File S2: The mutational dynamics of binding sites provides an upper bound on the incidence of misregulation.

Here I will examine the mutational dynamics of binding sites under the assumption that such sites are randomly and uniformly distributed in sequence space. Consider first the G_{off} genes that need to be off for optimal adaptation. A gene that needs to be off requires an inactive binding site. If a mutation converts such a site into an active binding site, the gene becomes wrongly active and thus contributes to f_{01}. The probability that a randomly chosen sequence S from sequence space mutates into an active binding site can be calculated as follows. Any one random mutation creates a randomly chosen sequence among all 1-mutant neighbors of S, and if binding sites are randomly and uniformly distributed, then a fraction p_B of the sequence's neighbors will be an active binding site. Thus, the probability μ^+ that a mutation creates an active binding site from a random sequence is given by

$$\mu^+ = L\mu p_B, \tag{6}$$

By the same argument, the likelihood that a mutation destroys an existing binding site is given by

$$\mu^- = L\mu(1 - p_B). \tag{7}$$

Elementary population genetic theory shows (Hartl and Clark, 2007) that in mutation equilibrium, the fraction of active binding sites associated with genes supposed to be off is given by $\mu^+/(\mu^+ + \mu^-) = p_B$. This value yields an equilibrium fraction of incorrectly active genes $\hat{f}_{01} = p_B(1 - f^O)$, where the factor $1 - f^O$ ensures proper normalization of $\hat{f}_{01}$.

For the G_{on} genes that should be on, the same line of reasoning applies, except that now the inactive sites lead to an undesirable (wrongly off) gene expression state. The mutation equilibrium of defective binding sites is given by $\mu^-/(\mu^- + \mu^+) = (1-p_B)$, which leads to an equilibrium fraction of wrongly inactive genes $\hat{f}_{10} = (1 - p_B)f^O$.

Altogether, in mutation balance a simple condition for $\hat{\Delta}_m = \hat{f}_{01} - \hat{f}_{10} > 0$ holds, namely that $p_B > f^O$. In other words, the fraction of sequence space filled with active binding sites must be greater than the fraction of genes that must be expressed. If that is the case, then mutation pressure alone will create more wrongly active genes than wrongly inactive genes. I note that this mutation equilibrium may also hold for finite populations, where expected allele frequencies under mutation and drift are identical to those under mutation alone (Ewens, 2012, 3.25).

References

Ewens, W. J. (2012). *Mathematical population genetics 1: Theoretical introduction*, Volume 27. New York, NY: Springer Science & Business Media.

Hartl, D. and A. Clark (2007). *Principles of population genetics* (4th ed.). Sunderland, MA: Sinauer Associates.