Supplementary Information: An Example of Integrating Modeling to Explore Endocrine Flexibility

Suppose a researcher is studying endocrine flexibility using a mouse model system. The researcher generally wants to understand how corticosteroid binding globulin (CBG) capacity influences endocrine speed (i.e., the rate at which corticosterone levels change). An empirical study could certainly be done, though it would likely necessitate repeated sampling of individuals. Doing this may be difficult without causing additional stress to the mice, which may confound the researcher's results. Modeling could provide insight in this situation where robust experimentation is problematic or be used to complement the researcher's empirical data.

Where does one begin if they are interested in integrating modeling approaches to study endocrine flexibility in their system? The type of modeling to be used depends on the question of interest. In this paper we have reviewed modeling approaches to examine the existence, causes, and consequences of variation in endocrine flexibility. In this section, we will discuss the conceptual steps that our hypothetical researcher might take to begin using modeling to explore endocrine flexibility from each of these perspectives.

Modeling the existence of endocrine flexibility

If our researcher is interested in determining if a relationship exists between CBG and endocrine speed, the researcher could conduct a meta-analysis and use statistical modeling to evaluate the existing strength of evidence for this relationship.

1. *Conduct a literature search to identify published studies that are relevant to the question*. In this particular instance, our researcher might search for papers that concurrently and repeatedly measure CBG and glucocorticoid levels.

2. *Determine the criteria that will be used to include (or exclude) papers*. Perhaps to be included, studies must have been done on rodent systems.

3. *Collect data from the papers*. Depending on the study, the researcher might include estimates of effect sizes, as well as potential covariates, such as taxonomic group or method of sample collection.

4. *Analyze the data*. There are several ways to analyze the data that may depend on the specific question. We recommend the following resources to help guide this step: Hedges and Olkin, 2014; Hunter and Schmidt, 2004; Balduzzi et al., 2019; Schwarzer, 2007; Schwarzer et al., 2019; Schwarzer et al., 2015.

5. *Identify patterns.* Our researcher can now identify large scale patterns of CBG and endocrine flexibility across taxonomic groups, or other covariates of interest.

Modeling the causes of endocrine flexibility

If our researcher is interested in modeling the causal relationship between CBG level and endocrine speed, they could approach this question at either the proximate or ultimate level. At the proximate level, the researcher could develop a set of differential equations to mathematically model the regulatory pathways governing the relationship between CBG and glucocorticoid levels and make predictions about the effect of CBG levels on the rate at which glucocorticoids change. At the ultimate level, the researcher could develop a state-dependent dynamic model with evolutionary simulations (e.g., Taborsky et al., 2020) to explore how CBG may optimally govern the speed of endocrine responses.

Proximate level: Differential Equations

1. *Decide on a mathematical approach*. Commonly used approaches include ordinary differential equations and time-delay differential equations which can be either stoichiometric or non-stoichiometric. The pros and cons of each of these approaches are outlined in the text. For example, our researcher could decide to use a stoichiometric network modelling approach using ordinary differential equations.

2. *Select appropriate models and relationships to describe the system*. A variety of alternative model structures exist for the HPA axis, each with different assumptions regarding the core feedback mechanisms underlying the system. Our researcher should review published mathematical models of their system and determine which models (equations) and assumed structures best fit their system and question.

3. *Parameterize model with data from study system*. Our researcher should use values from their study system, or a closely related system if the necessary data are not available for their specific study organism, to parameterize the equations used in their model.

4. *Explore in silico*. The researcher can modify aspects of the system by altering values in their model to determine how CBG may influence the speed of glucocorticoid responses.

Ultimate level: Evolutionary Optimality Analysis

1. *Construct a basic scenario in which to frame the model*. This may involve deciding on environmental conditions that the researcher thinks may shape the relationship between CBG and endocrine speed or to provide scenario details that set the model in a simplified version of reality to facilitate understanding by other researchers.

2. *Construct a model using state-dependent dynamic programming*. The researcher will construct equations that describe the relationship between changes in state variables, which, in our researcher's case, may be CBG levels and endocrine speed, and an arbitrary fitness metric. The researcher must also define a terminal fitness function as well as equations to determine how state variables change over time.

3. *Find an optimal response that maximizes expected fitness*. By calculating backwards from the terminal fitness function, the researcher can find the optimal state variables (e.g., CBG level and endocrine speed) that result in the highest fitness.

4. *Forward simulations*. The responses that led to the highest possible fitness are stored and the researcher can run forward simulations to explore how the model behaves when aspects of the model are altered. Our researcher, for example, may alter the frequency of simulated stressful events and determine the influence on the relationship between CBG levels and the speed of glucocorticoid responses.

5. *Simulate evolution of physiological mechanisms*. The researcher can then determine which traits are evolvable (e.g., the strength of the relationship between CBG levels and endocrine speed), what the evolutionary dynamics of those traits are (e.g., they are unlinked,

there is no dominance, the probability that an allele mutates, etc.), and simulate the population forward in time to determine how traits might evolve in that system.

Modeling the consequences of endocrine flexibility

If our researcher is interested in exploring the repercussions at the population level of the relationship between individual variation in CBG and endocrine speed, then they may construct an integral projection model or an individual-based simulation. The exact population model construct will depend on available data.

1. *Gather population parameter data on the study organism*. Data may include age- or size-specific vital rates (per capita birth and death rates), how endocrine flexibility influences population vital rates, and other factors that might play an important role in determining population size (e.g., seasonal variation in population vital rates).

2. *Construct population model*. This step will depend on available data and the specific aim of the researcher. If our researcher has data available at the individual level, an individual-based model may provide the most insight. If data are only available at higher resolution (e.g., by age class), then something like an integral projection model may be more appropriate.

3. *Run baseline model simulations*. In this, the researcher is running the population model to validate that the population dynamics are logical (e.g., the mouse population size does not increase from 100 to 1 million individuals in a month). The researcher can troubleshoot programming and model structure if predictions are nonsensical.

4. *Alter model parameters to explore effects of parameters on population size and/or run sensitivity analysis to determine parameters that have the largest effect on population size*. This may allow researchers to explore which specific aspect of endocrine flexibility (e.g., the speed of up- or down-regulation) or CBG levels has the largest relative impact on population size.

References (Supplementary Info)

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