**Linking modularity and conspicuous asymmetry in the insect head and mandibles.**

**Running head.** Modularity and conspicuous asymmetry

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Data availability. Landmark data is available as TPS files, as well as other raw data and R code on Github, at https://github.com/sginot/asym-real. Original 3D reconstructions of grasshopper heads are available on demand from the corresponding author, or at the Bonn Net repository.

**Abstract.** Bilateral symmetry is widespread across animals. Yet, even among Bilaterians, cases of conspicuous asymmetries are frequent. We propose that this breaking of the symmetrical *bauplan* may be related to the presence of modularity between symmetrical and asymmetrical structures, allowing co-existence of symmetrical and asymmetrical structures, as well as between the left and right sides of asymmetrical structures, allowing each side to develop a different morphology. To test this, the patterns of asymmetry and modularity in the head of the grasshopper *Schistocerca gregaria* (Forskål, 1775) were scrutinized. Results confirm the presence of conspicuous directional asymmetry, in particular in the mandibles, and show significant modularity between mandibles and the rest of the head. Modularity was also found, to a lesser degree, between left and right mandibles, and between the ventral and dorsal structures of the head capsule. These results constitute some of the first evidence that modularity may be involved in the appearance of conspicuous asymmetry. Finally, we hope to motivate more research in this direction, by proposing ways in which future studies could confirm or infirm this putative link at larger evolutionary scales.

**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 generally *not strictly* symmetrical, since many structures such as internal organs can be obviously asymmetrical or even unilateral (Babcock, 2005; Palmer, 1996; Van Valen, 1962). The breaking of symmetry can however take many other forms, sometimes less evident, with different biological implications (Klingenberg, 2022; Palmer, 1994; Van Valen, 1962). The differences between left and right sides can be subtle, requiring precise quantitative measurements to be detected, or conspicuous, i.e. visible directly upon observation. Among subtle asymmetry types, fluctuating asymmetry (FA) is arguably 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 stresses lead to small random left-right differences, the distribution of which should be normal and centered on 0 (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 0, or of random direction in antisymmetry, leading to a platykurtic or bimodal distribution of differences. Directional asymmetry and antisymmetry are also found, and generally characteristic for conspicuous asymmetries (Palmer, 2004). In this case again, a symmetric phenotype is not the norm, and the target phenotype is visibly different between left and right sides.

Because directional asymmetry and antisymmetry entail systematic differences between sides, they are generally considered to be inherited, and sometimes to be adaptive. The latter point is however 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 Cetacean skull has been argued to be related to feeding or biosonar function (Churchill et al., 2019; del Castillo et al., 2016; Huggenberger et al., 2017; Lanzetti et al., 2022; C. D. 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 few 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, (Brown & Wolpert, 1990)), or environmentally (e.g. lobster claws (Govind, 1989)). 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 related, 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. In addition to being developmentally modular, each side may also represent a quasi-autonomous functional module, 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). Although the modularity of crab claws has to our knowledge not directly been assessed, studies on the Cetacean skull 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 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, among which many Orthopterans, i.e. grasshoppers, crickets and relatives (Ball, 1992; Chapman, 1964; Clissold, 2007). Mandibles are used to shear and crush food items, and their asymmetric shapes allow the distal parts (incisivi) to cross and act as double blades, and the proximal parts (molar) to occlude (Clissold, 2007). The left and right mandibles each rotate around an axis formed by two articulation points with the head. They can move independently from each other and, in Orthopterans, 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 some extant physically independent from each other, while contrary to the fiddler crab claws, both mandibles must work together to achieve their feeding function. The Orthopteran head (including mandibles) therefore constitutes a somehow intermediate study case of an integrated structure, or *tagma* (Minelli et al., 2013), combining symmetric and asymmetric components, with the left and right asymmetric structures being to some extent physically independent, but sharing a common function. In addition to a common function, left and right mandibles share a common developmental origin, being derived from one of the highly modified head segments (Posnien & Bucher, 2010).

We propose that (i) to achieve conspicuous asymmetry, by which we mean visibly different morphologies on the right and left sides of an animal, the opposite medio-lateral axes of development must have some degree of autonomy from each other, possibly forming modules. At the same time, because overall body or tagma symmetry should be generally maintained, we may expect that (ii) asymmetric structures should be to some degree autonomous from symmetric ones, possibly *requiring* some pre-existing level of modularity to allow for asymmetry to arise evolutionarily. (iii) In the case where the left and right asymmetric components must work together to achieve a given function (like the Orthopterans mandibles) and/or when they are physically tightly linked (as in the Cetacean skull), the functional or architectural constraints may also counteract the potential right-left developmental modularity suggested in point (i). Another possibility is that (iv) asymmetries may “spill-over” from conspicuously asymmetrical modules to neighboring areas, for example through asymmetric mechanical loads producing plastic development or reinforcement preferentially on one side or through correlated growth (Levinton, 2016; Pratt & Mclain, 2002; Tiwari et al., 2017), which would increase correlations (and reduce modularity) between the conspicuously asymmetric structures and the anatomically linked structures.

To test these hypotheses, we focus here on the Orthopteran head morphology, which we quantify using geometric morphometrics. We measure morphological 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 covariation patterns between traits to test different variational modular partitions (Klingenberg et al., 2001; Wagner et al., 2007; Zelditch & Goswami, 2021) and compare them to each other. We expect that mandibles should show strong directional asymmetry, as shown qualitatively in the literature, and that their asymmetrical mechanical actions may induce asymmetry in the neighboring regions, but less so in more distant regions of the head, especially in sensory structures for which symmetry may be functionally advantageous (e.g. compound eyes). Second, we expect that, according to point (i) in the previous paragraph, the left and right mandible may constitute separate modules, allowing their divergent morphologies to emerge, and that they are also quasi-autonomous with regard to the head capsule structures, in which symmetry should be maintained (point (ii)). Alternatively, because the mandibles should enable proper shearing and occlusion, it may be expected as suggested by point (iii), that they are tightly integrated, forming one functional and variational module. If this is the case, it may also be expected that the level of asymmetry is tightly controlled, showing strong adaptive accuracy (Hansen et al., 2006; Pélabon & Hansen, 2008), with individuals deviating from the optimal level of asymmetry having worse biting performance.

**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. The sex of individuals was determined, and five linear measurements were obtained: body length (BL), pronotum width (PW), head width (HW), head length (HL) and head height (HH).

*Fixation and microCT scanning*. Specimens were then fixed in Bouin solution for around 3 days, 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 (Autosamdri 931.GL) before microCT scanning 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.

*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 used, meaning we considered here the object symmetry of the head and mandibles as a whole. Individual shapes and their replicates were aligned by partial Generalized Procrustes Analysis, using functions 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 fluctuating asymmetry (iFA) and directional asymmetry (iDA) at the individual level. 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 resulting PCA, the center (coordinates [0, 0]) has a biological meaning, since it represents perfect symmetry (i.e. no difference between a configuration and its reflection). 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. Individual positions (averaged across replicates) along this axis can therefore be used as a proxy for “individual directional asymmetry” (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 “individual fluctuating asymmetry” (iFA). In addition, individual total asymmetry (iTA) was also computed as the distance between a configuration and its reflection, averaged across replicates for the same individual. 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.

*Modularity and integration analyses*. Replicated landmark configurations for each individual were averaged, and the resulting shapes were used in the following analyses. In this study, we test strictly only variational modularity (i.e. whether the grasshopper head shows stronger covariation within than between given regions) (Zelditch & Goswami, 2021). We did not use exploratory but confirmatory approaches to test for modularity. This entails defining *a priori* different modular partitions to be tested against the null hypothesis of no modularity, and tested against each other. The landmarks were therefore sorted into six different partitions (Fig. 2): (i) a 2 modules “Head-Mandibles” partition, in which all landmarks placed on both left and right mandibles were gathered into one module, while all other landmarks, placed on the head capsule and sensory structures, formed the second module. (ii) a 3 modules “Head-Mandible-Sensory” partition, in which mandible landmarks form one module, landmarks from sensory structures (eyes, antennae, ocelli), which are in the dorsal half of the head form the second module, and landmarks from the ventral half of the head capsule and frons/clypeus form the third module. (iii) a 3 modules “Head-Mandibles asymmetric” partition in which landmarks from left and right mandible form two separate modules, while the rest (entire head) constitute the third module. (iv) a 4 modules “Head-Mandible asymmetric-Sensory” partition, in which left and right mandible constitute separate modules, and head landmarks are split between the dorsal (i.e. sensory) and ventral regions. (v) a 2 modules “Ventral-Dorsal” partition, in which landmarks from both mandibles and the ventral part of the head form a single module, while the dorsal head (i.e. sensory) landmarks constitute the second module. (vi) a 2 modules “Half-Half” partition, in which the left half and right half of the head (with their respective mandible) constitute separate modules, with midline landmarks excluded from the analysis. In addition, mandibles alone were also tested for left-right modularity. These different partitions were tested against the null hypothesis of no modularity, and ranked against each other using two of the most widely used current approaches: EMMLi (Evaluating modularity with maximum likelihood), implemented in the EMMLi R package (Goswami & Finarelli, 2016), and CR (Covariance ratio), implemented in the geomorph R package (Adams, 2016; Adams & Collyer, 2016). Because EMMLi has been shown to have high false positive rates and to strongly favor models with more parameters (Adams & Collyer, 2019), we only show these results in details in the Supplementary Material. Integration between modules was also tested pairwise for each partition using two-block partial list squares (PLS), as implemented in geomorph. Because discussions are ongoing about the impact of Procrustes superimposition on results of modularity analyses (Cardini, 2019, 2023; Zelditch & Swiderski, 2023), we ran our modularity and integration tests and comparisons a second time, while applying a second "local" superimposition module by module. In other terms, the globally superimposed coordinates array was split into "module by module" arrays, which were individually superimposed again. These "module by module" coordinates arrays were then concatenated back together in an array of the same dimensions as the original one, and with landmarks in the same order, before running modularity tests and comparisons once more. Although this process entirely removes the biological spatial relationship between modules, it does not preclude analyses of covariances. We then compare results from both approaches (i.e. global superimposition and module by module superimposition).

*Variability and correlations between bite force and asymmetry.* To test whether the degree of asymmetry in the head and mandibles was functionally constrained, 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 a 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 relate to worse fitness, therefore possibly leading to a negative relationship between iFA and BF. Finally, to test whether these traits may be 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).

**Results**

*Head shape asymmetry*. As expected, head shape at the population level was strongly directionally asymmetric (Table 1, Fig. 3). This directional asymmetry (DA) is however located mostly on the mandibles, with the incisivi (landmarks 18 to 23) being 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 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, although its magnitude is much less than DA. FA is more spread out across the head than DA, however the incisivi also appear to be the structure with the larger magnitude for FA (Supp. Fig. 1).

When using Neubauer et al.'s approach, it appears clearly that the major asymmetric component, explaining over 90% of asymmetric variance, is directional (Fig. 4). The second axis, which represents about 2% of asymmetric variance is centered around 0 (One sample t-test, mean = 0.0021, t = 0.5208, df = 48, P = 0.6049), and normally distributed (Shapiro-Wilk normality test, W = 0.9885, P = 0.9105), suggesting it represents an FA component. Further axes, representing ≤1% of asymmetric variance were not explored. Unexpectedly, iFA and iDA (i.e. positions of individuals along these two asymmetric axes) appear correlated with each other (Fig.4, Pearson's correlation, r = 0.3569, t = 2.6191, df = 47, P = 0.0118). Similar results were obtained when restricting these analyses to landmarks from the mandibles only.

*Modularity and integration*. Both CR and EMMLi do not support the null hypothesis of no modularity in the grasshopper's head (Table 2, Supp. Table 1). EMMLi suggests the most likely modularity partition is the "Head-Mandibles asymmetric-Sensory" one (Fig. 2D), followed by the "Head-Mandibles asymmetric" one (Fig. 2C). On the other hand, the comparison between CR tests suggests the strongest modular signal is found in the simple 2 modules "Head-Mandibles" partition (Fig. 2A). CR suggests that all modular partitions are significantly different from the null hypothesis of no modularity, but also that differences between modular signals in all those partitions are not significantly different from each other (Table 2). It should also be noted that the partition with the second largest modular effect is the most complex model, the 4 modules "Head-Mandibles asymmetric-Sensory" partition. When running the comparison of CR tests again after module-by-module superimposition, the ZCR values get lower (i.e. modular signal gets stronger) overall. This is expected, as these "local" superimposition mathematically tend to add intra-module covariance, and reduce inter-module covariance. Again, the all partitions are significantly different from the null hypothesis of no modularity (all P < 0.001), and again the differences between partitions in terms of modularity signal are not significant (all P > 0.1). The strongest modular signal (lowest ZCR) is found, as with the global superimposition, in the 2 modules "Head-Mandibles" partition, and the 4 modules "Head-Mandibles asymmetric-Sensory" partition also has strong modular signal (Fig. 5). It is however striking that the "Half-Half" partition, which has the weakest modular signal with the global superimposition, has on the other hand the second strongest signal in the module-by-module superimposition analysis.

Pairwise between-module integration analyses (Table 3, Fig. 6) show significant integration between modules in all partitions (all P < 0.02). Differences in integration signal between partitions are generally non-significant (Table 3). The exceptions are the "Half-Half" and "Mandibles only". The latter has significantly stronger integration than the "Head-Mandible", "Head-Mandibles-Sensory", "Head-Mandibles asymmetric", and "Head-Mandibles asymmetric-Sensory" partitions. The former is only significantly different from the "Head-Mandibles asymmetric" partition. Pairwise r-PLS correlation values from the different partitions (Fig. 6) are generally stronger between spatially close structures. Integration between the left and right mandibles, as well as with the ventral half of the head. The "Ventral-Dorsal" partition clearly shows the weakest between-module correlation, while the "Half-Half" partition has the strongest one. It should however be noted that the latter excludes midline landmarks, meaning the r-PLS value cannot be directly compared to other partitions (conversely, ZPLS values are standardized and can be compared). When using module by module superimposition, results are globally similar, with a fairly strong positive relationship between pairwise rPLS values computed after global vs. module-by-module superimposition (Fig. 6, Supp. Fig. 2, R² = 0.6783, P < 0.0001). Integration signal, is systematically weaker after module-by-module superimposition than after global superimposition. More importantly, these differences lead also to differences in significance (Supp. Fig. 2): while all tests were significant when using the global superimposition, with module-by-module superimposition, integration was not found to be significant in the "Head-Mandibles", the "Head-Mandibles-Sensory", and the "Ventral-Dorsal" partitions. All other partition had significant integration (all P = 0.001), but even in these cases, pairwise integration relationships between individual modules were not always the same as when using global superimposition.

*Variability and correlation in bite force and asymmetry*. *In vivo* bite force is correlated neither to iDA (Pearson's correlation, r = -0.0102, t = -0.0691, df = 46, P = 0.9452), nor to iFA (r = 0.1138, t = 0.7771, df = 46, P = 0.4411), nor to iTA (r = 0.0244, t = 0.1657, df = 46, P = 0.8691). Quadratic and linear models fit to the data were all non-significant (all R² < 0.1, all P > 0.2, Fig. 7). This result held whether we used iDA, iFA, iTA, or iDA restricted to only the mandibles. The coefficients of phenotypic variation (CVp) were rather low for iTA (CVp = 0.1716) and iDA (CVp = 0.2128), slightly higher for bite force (CVp = 0.3147), and very high for iFA (CVp = 1.2334). For reference we also computed CVp for head centroid size, which had a much lower value of CVp = 0.0506.

**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 also in surrounding head structures (Fig. 3). Fluctuating asymmetry (FA) was also significant, although accounting for much less variation as DA. Despite being more spread out than DA, the largest FA was also found in the mandibles. We also showed that the level of individual head asymmetry does not appear to influence individual biting performance (Fig. 7). Significant modularity was found, with the strongest modular signal in the 2 modules partition separating the head capsule (including sensory structures and the clypeus and labrum) from the mandibles (both sides combined) (Table 2, Fig. 5). While left and right mandibles are strongly correlated (Fig. 6), there appears nevertheless to be support for some degree of modularity between them (Table 2, Supp. Table 1). There is also support for significant modularity between the dorsal half of the head, holding the sensory structures, and the ventral half of the head, which appears more correlated to the mandibles (Fig. 6).

*Asymmetry "spill-over" and modularity within the head*.

The 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 not 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 correlation between the ventral half of the head and the mandibles, 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) (Ginot & Blanke under review). The limited DA observed in this dorsal region may therefore be explained by selection for maintenance of symmetry, related to the sensory organs, which could be achieved by reinforcements of the cuticle, as observed for example around the eyes. Such selective constraint may not be as strong in the ventral half of the head, which does not have symmetrical sensory organs.

*Linking conspicuous asymmetry with modularity.*

Our results support that, despite being recognized as a *tagma*, and therefore being an integrated anatomical unit (Minelli et al., 2013), the insect head is also to some extent modular. This result fits with the fact that different parts of the head derive developmentally from various specialized segments (Posnien & Bucher, 2010), which are then used for different functions. As such, we proposed that the mandibles would form a functional module for feeding, while the dorsal half of the head would make up a functional module for sensing. Within the feeding module formed by mandibles, we also proposed that the conspicuously asymmetric mandible shapes could be explained by developmental modularity between left and right.

Both approaches used to test and compare modularity partitions of the head lend some support to the fact that the left and right mandibles each constitute a variational module (hypothesis (i) of the Introduction). While EMMLi strongly favors partitions in which left and right mandibles are separate modules (Fig. 2C-D), it should be noted that these are also partitions which have a large number of parameters to estimate (Supp. Table 1). As the EMMLi approach was shown to artificially favor partitions with more parameters (Adams & Collyer, 2019), this result may therefore be spurious. On the other hand, the CR modularity analyses also showed significant modularity in partitions which separate left and right mandibles (Table 2). Although these partitions are not the ones showing the strongest modularity scores, differences with other partitions are not significant. It therefore appears there is a degree, albeit limited, of variational autonomy between the left and right mandibles. Several factors may play a role in this autonomy: as suggested in the Introduction, to achieve conspicuously different morphologies, left and right mandible developmental pathways must be to some extent divergent (Meinhardt, 2001; Palmer, 2004). This divergence may in turn be a source of decorrelation between mandibles, allowing variable left-right differences, and causing slight variational modularity. Another, non-exclusive possibility is revealed by the relatively large magnitude of FA located at the mandible incisivi (Supp. Fig. 1). We propose that this FA, which correlates to the mandibles DA (Fig. 4), may be mostly caused by mandible wear. Because FA is of random direction, it may in turn reduce the correlation between left and right mandible shapes.

Although mandibles have some degree of autonomy from each other, they remain strongly integrated (Fig. 6C-D, Table 3), and the strongest modular signal retrieved by the CR analysis is in the two modules partition which combines both mandibles into one module (Fig. 2A, Table 2), and the head structures in a second module. This suggests that the functional selection for matching left and right mandible shapes is limiting deviations from the left and right respective target phenotypes. This is corroborated by the relatively small CVP of iTA and iDA, which fall in the range of adaptively accurate 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. Here, we propose that modularity between the head and the mandibles may allow conspicuous asymmetry to appear in the mandibles while maintaining the head's global symmetry. This may in fact 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. One study in Cetacean skulls (Churchill et al., 2019) suggested that the link between asymmetry and modularity is reversed compared to what we propose, suggesting that “asymmetry drives modularity”. We instead propose that modularity favors, or may be a prerequisite for conspicuous asymmetry to start evolving. This fits in our opinion better with the idea that modularity allows traits to evolve independently (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, reinforcing integration within left and right structures and increasing modularity between them.

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 modularity between left and right sides. In other cases, such as pincers of lobsters or fiddler crabs, left and right functions are divergent, and one might therefore expect even stronger left-right autonomy. 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 more symmetrical species, e.g. insects with symmetrical mandibles, Mysticetes Cetaceans which have symmetrical skulls, or crustaceans with symmetric pincers, modularity should be smaller both between left and right sides, and between the studied structure and its anatomical surroundings.

*Effect of module-by-module superimposition on modularity analyses.*

As mentioned earlier in the text, there is ongoing discussion in the morphometrics community about the impact of Procrustes superimposition on modularity and integration results (Cardini, 2019, 2023; Zelditch & Swiderski, 2023). Studies tackling this problem have been relying heavily on simulations. However, empirical studies have generally not inquired those possible problems. Here, we ran CR analyses twice, first in the "usual" way, with one global superimposition, and second after applying a module-by-module superimposition. Reassuringly, the results are generally in agreement using both approaches, with all partitions showing significant modularity, and no significant differences between partitions. Furthermore, partitions with the highest modular signal are similar between both approaches, with one major difference in the "Half-Half" partition (Fig. 5) which has the lowest modular signal when using the global superimposition, but the second highest when using module by module superimposition. This phenomenon is most probably explained by the loss of size relationships between halves in the module-by-module approach. Indeed, since one mandible is shorter than the other, but both halves of the head capsule are of similar size, when splitting the halves, the scaling step of Procrustes superimposition will therefore mostly be affected by the degree of asymmetry in the mandibles, causing strong decorrelation, and increasing modularity. This phenomenon had been anticipated (Cardini, 2019), and the loss of spatial and size relationships between modules is also what explains that ZCR values are almost always higher with the module-by-module superimposition. Regarding the integration results, although the degree of integration computed for the different partitions is correlated between the global and module by module approaches (Supp. Fig. 2), the agreement does not appear as good as for modularity. Indeed, while integration is always significant using the global superimposition, it is only so in only around half of the cases when using module by module superimposition. Once again, this is certainly due to the fact that module by module superimposition necessarily removes spatial and size covariance between modules. However, it remains unknown how much of true biological covariance may also be lost in that process. We would argue that using module by module superimposition before integration analyses can therefore be taken as a conservative approach, lending strong confidence in the cases in which integration remains significant, although it may at the same time entail some false negative results.

*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 whatsoever 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 some large amount of variation in the fit of mandible shapes, we computed CVP, which appear 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, which could explain why CVP is higher for bite force than for iTA of iDA.

*Conclusion and perspectives*.

Modularity is often referred to as a mechanism which may favor phenotypic diversification by allowing different anatomical parts to evolve to some extent independently (Zelditch & Goswami, 2021). Our results constitute first evidence that modularity may indeed 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 evolved 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 further is of interest in at least two broad evolutionary biology questions. First, conspicuous asymmetries and their evolution remain largely understudied compared to subtle asymmetries, and in particular 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 unexplored aspect of phenotypic diversification (Palmer, 1996). Second, conspicuous asymmetries, which constitute a kind of "internal" diversification of homologous structures, would therefore be a good model to test the idea that modularity is key in phenotypic diversification. We suggest three possible avenues for future studies to test this: (i) By comparing modularity levels of the same structures between related species either showing conspicuous asymmetry or not, with the expectation that "asymmetric species" should show stronger left-right modularity as well as stronger modularity between symmetrical and asymmetrical structures, compared to "symmetric species". (ii) In species which have segmentally 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 left-right modularity compared to their symmetrical homologs. (iii) Across clades and across structures, the strongest left-right modularity should be found in structures in which the left and right sides achieve different functions, intermediate modularity should be found in structures showing left-right differences allowing them to achieve a common function, and the lowest left-right modularity may be found in structures in which the left and right sides are physically tightly connected. We hope that this study, which is restricted in terms of taxonomic scope and conspicuous asymmetry type, may in the future motivate other groups to also explicitly look at the link between modularity and conspicuous asymmetries.

**Figure legends**

**Figure 1.** 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. C.-E. 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). C. Posterior view. D. Medial view. E. Anterior view.

**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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