**Using the R package “equalCovs” to quantify the change in overall behavioral and physiological integration *(Accompaniment to Chapter 4)***

**Background**

In Chapter 4 (“*Social ascent affects cognition, behavior, and physiology in a highly social cichlid fish”*) I explored the influence of social ascent on a diverse set of characteristics in the social cichlid *Astatotilapia burtoni*. I identified a variety of correlations between dominance status, physiology, behavior, and cognitive performance. To integrate these correlations into a broader multidimensional view I conducted a set of principal component analyses and hierarchical clustering analyses. These analyses yielded emergent insights, statistically differentiating ascending and nonascending males and identifying key relationships (hierarchical clusters). The principal component analysis on data collected *prior* to perturbation statistically differentiated males that would eventually ascend from males that would eventually not ascend, suggesting that certain males (eventual nonascenders) may be “proactively” engaging in ascent-associated behavior. Interestingly, the analysis on data collected *after* perturbation did not differentiate ascenders and nonascenders.

Why might the multidimensional analysis prior to perturbation distinguish these two groups, but the data after perturbation not? One possibility is that following the perturbation, ascending males diverge in their behavioral and cognitive repertoires (e.g. individuals diverge into a variety of dominance “styles”, which reflects observed variation in dominant *A. burtoni* males). At a group level, this would mean greater variation and a weakening of the overall degree of behavioral and physiological integration. Conversely, nonascending males, some of which prior to the perturbation may have been engaging in ascent-like behaviors to a minor degree, subsequently do not ascend and cease engaging in these behaviors, coalescing around a more consistent subordinate behavioral and physiological phenotype. At a group level, this would mean reduced variation and a strengthening of the overall degree of behavioral and physiological integration.

This hypothesis is certainly not the only possible explanation for the difference in the principal component analyses before and after the perturbation, but here I explore this further. In this appendix I employ a relatively new covariance analysis to statistically test if social ascent changed the overall degree of behavioral and physiological integration in ascending and nonascending males.

**Methods**

To compare the overall degree of behavioral, cognitive, and physiological integration before and after the perturbation, I generated a total of four covariance matrices on the data from the novel object recognition task (NOR), spatial task (SPA), social opportunity and challenge assay (SOCA), and physiology (CORT & T): two matrices for ascending males (prior to and following the perturbation), and two matrices for nonascending males (prior to and following the perturbation). Each matrix thus had a distribution of covariance scores, and these distributions could be statistically compared. To compare these covariance distributions I used the R package “equalCovs” (Li et al. 2018), which tests for global differences in covariance structure (Li & Chen 2012). Note that variables included in these four matrices were first filtered via a separate covariance matrix on the data from all individuals: any two variables with a Pearson’s correlation coefficient above 0.8 in either the baseline testing or the post-perturbation retesting were considered redundant. Thus, for subsequent analysis I only included the variable which preserved the most variables between the baseline and retesting dataset, and, if necessary, to decide between two equal variables in this regard, the variable more commonly used in previous literature. Raw data and analysis code can be found at *github.com/kellyjwallace/Wallace\_Hofmann\_Dissertation\_Appendix\_EqualCovs\_2021*.

**Results**

To test if there was a change in overall covariance across behavioral and hormonal variables, I calculated four covariance matrices on variables across all three tasks (SPA, NOR, SOCA) and the hormone measurements (CORT & T) on ascending then nonascending males: one on the pre-perturbation measurements, the other on the post-perturbation measurements. I identified significantly different and opposing patterns of covariance by status: in ascending males, the distribution of Pearson covariance values *decreased* (i.e. a looser covariance structure) following the perturbation in ascending males (number of pairwise comparisons = 351, p = 3.037 x 10-7, reported test statistic = 4.989, Figure 1A) and *increased* (i.e. a tighter covariance structure) following the perturbation in nonascending males (number of pairwise comparisons = 351, p = 6.482 x 10-10, reported test statistic = 6.068, Figure 1B). Because the sample size in the ascending male dataset is small, I additionally viewed the distribution of Pearson correlation coefficients, which reflected this same pattern (Figure 1C,D).

**Discussion**

In Chapter 4, I identified a variety of correlations between dominance status, physiology, behavior, and cognitive performance. I further explored the data from Chapter 4 by conducting a multi-variate approach to understand changes in overall integration before and after the perturbation. Interestingly, the covariance structure of the data significantly *strengthened* in nonascenders following the perturbation but significantly *weakened* in ascenders. Individual variation between ascending males is potentially the most dramatic at the timepoint we assessed, in which ascending males are first attaining dominance. Typically, the full transition to dominance, including changes in gonad size, involves longer-scale changes in physiology, morphology, and neurobiology (with full social ascent taking roughly two weeks, Maruska 2014), though behaviorally males change much more quickly. Our experiment characterized males at the beginning of social ascent in an unstable environment, a time where there may be a large amount of variation between individuals and thus lower behavioral and cognitive covariance. Conversely, the nonascending males strengthened behavioral covariance structures following the perturbation, potentially reflecting a convergence of subordinate behavior in unstable social environments. This community level instability may also explain why our principal component analysis of the post-perturbation data did not yield any significantly different PC axes by ascent status and why the hierarchical clustering analysis of the PCA eigenvalues did not find any highly-supported clusters. Phenotypic integration vs independence (the connectedness of multivariate relationships across biological levels) is influenced by physiology and can be coordinated by physiology (Ketterson et al. 2009), thus I encourage future studies to more specifically investigate the role of physiology in strengthening/weakening overarching covariance structures across state transitions like social ascent.

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**References**

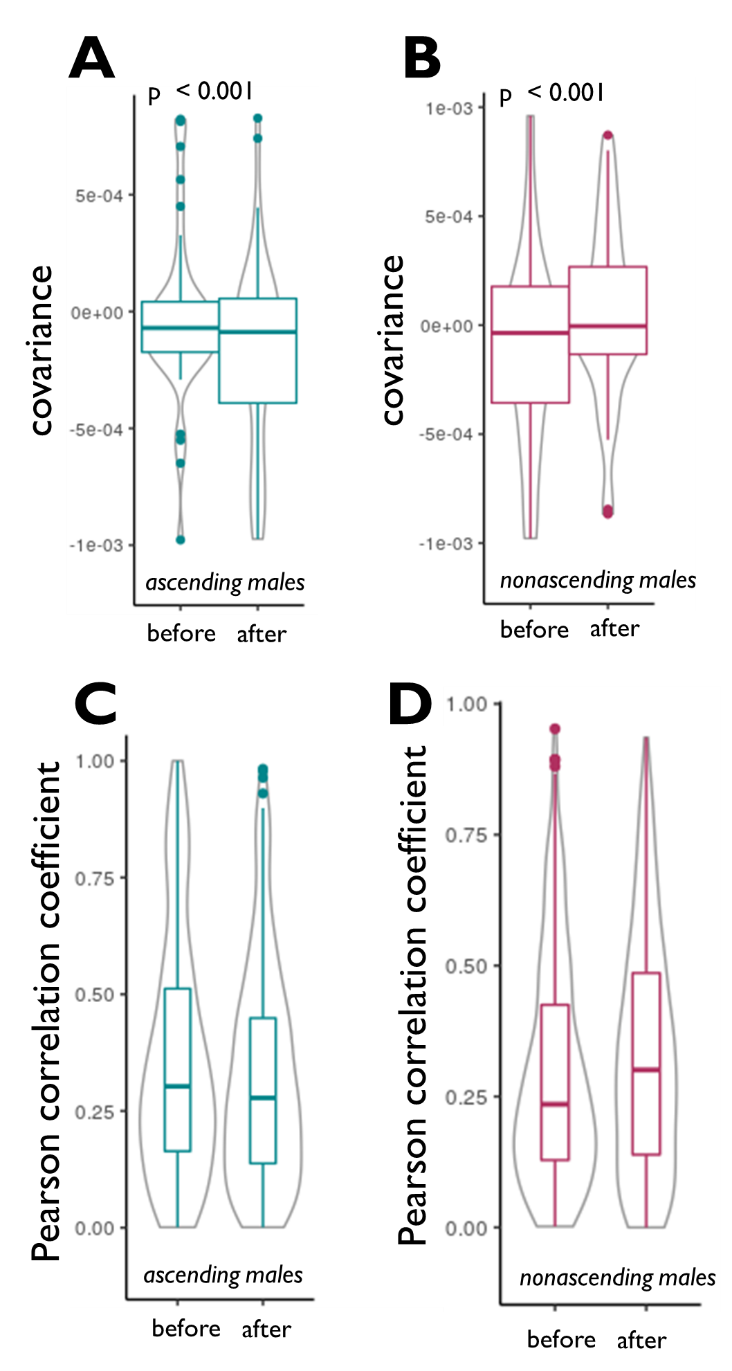
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**Figure 1.** When comparing before and after the perturbation, ascending males’ overall behavioral and physiological covariance significantly decreases (p < 0.001, A), whereas nonascending males’ overall covariance significantly increases (p < 0.001, B). This is visible when viewing the distribution of Pearson covariance scores (subsetted between -0.001 and 0.001 for visual purposes) (A,B) as well as when viewing the distribution of Pearson correlation coefficients (C,D).

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