**Guidelines for the analysis of S. Sommerfeld’s grasshopper data in terms of asymmetry**

1. Fluctuating asymmetry of non-mandibular head structures.
2. Directional asymmetry of mandibles.
3. Gradient of directional asymmetry from mandibles to structures that are further away.
4. Limitations and methodological challenges.

**1) Fluctuating asymmetry of non-mandibular head structures.**

Fluctuating asymmetry (FA) is supposed to be caused by small perturbations in the development of bilaterian animals, for example produced by environmental stresses during ontogeny. It is expected that fitter individuals will show less fluctuating asymmetry than less fit individuals, because they are better able to buffer against environmental stressors, thereby maintaining symmetrical development. Therefore, it has been suggested in the literature that FA could be used either as a proxy for quantifying different levels of stress in different environments, and/or that it should correlate to individual fitness within one population.

Since one can expect that less fit individuals also have poorer whole-organism performance, there should also be a negative link between performance and FA, here between bite force (BF) and FA. Because the symmetry of head capsule structures is *a priori* not *mechanically* related to biting (e.g. eyes are not used in biting), one may conclude that, if there is a correlation between BF and FA, it must be explained by the condition/fitness of individuals.

H0: Negative correlation between FA and BF => less fit individuals have higher FA and weaker BF.

**2) Directional asymmetry of mandibles.**

Left and right mandibles in grasshoppers are known to be directionally asymmetric, with the right mandible being longer, and overlapping the shorter left mandible. This allows the grasshoppers to use their mandible as scissors, using shearing to cut pieces of plants. Directional asymmetry (DA) is also present in the molar region, which ensures correct occlusion for chewing down the food. Two perfectly symmetric mandibles could not cut or occlude properly, therefore one may expect that more asymmetrical individuals have better performance, here better bite force. However, extremely asymmetric mandibles may also compromise cutting (adding space between the “blades” of scissors make them cut less well), and chewing, if molar regions do not come in contact anymore. Therefore, it is expected that the directional asymmetry of mandibles will maximize occlusion and shearing forces, with an optimum at intermediate values, and worse performance at smaller and larger amounts of asymmetry.

However, we are only measuring bite forces at the tips of mandibles. We are neither measuring shearing forces, nor chewing forces, which are the actual traits expected to be impacted by DA.

H0: Non-linear relationship between DA and BF, with one optimal amount of DA for perfect occlusion.

**3) Gradient of directional asymmetry from mandibles to structures further away.**

Because mandibles are directionally asymmetric, their mechanical action is also expected to be directionally asymmetric. Therefore, the mechanical constraints applied to the surrounding structures may also be directionally asymmetric, and plasticity may produce DA in these structures. This effect would be expected to diminish further away from mandibles, since mechanical constraints will apply less strongly.

However, one should be careful with areas of muscle insertion, which are distant from mandibles, may be under asymmetrical constraints nevertheless.

H0: landmarks closer to mandibles will show higher DA.

OR (more adequately)

H0: landmarks more *mechanically integrated* with mandibles will show higher DA.

**4) Limitations and methodological challenges.**

**FA and DA** ***CANNOT*** **be calculated at the individual level**, they are population-level traits. One cannot discriminate between the components of asymmetry within one individual. Therefore, correlation tests will not be applicable across individuals. However, this can be circumvented by splitting the sample into categories, for example “weak”, “intermediate”, and “strong” biters, then comparing their FA and DA. This does not apply to point 3), because in that part, we will be computing FA and DA for the entire sample, and then testing individual landmarks and/or structures against each other.

Furthermore, we have a safe assumption that most of the asymmetry in mandibles will be DA, while FA may be more or less important in other structures, which would make it worth to test the global asymmetry of the mandibles against bite force *at the individual level*. In fact, we could start the study by part 3), to first assess the relative importance of FA and DA in each landmark or structure, then compute an approximation of FA and DA at the individual level by multiplying individual global asymmetry for each landmark by the respective proportions of FA and DA for that given landmark. To my knowledge this kind of approach has never been done before, and may be a nice methodological advancement in the field.

Size is an important factor in bite forces, so we should make sure this is accounted for, especially if we are making categories like “weak”, “intermediate” and “strong” biters. In such context, we may use the residuals of a regression of raw size against bite force to produce our categories.