**Linking shape conspicuous asymmetry with shape covariation patterns and performance in the insect head and mandibles.**

**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 between landmark partitions in a grasshopper withconspicuously asymmetric mandibles. Our morphometric data confirms the presence of strictly directional conspicuous asymmetry in the mandibles, and surrounding structures. Covariance patterns and tests hint at a strong integration between mandibles despite their divergent morphologies, and variational modularity with the head capsule While mandibles have been selected to achieve a key-and-lock morphology by having interlocking shapes, the developmental modularity required to achieve this seems 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. Finally, the degree of directional asymmetry appears to be under selection, although we find no relationship between bite force and mandible shape or asymmetry.

**Keywords.** Bite force, Covariance ratio, Integration, Modularity, 3D geometric morphometrics, 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 was 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) while extreme conspicuous directional asymmetry in the skulls of flatfishes relates to their benthic ecology (Evans et al., 2021), conspicuous antisymmetry in many Crustacean appendages is 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 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 related but 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 slightly different ontogenetic pathways on both sides, leading to the same structure identity (e.g. claw, limb, mandible) but with different morphologies. 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), and may appear through a reduction of pleiotropic factors across both sides (i.e. parcellation), and/or by increasing integration within left and right structures (Wagner & Altenberg, 1996). Although the modularity of crab claws has, to our knowledge, not directly been assessed, studies on the skull of toothed whales suggest a link between the presence of directional asymmetry and a modification of the modularity partition 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 are at the same time involved in feeding and 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.

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 morpho-functional modules, and, in Orthopterans, they are each actuated by one adductor and one abductor muscle which attach to the inside of the head capsule (Clissold, 2007). Contrary to the bones of the Cetacean skull, Orthopteran mandibles are 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 interlock. If they were bilaterally symmetric, they would not function, since the various ridges and cusps would simply hit each other without shearing. Therefore, contrary to the assumption made for fluctuating asymmetry studies, a certain degree of asymmetry is expected to be the functional and selective optimum. On the other hand, *extremely* asymmetric mandibles, for instance one very long and one very short, would also not function properly to shear and crush. Therefore, we hypothesize that each mandibles’ shape is influenced by 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 good 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 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 a 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 induce 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.

Given above aspects, we expect that (i) mandibles show strong directional asymmetry, as already visible qualitatively (Fig. 1), and that the left and right mandibles should constitute *variational* modules, with large covariation *within* each mandible and low covariation *between* them.

However, (ii) 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 modularity mentioned in point (i). If so, high covariation between left and right mandibles should be expected, and the degree of asymmetry between them should be tightly controlled, with relatively high adaptive accuracy (i.e. average asymmetry different from zero, but with limited variation; Hansen et al., 2006; Pélabon & Hansen, 2008). Mandible shape variation and the degree of mandible asymmetry should also relate to performance, as measured here with bite forces, with an optimal degree of asymmetry leading to maximum performance.

At the same time, head symmetry should be maintained, and we may assume that (iii) 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. We therefore expect that mandibles will show low levels of covariation with head structures, and that the head will not show directional asymmetry.

Alternatively, (iv) anatomical connections and mechanical loads from the mandibles may induce changes in the head shape, in which case we should expect increased covariation with the head structures closely related to mandibles, and possibly significant directional asymmetry “spill-over” to these structures (Tiwari et al., 2017), while head structures more spatially distant from the mandibles should not display directional asymmetry or significant covariation with mandibles.

To test these hypotheses, we focus here on the Orthopteran head and mandibles shape, which we quantify using 3D 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 head-mandible system and use those covariation patterns within and between parts as evidence for *developmental* and *functional* modularity and integration (Klingenberg et al., 2001; Wagner et al., 2007; Zelditch & Goswami, 2021). We further test the relationship of asymmetry and shape variation on performance using *in vivo* measurements of bite forces.

**Materials and Methods**

*Specimens and measurements*. Forty-nine live adult specimens of *Schistocerca gregaria* (Forskål, 1775) were purchased from Fressnapf© (Krefeld, Germany). Their bite forces were measured on the same day or the next, using the setup developed by Rühr & Blanke (2022). 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 physiological 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, followed by an ascending ethanol series to 100% ethanol. Afterwards, heads were critical-point dried (Tousimis Autosamdri 931.GL), imaged using microCT (Bruker SkyScan 1272; Voltage = 50 kV, current = 200 µA, image pixel size = 6.0 µm or 7.5 µm), and digitally reconstructed (NRecon, Bruker). All heads had fully closed mandibles when scanned, to ensure anatomical comparability between specimens while landmarking.

*Landmarking*. Reconstructed heads were rendered in the software MorphoDig v. 1.6.7 (Lebrun, 2018). Each 3D model was oriented anatomically (x-axis: antero-posterior, y-axis: mediolateral, z-axis: dorso-ventral) and centered in the global coordinate system. In total, 35 homologous landmarks were placed across the head (Figs. 1-2). 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).

*Decomposition of shape variation and analysis of asymmetry*. The full landmark set was separated into mandible landmarks and head capsule landmarks. Left mandibles were mirrored along the medio-lateral (y-)axis, while for the head capsule, each complete configuration was mirrored, also along the medio-lateral axis, 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 of asymmetry. 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 for 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) 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 (object symmetry, i.e., head capsule), or, alternatively, the matrix of differences between right and mirrored left object (matching symmetry, i.e., mandibles). In the resulting PCA, the center of the space has a biological meaning, since it represents perfect symmetry. 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 ‘bilat.symmetry’ in ‘geomorph’ (Adams et al., 2022; Baken et al., 2021; Collyer & Adams, 2018, 2021). The ANOVA has two explanatory factors, individual (representing inter-individual variance), and 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 (Fig. 3). 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 ‘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 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 by definition 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 warped 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 were superimposed separately. Because mandibles are anatomically separate with respect to each other and to the head capsule the head-mandible morphological system is formed by at least three modules. 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 more related to the mandibles. We then tested for *variational* modularity, i.e. whether covariation is stronger within modules than between modules (Zelditch & Goswami, 2021), separately for the head and for the mandibles, using ‘modularity.test’ from ’geomorph’. In addition, pairwise correlations between each of the four proposed modules were tested via two-block partial least squares analyses, as implemented in ‘integration.test’ 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 a landmark correlation matrix, using congruence coefficients, as implemented in ‘dotcorr’ of ‘paleomorph’ (Lucas & Goswami, 2017). We then visualized representations of these matrices as heatmaps, with variables ordered according to the module they belong to, and in approximate dorso-ventral order (Fig. 2). 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, shape 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. To test whether these traits are individually 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 BF 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, Fig. S2). This directional asymmetry (DA) is located mostly at the incisivi which are the most conspicuously asymmetric structures, as well as the insertion area of the mandible closer muscle (Fig. 3, Fig. S2). Basically, the asymmetrical difference in mandible shapes can be summarized as a lengthier and less broad left mandible, with a more medio-laterally compressed mandibular rim (i.e. area of muscle insertion and articulation with the head), while the right mandible is bulkier, and makes a sharper angle between its dorsal and ventral parts leading to a more “bent” shape. DA can also be noticed in head capsule structures which are located close to the mandibles, notably the clypeus-labrum, and the tentorial bridge (Fig. 3, Fig. S2). The major directionally asymmetric patterns are the tilting of the clypeus-labrum region towards the right side, but when differences are amplified, the eye and antenna region also reveal subtle directional asymmetry, with the right side projecting more posteriorly and dorsally than the left side. Fluctuating asymmetry (FA) is also significant, in the mandibles and in the head capsule, although its magnitude is much less than DA (Table 1, Fig. 4). FA is more spread out across the head than DA, however, the incisivi also show a high FA variation component (Fig. S1).

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, and 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 and variational modularity*. Covariance ratio tests, as implemented in ‘modularity.test’, were significant for a partition between left and right mandible (CR = 0.8739, [95%CI: 0.8161, 0.9797], P = 0.001, Effect size = -3.1302), as well as between ventral and dorsal halves of the head (CR = 0.8900, [95%CI: 0.8514, 0.9834], P = 0.006, Effect size = -2.3246). 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-block partial least squares analyses between the four modules show significant correlations between both head halves and between left and right mandibles, as well as between the ventral head module and the left mandible (Fig. 2C).

Covariance and correlation heatmaps (Fig. 2B-C) display additional details: overall, covariances and correlations are higher within and between mandibles than across the head (Fig 2B and C). In the head, the dorsal half, supporting the major sensory structures, appears to have greater covariances and correlations compared to the ventral half of the head. On average, covariances are higher within modules than between them, with the exception of the ventral head which shows the lowest average covariance. 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 (i.e. along the diagonal of the between mandible square), however, strong covariances and correlations are also observed between combinations of the points representing the muscle insertions both within and between mandibles.

Patterns of within-mandible covariation are similar across the left and right mandible, with higher values across landmarks representing insertions of adductor muscles and condyles. However, there is also some asymmetry in these patterns, with a lower average covariance in the left mandibles, driven at least in part by lower covariance of incisivi landmarks with the rest of the mandible.

Although anatomical distance between landmarks appears to play a role in the high covariation between some of the landmarks (e.g. anterior and posterior points of insertion of the adductor muscle), this is not a general rule, as seen for example in the high covariation between adductor muscle/condyle landmarks and some of the incisivi landmarks in the right mandible, or the low covariation between dorsal eye landmarks and left and right ocelli (Fig. 2).

*Variation and correlation in bite force, shape and asymmetry*. None of the shape components or their symmetrical or asymmetrical components is correlated to *in vivo* bite force (r-PLS = [0.45; 0.54], all P > 0.1). *In vivo* bite force is also not correlated to iDA, iFA, or 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. 5). 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) and the head (CVp = 0.1909). On the other hand, coefficient of phenotypic variation of 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, as well as 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. 3-4). 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 capsule structures (Fig. 3, Fig S2). Fluctuating asymmetry (FA) was significant too, although accounting for much less variation than DA (Table 1, Fig. 4, Fig. S1). Despite being more spread out across the mandibles and head capsule 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. 5), nor did the limited mandible or head shape variation found in our sample. Despite their asymmetry, strong covariation was found within and between left and right mandibles, while the head capsule showed weaker within-covariation overall. Significant variational modularity was found between the dorsal part of the head capsule, holding sensory structures, and the ventral part, functionally and spatially related to the mandibles. Nevertheless, significant correlation between these head parts was highlighted, and the ventral part was also correlated to the left mandible shape. (Fig. 2). Variational modularity was highlighted between left and right mandibles, which at the same time show strong correlation with each other.

*Conspicuous asymmetry, modularity and the palimpsest model.* Despite being recognized as a *tagma*, and therefore being considered as an integrated anatomical unit (Minelli et al., 2013), our data support that the insect head system is also a modular system. This result fits with the fact that different parts of the head, notably the head capsule and mandibles, derive developmentally from various segments (Posnien & Bucher, 2010) with different functions. Variation and covariation patterns support the idea that the mandibles form one functional module for feeding, with the left and right mandibles showing strong covariation. Within the feeding module, 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 support that the left and right mandibles each constitute a variational module, with strong within-mandible covariation (hypothesis (i)). The CR tests 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, however, 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 (CR = 0.87), despite high covariance between mandibles. This highlights the fact that it is possible for a developmental system to have at the same time several developmentally and anatomically defined modules, which can also be strongly integrated with each other developmentally and/or functionally, producing between-modules covariation, leading to CR values close (or possibly equal?) to one.

Such types of developmental systems 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 (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 between them (hypothesis (ii)), obscuring to some extent the original developmental modularity of the system. Such an increase in covariation could be due to plastic shape changes (layers of endocuticle can be added after the last moult in insects; Parle et al., 2016; Parle & Taylor, 2017), self-sharpening to achieve more performant shapes (Schofield et al., 2021), or the more usual wear linked to individual diet. With this in mind, we propose that the pattern of variational modularity between mandibles observed here derives from developmental modularity, but may actually be blurred 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, making the mandibular system a hierarchically modular system, with mandible modules nested withing the so-called feeding module (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 magnitudes of FA located at the mandible incisivi (Fig. S1). 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 importance of modularity for conspicuously asymmetric systems*. 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 from the head capsule. The observed pattern of weak covariation constitutes evidence corroborating modularity between the head capsule and the mandibles. Such disconnection may be critical to allow conspicuous asymmetry to appear in the mandibles while maintaining the head's global symmetry (hypothesis (iii)). Indeed, if the factors defining the left-right conspicuous asymmetry of mandibles were similarly expressed in the head capsule, one would expect equally conspicuous head asymmetry. Here, we instead observe significant but 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" (Evans et al., 2023; 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 to some extent 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 (i.e. reduction of pleiotropic effects between traits) *between* them (Wagner & Altenberg, 1996). More generally, parcellation may be an important process in the evolution of conspicuously asymmetric structures, by breaking down pleiotropic effects between left-right homologous structures.

The link we propose 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). Our data on the other hand appears to show some asymmetry of covariation patterns across sides (Fig. 2). In cases where asymmetric structures are embedded within symmetric structures, such as the Cetacean skull, one may expect a degree of covariation between them even higher compared to what we observe in our study. One may also expect that in typical 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, thus preserving pleiotropic effects 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 whale 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 the majority of 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), Fig. 3) may give us a hint. Indeed, asymmetrical mechanical loads resulting from the joint reaction forces of the mandibles 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 articulation of mandibles at the ventral half of the head, could participate in the significant correlation between the ventral half of the head and the left mandible, comparable to the correlation between the ventral and dorsal half of the head (Fig. 2C). 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 DA observed in this dorsal region may be explained by asymmetric muscle actions, but seems limited, possibly 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 circumocular ridge as a reinforcing structure. Such selective constraints may not be as strong in the ventral half of the head capsule, which does not have multiple sensory organs.

*Constraints on shape, shape asymmetry, adaptive accuracy, and the relationship with bite force.* The impact of functional selection, developmental integration and/or plasticity for interlocking left and right mandible shapes is apparent in the strong within-mandible covariation, constraining 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 controlled 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). Self-sharpening mechanisms have been suggested to play a role in maintaining precise interlocking of arthropod piercing, cutting or crushing structures through the life of individuals (Schofield et al., 2021).

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 exist an optimal degree of directional asymmetry, deviations from which would 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. 5). 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 a 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 influenced by other unrelated factors, which could explain why CVP is higher for bite force than for iTA of iDA. In addition to asymmetry itself, our results show that bite force is not impacted by left or right mandible shape variation observed here, which also supports the idea that variation in bite force may be driven by multiple other factors, among which size, muscle anatomy and physiology may be prominent (Ginot & Blanke, 2023; Püffel et al., 2021).

Combined, our results suggest that the conspicuous DA observed in the mandibles is of adaptive significance, since its CVP is small, and notably lower than that observed in cases of non-adaptive DA (Pélabon & Hansen, 2008). However, this adaptive significance might have more to do with the interlocking of mandibles and dynamic shearing forces, rather than with the static equilibrium bite forces measured here.

*Conclusion and perspectives*. Modularity is often referred to as a property which favors phenotypic diversification within morphological systems by allowing different anatomical parts to evolve in relative independence (Hansen, 2003; Wagner & Altenberg, 1996; Zelditch & Goswami, 2021). Our results constitute some of the first evidence that modularity may have a role in the evolution of adaptively 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 was never tested using separate left-right modules. Genetic assimilation has been suggested (Palmer, 1996, 2004) as another avenue for appearance of conspicuous asymmetry, which would not necessarily prerequire modularity. Our results also highlight that modular biological systems can have a hierarchical structure, and that combined developmental, functional and plastic processes can lead to strong covariation between well-identified developmental and anatomical modules. With this in mind, covariation patterns observed in an adult population should be seen, not as the direct result of developmental processes, but as a palimpsest of (possibly opposing) processes throughout the life history of individuals. Despite this, those covariation patterns, and the variational modularity of the system, do relate to its functions and adaptive accuracy, making them relevant for evolutionary biology, even though their strict link with development can hardly be fully assessed.

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 asymmetrical and neighbouring symmetrical 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.** A. Two-dimensional representation of landmarks used, approximately overlaid on the anterior view of the head, with abbreviations for individual landmarks, and a list of their detailed descriptions. The landmarks are coloured and ordered in the list according to their putative module, and in approximate dorso-ventral order. B. Heatmap representing the coordinates covariance matrix (absolute values). The upper triangle and diagonal have been removed because the matrix is symmetrical. Warmer colours correspond to larger covariance values. Each row and column represent a given coordinate for a given landmark, as ordered in the list in A. Note that each successive triplet of rows/columns corresponds to the x, y and z coordinates of the same landmark (e.g. the first three rows and columns are the x, y and z coordinates for landmark ‘topEyer’). The large-squared grid of thick grey lines is a visual help to delineate blocks of variables belonging to each module. Values written in the upper triangle correspond to average covariances for a given combination of modules. C. Heatmap representing the so-called congruence matrix, i.e. matrix of landmark by landmark correlations. Each row and column correspond to an individual landmark, as ordered in the list in A. and along the upper and right axes of the plot. Warmer colours show higher correlation values, as shown by the legend to the right of the plot (note this scale does not apply to B.). Values written in the upper triangle show the results of module pairwise two-block partial least squares analyses. Abbreviations: LM, left mandible; RM, right mandible.

**Figure 3.** Three-dimensional representations of directionally asymmetric variation. First row: anterior view of the head capsule. Second row: posterior view. Third row: dorsal view. Grey head morphs represent a perfectly symmetrical shape (average between original and mirrored mean shapes). Blue and red morphs represent the symmetrical shape plus or minus twice the directionally asymmetric difference. Fourth row: dorsal view of the right (blue) and mirrored left (red) mandibles (left and right mean shape), with dark red and dark blue meshes corresponding to left and right mean shape, plus or minus twice the left-right difference. Fifth row: anterior view. Sixth row: posterior view. Note that the original head capsule and mandible meshes belong to an arbitrarily selected individual and that their deformations are purely interpolations based on the amplified directionally asymmetric differences. Therefore, these deformations should not be taken as biologically realistic, but only as visual help to understand shape changes. Furthermore, for the mandibles, a mirrored left and right mandible meshes were used, given that left and right mandibles are conspicuously asymmetric, to achieve more biologically correct deformations. Therefore many of the observed differences correspond, not to deformations but actual left-right differences.

**Figure 4.** Non-centred PCAs computed from difference matrices between landmark configurations and their respective mirror or opposite side configurations, for the head capsule (A.) and mandibles (B.). 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). Both PC1s represent purely directional variation (average largely different from 0), accounting for most of the asymmetric variation, while the PC2s axes represent the first fluctuating asymmetry (FA) component. Other axes were not explored.

**Figure 5.** Individual *in vivo* bite forces plotted against the various indices of individual asymmetry computed in our study. Dashed lines represent fitted linear regressions, while plain lines represent fitted quadratic regressions. None of these were significant, suggesting no impact of any type of asymmetry on bite force.

**Figure S1.** Lollipop graph illustrating fluctuating asymmetry (FA) 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 FA. Left panel shows the frontal view, while the right panel shows the ventral view, both combined illustrating FA patterns in all three dimensions. Numbers close to landmarks are here to help the reader matching corresponding landmarks in frontal and ventral view.

**Figure S2.**

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



|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Df | SS | MS | Rsq | F | Z | Pr(>F) |
| A. Head shape asymmetry ANOVA | | | | | | | |
| Ind | 48 | 0.133798 | 0.002787 | 0.46374 | 2.7635 | -3.1724 | 1.000 |
| Side | 1 | 0.071382 | 0.071382 | 0.24741 | 70.7676 | 4.1427 | 0.001 \*\* |
| Ind:side | 48 | 0.048417 | 0.001009 | 0.16781 | 2.8304 | 13.4276 | 0.001 \*\* |
| Ind:side:replic | 98 | 0.034925 | 0.000356 | 0.12105 |  |  |  |
| Total | 195 | 0.288521 |  |  |  |  |  |
| B. Mandible shape asymmetry ANOVA | | | | | | | |
| Ind | 48 | 0.09468 | 0.001973 | 0.23351 | 2.5681 | -5.1380 | 1.000 |
| Side | 1 | 0.24425 | 0.244246 | 0.60239 | 317.9870 | 3.9022 | 0.001 \*\* |
| Ind:side | 48 | 0.03687 | 0.000768 | 0.09093 | 2.5372 | 10.5743 | 0.001 \*\* |
| Ind:side:replic | 98 | 0.02967 | 0.000303 | 0.07317 |  |  |  |
| Total | 195 | 0.40546 |  |  |  |  |  |
| C. Mandible size asymmetry ANOVA | | | | | | | |
| Ind | 48 | 1.2606e-05 | 2.6263e-07 | 0.38319 | 7.5554 | 8.3059 | 0.001 \*\* |
| Side | 1 | 3.4400e-07 | 3.4404e-07 | 0.01046 | 9.8976 | 2.4929 | 0.002 \*\* |
| Ind:side | 48 | 1.6541e-05 | 3.4460e-07 | 0.50280 | 9.9136 | 9.0728 | 0.001 \*\* |
| Ind:side:replic | 98 | 3.4060e-06 | 3.4760e-08 | 0.10355 |  |  |  |
| Total | 195 | 3.2897e-05 |  |  |  |  |  |







Adams, D. C. (2016). Evaluating modularity in morphometric data: Challenges with the RV coefficient and a new test measure. *Methods in Ecology and Evolution*, *7*(5), 565–572. https://doi.org/10.1111/2041-210X.12511

Adams, D. C., & Collyer, M. L. (2016). On the comparison of the strength of morphological integration across morphometric datasets. *Evolution*, *70*(11), 2623–2631. https://doi.org/10.1111/evo.13045

Adams, D. C., & Collyer, M. L. (2019). Comparing the strength of modular signal, and evaluating alternative modular hypotheses, using covariance ratio effect sizes with morphometric data. *Evolution*, *73*(12), 2352–2367. https://doi.org/10.1111/evo.13867

Adams, D. C., Collyer, M. L., Kaliontzopoulou, A., & Baken, E. K. (2022). *geomorph: Software for geometric morphometric analyses* (4.0.4) [R]. https://cran.r-project.org/package=geomorph

Babcock, L. E. (2005). Asymmetry in the fossil record. *European Review*, *13*(S2), 135–143. https://doi.org/10.1017/S1062798705000712

Baken, E. K., Collyer, M. L., Kaliontzopoulou, A., & Adams, D. C. (2021). geomorph v4.0 and gmShiny: Enhanced analytics and a new graphical interface for a comprehensive morphometric experience. *Methods in Ecology and Evolution*, *12*(12), 2355–2363. https://doi.org/10.1111/2041-210X.13723

Ball, G. E. (1992). The Tribe Licinini (Coleoptera: Carabidae): A Review of the Genus-Groups and of the Species of Selected Genera. *Journal of the New York Entomological Society*, *100*(2), 325–380.

Benítez, H., Lemic, D., Villalobos-Leiva, A., Bažok, R., Órdenes-Claveria, R., Pajač Živković, I., & Mikac, K. (2020). Breaking Symmetry: Fluctuating Asymmetry and Geometric Morphometrics as Tools for Evaluating Developmental Instability under Diverse Agroecosystems. *Symmetry*, *12*(11), 1789. https://doi.org/10.3390/sym12111789

Brown, N. A., & Wolpert, L. (1990). The development of handedness in left/right asymmetry. *Development*, *109*(1), 1–9. https://doi.org/10.1242/dev.109.1.1

Cardini, A. (2019). Integration and Modularity in Procrustes Shape Data: Is There a Risk of Spurious Results? *Evolutionary Biology*, *46*(1), 90–105. https://doi.org/10.1007/s11692-018-9463-x

Cardini, A. (2023). *Shall we all adopt, with no worries, the ‘within a configuration’ approach in geometric morphometrics? A comment on claims that the effect of the superimposition and sliding on shape data is “not an obstacle to analyses of integration and modularity”*. EcoEvoRxiv.

Chapman, R. F. (1964). The structure and wear of the mandibles in some African grasshoppers. *Proceedings of the Zoological Society of London*, *142*(1), 107–122. https://doi.org/10.1111/j.1469-7998.1964.tb05157.x

Churchill, M., Miguel, J., Beatty, B. L., Goswami, A., & Geisler, J. H. (2019). Asymmetry drives modularity of the skull in the common dolphin ( *Delphinus delphis* ). *Biological Journal of the Linnean Society*, *126*(2), 225–239. https://doi.org/10.1093/biolinnean/bly190

Claude, J. (2008). *Morphometrics with R*. Springer.

Clissold, F. J. (2007). The Biomechanics of Chewing and Plant Fracture: Mechanisms and Implications. In *Advances in Insect Physiology* (Vol. 34, pp. 317–372). Elsevier. https://doi.org/10.1016/S0065-2806(07)34006-X

Clune, J., Mouret, J.-B., & Lipson, H. (2013). The evolutionary origins of modularity. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1755), 20122863. https://doi.org/10.1098/rspb.2012.2863

Collyer, M. L., & Adams, D. C. (2018). RRPP: An R package for fitting linear models to high‐dimensional data using residual randomization. *Methods in Ecology and Evolution*, *9*(7), 1772–1779. https://doi.org/10.1111/2041-210X.13029

Collyer, M. L., & Adams, D. C. (2021). *RRPP: Linear Model Evaluation with Randomized Residuals in a Permutation Procedure* [R]. https://cran.r-project.org/web/packages/RRPP

del Castillo, D. L., Segura, V., Flores, D. A., & Cappozzo, H. L. (2016). Cranial development and directional asymmetry in Commerson’s dolphin, *Cephalorhynchus commersonii commersonii*: 3D geometric morphometric approach. *Journal of Mammalogy*, *97*(5), 1345–1354. https://doi.org/10.1093/jmammal/gyw101

del Castillo, D. L., Viglino, M., Flores, D. A., & Cappozzo, H. L. (2017). Skull ontogeny and modularity in two species of *Lagenorhynchus*: Morphological and ecological implications. *Journal of Morphology*, *278*(2), 203–214. https://doi.org/10.1002/jmor.20629

Evans, K. M., Buser, T. J., Larouche, O., & Kolmann, M. A. (2023). Untangling the relationship between developmental and evolutionary integration. *Seminars in Cell & Developmental Biology*, *145*, 22–27. https://doi.org/10.1016/j.semcdb.2022.05.026

Evans, K. M., Larouche, O., Watson, S.-J., Farina, S., Habegger, M. L., & Friedman, M. (2021). Integration drives rapid phenotypic evolution in flatfishes. *Proceedings of the National Academy of Sciences*, *118*(18), e2101330118. https://doi.org/10.1073/pnas.2101330118

Felice, R. N., Randau, M., & Goswami, A. (2018). A fly in a tube: Macroevolutionary expectations for integrated phenotypes. *Evolution*, *72*(12), 2580–2594. https://doi.org/10.1111/evo.13608

Ginot, S., & Blanke, A. (2023). A comparison of dissection and 3D approaches to estimate muscle physiological cross-sectional area, validated by *in vivo* bite forces. *Journal of Experimental Biology*, jeb.246341. https://doi.org/10.1242/jeb.246341

Goswami, A., & Finarelli, J. A. (2016). EMMLi: A maximum likelihood approach to the analysis of modularity. *Evolution*, *70*(7), 1622–1637. https://doi.org/10.1111/evo.12956

Govind, C. K. (1989). Asymmetry in Lobster Claws. *American Naturalist*, *77*(5), 468–474.

Govind, C. K., & Blundon, J. A. (1985). Form and function of the asymmetric chelae in blue crabs with normal and reversed handedness. *The Biological Bulletin*, *168*(2), 321–331. https://doi.org/10.2307/1541244

Graham, J. H., Freeman, D. C., & Emlen, J. M. (1994). Antisymmetry, directional asymmetry, and dynamic morphogenesis. In T. A. Markow (Ed.), *Developmental Instability: Its Origins and Evolutionary Implications* (Springer Netherlands, Vol. 2, pp. 123–139). Kluwer Academic Publishers. https://doi.org/10.1007/978-94-011-0830-0\_10

Hallgrímsson, B., Jamniczky, H., Young, N. M., Rolian, C., Parsons, T. E., Boughner, J. C., & Marcucio, R. S. (2009). Deciphering the Palimpsest: Studying the Relationship Between Morphological Integration and Phenotypic Covariation. *Evolutionary Biology*, *36*(4), 355–376. https://doi.org/10.1007/s11692-009-9076-5

Hansen, T. F. (2003). *Is modularity necessary for evolvability? Remarks on the relationship between pleiotropy and evolvability*.

Hansen, T. F., Carter, A. J. R., & Pélabon, C. (2006). On Adaptive Accuracy and Precision in Natural Populations. *The American Naturalist*, *168*(2), 168–181. https://doi.org/10.1086/505768

Huggenberger, S., Leidenberger, S., & Oelschläger, H. H. A. (2017). Asymmetry of the nasofacial skull in toothed whales (Odontoceti). *Journal of Zoology*, *302*(1), 15–23. https://doi.org/10.1111/jzo.12425

Klingenberg, C. P. (2022). Shape asymmetry—What’s new? *Emerging Topics in Life Sciences*, *6*(3), 285–294. https://doi.org/10.1042/ETLS20210273

Klingenberg, C. P., Badyaev, A. V., Sowry, S. M., & Beckwith, N. J. (2001). Inferring Developmental Modularity from Morphological Integration: Analysis of Individual Variation and Asymmetry in Bumblebee Wings. *The American Naturalist*, *157*(1), 11–23. https://doi.org/10.1086/317002

Klingenberg, C. P., McIntyre, G. S., & Zaklan, S. D. (1998). Left-right asymmetry of fly wings and the evolution of body axes. *Proceedings of the Royal Society B: Biological Sciences*, *265*, 1255–1259. https://doi.org/10.1098/rspb.1998.0427

Laeta, M., Oliveira, J. A., Siciliano, S., Lambert, O., Jensen, F. H., & Galatius, A. (2023). Cranial asymmetry in odontocetes: A facilitator of sonic exploration? *Zoology*, *160*, 126108. https://doi.org/10.1016/j.zool.2023.126108

Lanzetti, A., Coombs, E. J., Miguez, R. P., Fernandez, V., & Goswami, A. (2022). The ontogeny of asymmetry in echolocating whales. *Proceedings of the Royal Society B: Biological Sciences*, *289*, 20221090. https://doi.org/10.1098/rspb.2022.1090

Lebrun, R. (2018). *MorphoDig, an open-source 3D freeware dedicated to biology* [Computer software].

Levinton, J. S. (2016). Bilateral linkage of monomorphic and dimorphic limb sizes in fiddler crabs. *Biological Journal of the Linnean Society*, *119*(2), 370–380. https://doi.org/10.1111/bij.12809

Lucas, T., & Goswami, A. (2017). *paleomorph: Geometric Morphometric Tools for Paleobiology* (0.1.4) [R]. https://CRAN.R-project.org/package=paleomorph

Macleod, C. D., Reidenberg, J. S., Weller, M., Santos, M. B., Herman, J., Goold, J., & Pierce, G. J. (2007). Breaking symmetry: The marine environment, prey size, and the evolution of asymmetry in cetacean skulls. *The Anatomical Record*, *290*(6), 539–545. https://doi.org/10.1002/ar.20539

Meinhardt, H. (2001). Organizer and axes formation as a self-organizing process. *International Journal of Developmental Biology*, *45*, 177–188.

Minelli, A., Boxshall, G., & Fusco, G. (Eds.). (2013). *Arthropod Biology and Evolution: Molecules, Development, Morphology*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-36160-9

Møller, A. P. (1990). Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. *Animal Behaviour*, *40*(6), 1185–1187. https://doi.org/10.1016/S0003-3472(05)80187-3

Neubauer, S., Gunz, P., Scott, N. A., Hublin, J.-J., & Mitteroecker, P. (2020). Evolution of brain lateralization: A shared hominid pattern of endocranial asymmetry is much more variable in humans than in great apes. *Science Advances*, *6*(7), eaax9935. https://doi.org/10.1126/sciadv.aax9935

Nogueira, C. S., da Silva, A. R., & Palaoro, A. V. (2022). Fighting does not influence the morphological integration of crustacean claws (Decapoda: Aeglidae). *Biological Journal of the Linnean Society*, *136*(1), 173–186. https://doi.org/10.1093/biolinnean/blac026

Palmer, A. R. (1994). Fluctuating asymmetry analyses: A primer. In T. A. Markow (Ed.), *Developmental Instability: Its Origins and Evolutionary Implications* (Vol. 2, pp. 335–364). Springer Netherlands. https://doi.org/10.1007/978-94-011-0830-0\_26

Palmer, A. R. (1996). From symmetry to asymmetry: Phylogenetic patterns of asymmetry variation in animals and their evolutionary significance. *Proceedings of the National Academy of Sciences*, *93*(25), 14279–14286. https://doi.org/10.1073/pnas.93.25.14279

Palmer, A. R. (2004). Symmetry Breaking and the Evolution of Development. *Science*, *306*(5697), 828–833. https://doi.org/10.1126/science.1103707

Palmer, A. R. (2016). What determines direction of asymmetry: Genes, environment or chance? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1710), 20150417. https://doi.org/10.1098/rstb.2015.0417

Parle, E., Dirks, J.-H., & Taylor, D. (2016). Bridging the gap: Wound healing in insects restores mechanical strength by targeted cuticle deposition. *Journal of The Royal Society Interface*, *13*(117), 20150984. https://doi.org/10.1098/rsif.2015.0984

Parle, E., & Taylor, D. (2017). The effect of aging on the mechanical behaviour of cuticle in the locust *Schistocerca gregaria*. *Journal of the Mechanical Behavior of Biomedical Materials*, *68*, 247–251. https://doi.org/10.1016/j.jmbbm.2017.02.008

Parr, W. C. H., Wilson, L. A. B., Wroe, S., Colman, N. J., Crowther, M. S., & Letnic, M. (2016). Cranial Shape and the Modularity of Hybridization in Dingoes and Dogs; Hybridization Does Not Spell the End for Native Morphology. *Evolutionary Biology*, *43*(2), 171–187. https://doi.org/10.1007/s11692-016-9371-x

Pélabon, C., & Hansen, T. F. (2008). On the adaptive accuracy of directional asymmetry in insect wing size. *Evolution*, *62*(11), 2855–2867. https://doi.org/10.1111/j.1558-5646.2008.00495.x

Pither, J., & Taylor, P. D. (2000). Directional and fluctuating asymmetry in the black-winged damselfly *Calopteryx maculata* (Beauvois) (Odonata: Calopterygidae). *Canadian Journal of Zoology*, *78*(10), 1740–1748. https://doi.org/10.1139/z00-130

Posnien, N., & Bucher, G. (2010). Formation of the insect head involves lateral contribution of the intercalary segment, which depends on Tc-labial function. *Developmental Biology*, *338*(1), 107–116. https://doi.org/10.1016/j.ydbio.2009.11.010

Pratt, A. E., & Mclain, D. K. (2002). Antisymmetry in male fiddler crabs and the decision to feed or breed. *Functional Ecology*, *16*(1), 89–98. https://doi.org/10.1046/j.0269-8463.2001.00605.x

Püffel, F., Johnston, R., & Labonte, D. (2023). A biomechanical model for the relation between bite force and mandibular opening angle in arthropods. *Royal Society Open Science*, *10*, 221066.

Püffel, F., Pouget, A., Liu, X., Zuber, M., van de Kamp, T., Roces, F., & Labonte, D. (2021). *Morphological determinants of bite force capacity in insects: A biomechanical analysis of polymorphous leaf-cutter ants*. BioRxiv. https://doi.org/10.1101/2021.08.06.453764

R Core Team. (2022). *R: A language and environment for statistical computing.* (4.2.1) [Computer software]. R Foundation for Statistical Computing,. https://www.R-project.org/

Rühr, P., & Blanke, A. (2022). ForceX and ForceR: A mobile setup and R package to measure and analyse a wide range of animal closing forces. *Methods in Ecology and Evolution*, *13*(9), 1938–1948. https://doi.org/10.1111/2041-210X.13909

Savriama, Y., Vitulo, M., Gerber, S., Debat, V., & Fusco, G. (2016). Modularity and developmental stability in segmented animals: Variation in translational asymmetry in geophilomorph centipedes. *Development Genes and Evolution*, *226*(3), 187–196. https://doi.org/10.1007/s00427-016-0538-3

Schofield, R. M. S., Bailey, J., Coon, J. J., Devaraj, A., Garrett, R. W., Goggans, M. S., Hebner, M. G., Lee, B. S., Lee, D., Lovern, N., Ober-Singleton, S., Saephan, N., Seagal, V. R., Silver, D. M., Som, H. E., Twitchell, J., Wang, X., Zima, J. S., & Nesson, M. H. (2021). The homogenous alternative to biomineralization: Zn- and Mn-rich materials enable sharp organismal “tools” that reduce force requirements. *Scientific Reports*, *11*(1), 17481. https://doi.org/10.1038/s41598-021-91795-y

Tiwari, S., Nambiar, S., & Unnikrishnan, B. (2017). Chewing side preference—Impact on facial symmetry, dentition and temporomandibular joint and its correlation with handedness. *Journal of Orofacial Sciences*, *9*(1), 22. https://doi.org/10.4103/jofs.jofs\_74\_16

Van Valen, L. (1962). A Study of Fluctuating Asymmetry. *Evolution*, *16*(2), 125–142.

Wagner, G. P., & Altenberg, L. (1996). PERSPECTIVE: COMPLEX ADAPTATIONS AND THE EVOLUTION OF EVOLVABILITY. *Evolution*, *50*(3), 967–976. https://doi.org/10.1111/j.1558-5646.1996.tb02339.x

Wagner, G. P., Pavlicev, M., & Cheverud, J. M. (2007). The road to modularity. *Nature Reviews Genetics*, *8*(12), 921–931. https://doi.org/10.1038/nrg2267

Zelditch, M. L., & Goswami, A. (2021). What does modularity mean? *Evolution & Development*, *23*(5), 377–403. https://doi.org/10.1111/ede.12390

Zelditch, M. L., & Swiderski, D. L. (2023). Effects of Procrustes Superimposition and Semilandmark Sliding on Modularity and Integration: An Investigation Using Simulations of Biological Data. *Evolutionary Biology*, *50*(2), 147–169. https://doi.org/10.1007/s11692-023-09600-9