions, possibly countered by selection for maintenance of symmetry, related to the maintenance of optimal sensory performance, which could be achieved by reinforcements of the cuticle, as observed for example around the eyes with the circumoccular ridge as a reinforcing structure. Such selective constraint may not be as strong in the ventral half of the head, which does not have large sensory organs as on the dorsal head capsule.

*Relationship between bite force performance and asymmetry*.

Because left and right mandible shapes must fit each other to achieve their proper function, it could be expected that there exists an optimal asymmetric shape producing the best feeding performance. We therefore expected that there should be an optimal degree of directional asymmetry, deviations from which should reduce performance. We aimed at measuring this performance by recording maximum bite forces at the incisivi. Our results, however, clearly show no relationship between bite forces and directional, total or fluctuating asymmetry (Fig. 7). Because it may be argued that proper feeding performance may in fact leave room for a larger amount of variation in the fit of mandible shapes, we computed CVP, which appears in accordance with the fact that iDA and iTA are indeed under selection, with values matching those for other selected characters reviewed by (Hansen et al., 2006). The absence of relationship revealed here may therefore have two explanations: (i) the degree of asymmetry may impact shearing forces and occlusion, but not static equilibrium bite forces at the tip of the incisivi, as we measure here, or (ii) because mandible shapes are selected to fit each other, variation in the degree of asymmetry is limited, while variation in bite force may be increased by other unrelated factors (for example environmental or experimental), which could explain why CVP is higher for bite force than for iTA of iDA. We also cannot rule out completely that bite force measurements ran by two different users (SG and SS) may add inter-user variation, although our preliminary analyses did not reveal differences on average.

*Conclusion and perspectives*.

Modularity is often referred to as a property which favors phenotypic diversification by allowing different anatomical parts to evolve in relative independence by reducing pleiotropic effects between them (Hansen, 2003; Zelditch & Goswami, 2021). Our results constitute some of the first evidence that modularity may have a role in the evolution of disrupted symmetry, here in the head and mandibles of grasshoppers. This potential link between conspicuous asymmetry and modularity had, to our knowledge, only been suggested once before (Churchill et al., 2019), but never tested using separate left-right modules. Interestingly, one study also reported an asymmetric modular pattern in the context of hybridization (Parr et al., 2016), in non-conspicuously asymmetric animals (dogs and dingoes). This may hint at one possible way for conspicuous asymmetry to start appearing, with hybridization disrupting integration and modularity patterns within each species. Genetic assimilation has been suggested (Palmer, 1996, 2004) as another avenue for appearance of conspicuous asymmetry, which would not necessarily require modularity.

It is our opinion that exploring the link between conspicuous asymmetry and modularity is of interest in at least two broad evolutionary questions. First, conspicuous asymmetries and their evolution remain largely understudied compared to subtle asymmetries, particularly compared to FA. Understanding how ancestrally symmetrical structures can evolve to break the classical bilaterian symmetrical *bauplan* locally, while maintaining overall symmetry seems like a major, yet underexplored, aspect of phenotypic diversification (Palmer, 1996). Second, conspicuous asymmetries, which constitute a kind of "internal" diversification of homologous structures, would be a good model to test the idea that modularity is key in phenotypic diversification. This could be tested in at least three complementary ways: (i) By comparing covariation patterns of the same structures between related species either showing conspicuous asymmetry or not, with the expectation that "asymmetric species" should show significant variational modularity between left-right structures as well as between symmetrical and asymmetrical structures. (ii) In species which have serially homologous structures, some of which are conspicuously asymmetric while others not (e.g. arthropod appendages), with the expectation that the asymmetrical structures would have stronger within-side covariation, and weaker between-side covariation, compared to their symmetrical serial homologs (with the possible exception of appendages like the mandibles which achieve a common function). (iii) Across clades and across structures, the weakest left-right covariation should be found in structures in which the left and right sides achieve different functions, intermediate covariation should be found in structures showing left-right differences allowing them to achieve a common function (such as our study), and the highest left-right covariation may be found in structures in which the left and right sides are physically tightly connected.

**Figure legends**

**Figure 1.** A.-B., G.-I.Location of the 35 landmarks used in the present study, illustrated on a colorized 3D reconstruction of a grasshopper head. A. Frontal view of the head as a whole. B. Ventral view of head capsule with mandibles, muscles and other internal organs removed. G.-I. Mandibular landmarks, illustrated for the left mandible (red), and its associated opener (pink), and closer (violet) muscles. Landmarks homologous to those shown here were also placed on the right mandible (not shown). G. Posterior view. H. Medial view. I. Anterior view. C.-F. Illustration of the mandibles in closed occluding position, displaying their key-and-lock morphology. C.-D. Posterior view, with and without transparency of the right mandible, respectively. E.-F. Anterior view, with and without transparency of the left mandible.

**Figure 2.** The various modularity partitions of landmarks tested and compared in this study. Dots represent landmarks, shown in frontal view, as reminded in A. by the underlaid picture of the grasshopper head. Landmark colours do not have any specific meaning, but distinguish between each individual module. White landmarks in F. are excluded from any module.

**Figure 3.** Lollipop graph illustrating directional asymmetry (DA) patterns in the grasshopper head. Red landmarks belong to the mandibles, while beige landmarks are placed on the head capsule and sensory structures. Black bars show the direction and magnitude of DA. Left panel shows the frontal view, while the right panel shows the ventral view, both combined illustrating DA patterns in all three dimensions. Numbers close to landmarks are here to help the reader matching corresponding landmarks in frontal and ventral view.

**Figure 4.** Non-centred PCA computed from difference matrices between landmark configurations and their respective mirror configurations. Each dot represents one individual, and the centre of the plot corresponds to perfect symmetry (i.e. no difference between a configuration and its mirror). The x axis represents purely directional variation, accounting for most of the asymmetric variation, while the y axis represents the first fluctuating asymmetry (FA) component. Other axes are not illustrated, but account for ≤1% of total asymmetric variation. Note the apparent correlation between the two axes.

**Figure 5.** Barplot comparing ZCR values (i.e. modularity effect size) across the different modularity partitions tested in this study, and between module by module Procrustes superimposition (black bars) or global Procrustes superimposition (grey bars). Note that more negative values correspond to stronger modularity.

**Figure 6.** Module pairwise integration, as represented by r-PLS values. Colours do not have a specific meaning but distinguish between individual modules. The width of the grey lines connecting the centroid of each module (large dots) is proportional to the r-PLS values, which are also displayed as numbers. Large font numbers show r-PLS values computed after global superimposition, while underlying small font numbers show corresponding r-PLS values computed after module-by-module superimposition. Note the latter values are always smaller than the former, which is explained by the loss of spatial and size covariance after module-by-module superimposition. Note that r-PLS values are not standardized effect sizes, and should therefore not be compared between the different partitions.

**Figure 7.** Individual *in vivo* bite forces plotted against the various indices of individual asymmetry computed in our study. Dashed grey lines show non-significant fitted quadratic regressions.

Table 1. Results from the bilateral object symmetry shape ANOVA (Type I), using 1000 Randomized Residual Permutations (RRPP) for significance testing.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Df | SS | MS | Rsq | F | Z | Pr..F. |
| ind | 48 | 0.14689 | 0.00306 | 0.24838 | 2.6422 | -7.25098 | 1 |
| side | 1 | 0.3648 | 0.3648 | 0.61686 | 314.97456 | 3.36624 | 0.001 |
| ind:side | 48 | 0.05559 | 0.00116 | 0.09401 | 4.70964 | 14.47838 | 0.001 |
| ind:side:replicate | 98 | 0.0241 | 0.00025 | 0.04075 |  |  |  |
| Total | 195 | 0.59138 |  |  |  |  |  |

Table 2. Comparison of effect sizes (ZCR) from CR modularity analyses.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **A. Effect sizes (ZCR). Negative values represent stronger modular signal.** | | | | | | | |
|  | No Modules | Head-Mandibles | Head-Mandibles-Sensory | Head-Mandibles asymmetric | Head-Mandibles asymmetric-Sensory | Ventral-Dorsal | Half-Half | Mandibles only |
|  | 0 | -4.5 | -3.34 | -3.25 | -4.13 | -3.5 | -2.21 | -3.23 |
|  | **B. Pairwise differences in effect size.** | | | | | | | |
| No Modules | 0 | 4.5 | 3.34 | 3.25 | 4.13 | 3.5 | 2.21 | 3.23 |
| Head-Mandibles | 4.5 | 0 | 0.16 | 0.01 | 0.13 | 0.19 | 1.59 | 0.43 |
| Head-Mandibles-Sensory | 3.34 | 0.16 | 0 | 0.12 | 0.26 | 0.31 | 1.24 | 0.24 |
| Head-Mandibles asymmetric | 3.25 | 0.01 | 0.12 | 0 | 0.12 | 0.18 | 1.31 | 0.35 |
| Head-Mandibles asymmetric-Sensory | 4.13 | 0.13 | 0.26 | 0.12 | 0 | 0.07 | 1.61 | 0.52 |
| Ventral-Dorsal | 3.5 | 0.19 | 0.31 | 0.18 | 0.07 | 0 | 1.51 | 0.54 |
| Half-Half | 2.21 | 1.59 | 1.24 | 1.31 | 1.61 | 1.51 | 0 | 1.03 |
| Mandibles only | 3.23 | 0.43 | 0.24 | 0.35 | 0.52 | 0.54 | 1.03 | 0 |
|  | **C. Pairwise P-values associated with pairwise differences in effect size.** | | | | | | | |
| No Modules | 1 | 0 | 0 | 0 | 0 | 0 | 0.03 | 0 |
| Head-Mandibles | 0 | 1 | 0.88 | 0.99 | 0.9 | 0.85 | 0.11 | 0.67 |
| Head-Mandibles-Sensory | 0 | 0.88 | 1 | 0.9 | 0.79 | 0.76 | 0.22 | 0.81 |
| Head-Mandibles asymmetric | 0 | 0.99 | 0.9 | 1 | 0.91 | 0.86 | 0.19 | 0.72 |
| Head-Mandibles asymmetric-Sensory | 0 | 0.9 | 0.79 | 0.91 | 1 | 0.94 | 0.11 | 0.6 |
| Ventral-Dorsal | 0 | 0.85 | 0.76 | 0.86 | 0.94 | 1 | 0.13 | 0.59 |
| Half-Half | 0.03 | 0.11 | 0.22 | 0.19 | 0.11 | 0.13 | 1 | 0.31 |
| Mandibles only | 0 | 0.67 | 0.81 | 0.72 | 0.6 | 0.59 | 0.31 | 1 |

Table 3. Comparison of effect sizes (ZPLS) from r-PLS integration analyses. Significant differences are highlighted in bold.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **A. Effect sizes (ZPLS) for each partition.** | | | | | | |
|  | Head-Mandibles | Head-Mandibles-Sensory | Head-Mandibles asymmetric | Head-Mandibles asymmetric-Sensory | Ventral-Dorsal | Half-Half | Mandibles only |
|  | 2.75624 | 4.18681 | 3.11687 | 4.01071 | 2.17762 | 5.15903 | 5.31365 |
|  | **B. Pairwise differences in ZPLS between partitions.** | | | | | | |
| Head-Mandibles | 0 | 0.18549 | 0.63877 | 0.00469 | 0.37715 | 1.57656 | **2.10529** |
| Head-Mandibles-Sensory | 0.18549 | 0 | 1.0292 | 0.23604 | 0.27588 | 1.63784 | **2.21155** |
| Head-Mandibles asymmetric | 0.63877 | 1.0292 | 0 | 0.80223 | 0.85547 | **2.55307** | **3.04515** |
| Head-Mandibles asymmetric-Sensory | 0.00469 | 0.23604 | 0.80223 | 0 | 0.41235 | 1.85817 | **2.41466** |
| Ventral-Dorsal | 0.37715 | 0.27588 | 0.85547 | 0.41235 | 0 | 0.79362 | 1.25629 |
| Half-Half | 1.57656 | 1.63784 | **2.55307** | 1.85817 | 0.79362 | 0 | 0.65754 |
| Mandibles only | **2.10529** | **2.21155** | **3.04515** | **2.41466** | 1.25629 | 0.65754 | 0 |
|  | **C. Pairwise P-values associated with pairwise differences in effect size.** | | | | | | |
| Head-Mandibles | 1 | 0.85284 | 0.52297 | 0.99626 | 0.70606 | 0.1149 | **0.03527** |
| Head-Mandibles-Sensory | 0.85284 | 1 | 0.30339 | 0.8134 | 0.78264 | 0.10146 | **0.027** |
| Head-Mandibles asymmetric | 0.52297 | 0.30339 | 1 | 0.42242 | 0.39229 | **0.01068** | **0.00233** |
| Head-Mandibles asymmetric-Sensory | 0.99626 | 0.8134 | 0.42242 | 1 | 0.68009 | 0.06314 | **0.01575** |
| Ventral-Dorsal | 0.70606 | 0.78264 | 0.39229 | 0.68009 | 1 | 0.42742 | 0.20901 |
| Half-Half | 0.1149 | 0.10146 | **0.01068** | 0.06314 | 0.42742 | 1 | 0.51083 |
| Mandibles only | **0.03527** | **0.027** | **0.00233** | **0.01575** | 0.20901 | 0.51083 | 1 |

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