Competitive binding of transcription factors drives Mendelian dominance in regulatory genetic pathways

Adam H. Porter\textsuperscript{a,b,1}, Norman A. Johnson\textsuperscript{a,b} and Alexander Y. Tulchinsky\textsuperscript{c}

\textsuperscript{a}Department of Biology, University of Massachusetts-Amherst, Amherst MA 01003
\textsuperscript{b}Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts-Amherst, Amherst MA 01003
\textsuperscript{c}Department of Biology, SUNY New Paltz, 1 Hawk Drive, New Paltz NY 12561

\textsuperscript{1}Corresponding author:
Adam Porter
Department of Biology
University of Massachusetts-Amherst
Morrill Science Center
211 Stockbridge Rd.
Amherst MA 01003
(413) 545-1036 (cell: 413-824-8192)
Email: aporter@bio.umass.edu

Keywords: dominance; gene expression; thermodynamics; transcription-factor binding
Abstract:
We report a new mechanism for allelic dominance in regulatory genetic interactions that we call binding dominance. We investigated a biophysical model of gene regulation, where the fractional occupancy of a transcription factor (TF) on the cis-regulated promoter site it binds to is determined by binding energy ($-\Delta G$) and TF concentration. Transcription and gene expression proceed when the TF is bound to the promoter. In diploids, individuals may be heterozygous at the cis-site, at the TF’s coding region, or at the TF’s own promoter, which determines allele-specific TF concentration. We find that when the TF’s coding region is heterozygous, TF alleles compete for occupancy at the cis sites and the tighter-binding TF is dominant in proportion to the difference in binding strength. When the TF’s own promoter is heterozygous, the TF produced at the higher concentration is also dominant. Cis-site heterozygotes have additive and therefore codominant phenotypes. Binding dominance extends to the expression of downstream loci and is sensitive to genetic background. While binding dominance is inevitable at the molecular level, it may be difficult to detect in the phenotype under some biophysical conditions, more so when TF concentration is high and allele-specific binding affinities are similar. A body of empirical research on the biophysics of TF binding demonstrates the plausibility of this mechanism of dominance, but studies of gene expression under competitive binding in heterozygotes in a diversity of genetic backgrounds are needed.

Introduction:
Mendel (1) coined the terms dominant and recessive to describe variants that respectively appear in 3:1 ratios in first-generation hybrid crosses. Wright (2) proposed a plausible mechanism, demonstrating theoretically that dominance can arise as a natural consequence of functional allelic differences among enzymes that play roles in metabolic pathways. Alleles with reduced function tended to be recessive, and variation in the genetic background could modify the degree of dominance. Kacser and Burns (3) cast Wright’s mechanism into the language of enzyme kinetics and metabolic flux, a mechanism we will call flux dominance, and several studies have extended and modified it (e.g., 4-6). Since then several other mechanisms have been found to produce dominance, including negative regulatory feedback (7), threshold-based reaction-diffusion systems (8), and epigenetic modifications (9, 10).

Empirical studies have shown that dominance is commonly found in loci involved in gene regulation. In particular, trans-acting alleles (e.g., transcription factors) commonly
show dominance, whereas the cis-acting sites they regulate only rarely do (11-13). The mechanism is unknown. We propose that this dominance is an inevitable consequence of differences in binding dynamics between trans-acting gene products as they compete for access to the cis sites they regulate. The degree of dominance thereby depends on differences in concentration and binding affinity of the trans-acting gene products. This form of dominance, which we term binding dominance, propagates through regulatory pathways and is modified by polymorphism at other loci in the pathway. Our findings apply to any trans-acting regulatory molecules interacting with cis-acting regulatory sites. Transcription-factor/promoter interactions meet these criteria well and we will develop the model using that language.

**Biophysical model of gene expression.** Biophysical models have long been used to study molecular interactions between DNA and molecules that bind to it (e.g., 14-16). The central premise of these models is that interactions between regulatory molecules and the sites they regulate behave according to the thermodynamic and kinetic principles that drive all molecular interactions. Consistent with empirical data (reviewed in ref. 17), gene expression only ensues while a transcription factor (TF) molecule is physically bound to the promoter of the regulated gene.

In our model, binding is a stochastic process determined by the free energy of association (\(-\Delta G\)) between a TF molecule and promoter, which we will call 'binding energy.' The fractional occupancy \(\theta\) — the proportion of time a promoter is occupied by a TF molecule, and therefore the gene-expression level — depends on \(-\Delta G\), and also on \(N_{TF}\), the number of free TF molecules available to bind when the promoter is unoccupied. We treat \(N_{TF}\) and TF concentration as synonyms, using the nucleus as unit volume.

The biophysical model represents interacting TF molecules and the promoter sequence as strings of bits of arbitrary length, an approach based in statistical physics and information theory (14). This method of abstraction permits characterization of molecular interactions at arbitrary scales, from the state space of electrostatic interaction among atoms to amino acid and nucleotide variation, and ultimately, to the genetic basis of variation in those molecules. The binding energy drops in steps of \(-\Delta G_1\) as \(m\), the
proportion of mismatched bits over the length of the bitstring, increases. The haploid model, a parameter-reduced form of our model in Tulchinsky et al. (15; see SI Appendix), is

\[ \theta = \frac{N_{TF}}{N_{TF} + \exp[-m \Delta G_1]} \]  

[1]

We use the following notational conventions throughout. Interacting loci are labeled with letters A and B, with C included for 3-locus pathways. Subscripts indicate allelic variants as in Fig. 1A; those before the letter (e.g., A, N_{TF,1A}) refer to promoter alleles and those after the letter (e.g., A_1) indicate gene-product alleles; subscripts are dropped for homozygotes (e.g., AA; N_{TF}). Arrows indicate allele-specific regulatory interactions (e.g., m_{A_1\rightarrow 1B} represents bitstring mismatches between TF allele A_1 and cis-site allele 1B).

**Diploid model.** In diploids, the allelic forms A_1 and A_2 of the TF molecule (Fig. 1) compete for occupancy at both promoter sites 1B and 2B independently (15) and the total number of TF molecules is the sum of those from each TF allele copy (N_{TF} = N_{TF,1A} + N_{TF,2A}). Under TF competition, the fractional occupancy of A_1 on promoter site 1B in the presence of A_2 is

\[ \theta_{A_1\rightarrow 1B} = \frac{N_{TF,1A}}{N_{TF,1A} + \alpha_{A_2\rightarrow 1B} \exp[-m_{A_1\rightarrow 1B} \Delta G_1]} \]  

[2a]

\[ \alpha_{A_2\rightarrow 1B} = 1 + N_{TF,2A} \exp[m_{A_2\rightarrow 1B} \Delta G_1] \]  

[2b]

where m_{A_1\rightarrow 1B} and m_{A_2\rightarrow 1B} are the proportion of mismatches between the bit strings of 1B vs. A_1 and A_2 respectively, and \( \alpha_{A_2\rightarrow 1B} \) is the coefficient of competition with A_2. Fractional occupancies of the other three interactions are calculated analogously.

The final expression level (\( \theta \)) is the sum of the fractional occupancies of the four TF-promoter pairs, scaled to a maximum of 1,

\[ \theta = \frac{1}{2\theta_{\text{max}}} \left( (\theta_{A_1\rightarrow 1B} + \theta_{A_2\rightarrow 1B}) + (\theta_{A_1\rightarrow 2B} + \theta_{A_2\rightarrow 2B}) \right). \]  

[3]

Maximum fractional occupancy \( \theta_{\text{max}} = N_{TF,\text{sat}}/(1+N_{TF,\text{sat}}) \) occurs when \( m = 0 \) for all TF-promoter pairs and both TF variants are at saturating concentration (i.e., \( N_{TF,1A} + N_{TF,2A} \geq N_{TF,\text{sat}} \)). As a baseline for scaling purposes, when \( m=0 \) for both alleles, we use \( N_{TF,1A} = N_{TF,2A} = N_{TF,\text{sat}}/2 \) as the allele-specific saturating concentration.

**Genotype-phenotype (G-P) map.** We treat the phenotype, \( P \), as being proportional to the expression level of the cis-regulated locus, such that \( P = k\theta \), and without loss of generality, treat that proportionality constant as \( k=1 \), such that \( P = \theta \).
In the biophysical model, the bit strings are abstract representations of information content that can characterize underlying genetic differences in the interacting molecules. Equations 1 and 3 therefore characterize the genotype-phenotype (G-P) map, the rules by which the phenotype is generated from the underlying genotype, as a function of the binding energies and TF concentrations.

**Dominance.** Competition between TF alleles for binding to their *cis*-regulated sites creates conditions for allelic dominance (15). Following Wright (2), we use \( d = (P_{11} - P_{12})/(P_{11} - P_{22}) \) as the dominance coefficient, where \( P_{12} \) is the heterozygote phenotype and \( P_{11} \) and \( P_{22} \) are homozygote phenotypes; allele ‘1’ of the respective locus is thereby the reference allele for which dominance is assessed. Allele 1 is codominant when \( d = \frac{1}{2} \), completely dominant at \( d = 0 \) and completely recessive at \( d = 1 \).

If fractional occupancy cannot be measured separately for each allele, then \( d \) must be assessed phenotypically. Even strong dominance becomes increasingly difficult to detect as \( \theta \)s for homozygotes and heterozygotes of both alleles approach equality because the three genotypes will have very similar phenotypes; the locus will appear monomorphic or the degree of dominance will be obscured by sampling and measurement error. Detectability \( (t) \) is proportional to the absolute difference between the two homozygote phenotypes, such that \( t = \kappa |P_{11} - P_{22}| \) with proportionality function \( \kappa \). In a constant genetic background, \( \kappa \) is some increasing function of the accuracy in the measurement of \( P \) (or \( \theta \)) and the sample size of the study. To graphically illustrate the effects of detectability, we overlay the genotype-dominance maps with white opacity masks, grading from opaque at \( t = 0 \) through translucency to transparency at \( t = 1 \), with the effect of making the underlying genotype-dominance map increasingly visible as detectability increases. As a heuristic, we treat \( \kappa \) as a constant arbitrarily set to 4 and a maximum of \( t = 1 \); i.e., dominance is undetectable when homozygote phenotypes are equal and always detectable when their difference equals or exceeds 1/4.

**Analysis.** We considered cases where fractional occupancy and therefore gene expression is maximal \( (\theta = P = 1) \) when binding is maximal \( (m = 0) \) and TF concentration is saturating, and that \( \theta = P = 0.5 \) when \( m = 0.5 \) at the same \( N_{TF,sat} \). Analysis of the role of TF
concentration requires scaling $-\Delta G_1$ to $N_{TF,sat}$ in order to meet these constraints (eqs. 1 and 2). Substituting eq. 2 into eq. 3 and solving for $-\Delta G_1$, we used

$$-\Delta G_1 = 2 \ln \left[ \frac{N_{TF,sat}}{1+N_{TF,sat}} \right].$$

We report results from the cases where $N_{TF,sat}$ takes the values 10, 100 and 1000. All analyses were done using Mathematica (18).

We compare three types of polymorphism (Fig. 1A). Polymorphism in the cis-regulated B locus is represented as $AA \rightarrow 1B_2B$; that in the TF protein-coding region is $A_1A_2 \rightarrow BB$, and variation in upstream TF expression (i.e., allele-specific $N_{TF}$) is $1A_2A \rightarrow BB$. In the SI Appendix, we consider the propagation of dominance at the A locus to loci further downstream, and further explore genetic-background effects, in the 3-locus $AA \rightarrow BB \rightarrow CC$ pathway.

**Results**

**Genotype-phenotype maps.** The shapes of the G-P maps differ depending on which site is polymorphic. In the $1A_2A \rightarrow BB$ case (Fig. 1B-D) with maximal TF binding ($m=0$), $\theta$ is low when both alleles have low expression ($N_{TF}$), climbing towards high expression as $N_{TF}$ of both alleles rises to $N_{TF,sat}$. The effect is very sensitive to $N_{TF,sat}$ such that the region of detectably lower $\theta$ is confined to the very bottom left corner of Fig. 1D when $N_{TF,sat}$ is high. The drop-off in $\theta$ is perpendicular to the $N_{TF,1A} = N_{TF,2A}$ diagonal, proportional to their sum, $N_{TF}$.

In the $A_1A_2 \rightarrow BB$ case (Fig. 1E-G) at $N_{TF,sat}$ $\theta$ depends on competitive binding of the TF variants to the cis-sites they regulate (eqs. 2 and 3). $\theta$ is high as long as either TF binds tightly ($m_{A_1A_2B}$ or $m_{A_2A_3B}$ is low), yielding a characteristic L-shaped ridge on the density plot, indicating dominance of the tighter-binding allele. Increasing $N_{TF,sat}$ (Fig. 1F and G) broadens and flattens the ridge.

In the $AA \rightarrow 1B_2B$ case (Fig. 1H-J), the expression of the two B-allele copies is additive (eq. 3) and at $N_{TF,sat}$, peak $\theta$ occurs when both alleles perfectly match the TF ($m_{A_31B} = m_{A_32B} = 0$). Expression falls away on both axes, leaving a characteristic arc on the density plot (Fig. 1H), curving opposite the direction of the $A_1A_2 \rightarrow BB$ case. Increasing $N_{TF,sat}$ produces a
more plateaued ridge that extends further out along the $m_{A_2}B = m_{A_2}B$ diagonal, visible as a more squared-off arc on the density plot (Fig. 1I and J).

**Dominance in fractional occupancy.** Dominance in $\theta$ emerges in the $A_2A \rightarrow BB$ and $A_1A_2 \rightarrow BB$ cases, with different patterns (Figs. 2A-F and 3F), but the $AA \rightarrow B_2B$ case is always codominant ($d = 0.5$) due to the additivity of the products of locus B (eq. 3).

In the $A_1A_2 \rightarrow BB$ case (Fig. 2A-C), the TF allele with higher binding affinity (lower $m$) has a competitive advantage and dominant expression. The isoclines follow the diagonal when $m$ is low but flare at higher $m$ such that the competitive binding effect becomes much weaker. In this range the occupancy of each allele is so low that the TF’s cease to compete and the phenotype becomes effectively additive (i.e., diploid $\theta$ of eq. 3 approaches haploid $\theta$ of eq. 1 as $m \rightarrow 1$). $N_{TF,sat}$ has a strong effect on dominance due to its effect on competition. When $N_{TF,sat}$ is high (Fig. 2C), small changes in binding affinity can produce large changes in $d$, particularly when $m < 0.5$, whereas much larger changes in $m$ are required for the same effect at $N_{TF,sat} = 10$ (Fig. 2A).

In the $A_2A \rightarrow BB$ case (Fig. 2D and E), the allele with higher $N_{TF}$ is dominant. The isoclines spread linearly from the bottom left corner of the density plot, where $N_{TF}$ is low for both alleles, continuing into the region beyond the dotted line where total TF concentration is saturating ($N_{TF,1A} + N_{TF,2A} \geq N_{TF,sat}$). This dominance pattern is not substantially altered by $N_{TF,sat}$, nor is it by $m < 1$ provided that the TF coding region and the cis-site are homozygous. These plots are therefore not shown.

When the promoter and coding region of the TF are both heterozygous (the $A_1A_2 \rightarrow BB$ case), the two sources of dominance interact cooperatively. Fig. 3F shows the effect of allelic variation $N_{TF,1A}$ and $N_{TF,2A}$ under conditions where $m_{A1}B = 0.1$, $m_{A2}B = 0.2$, and $N_{TF,sat} = 100$. For orientation, the circles in the centers of Figs. 2B and 3F represent the same conditions. At saturating concentration ($N_{TF,1A} = N_{TF,2A} = N_{TF,sat}/2$), allele $A_1$ is dominant with $d = 0.291$. Increasing $N_{TF}$ of the more tightly binding $A_1$ allele above $N_{TF,sat}/2$ increases its dominance, whereas decreasing its concentration pushes $d$ back towards codominance until ultimately $A_1$ becomes recessive. Increasing $N_{TF,2A}$ also counteracts dominance of the $A_1$ allele, but the rate of change is much slower, and is only able to reverse the direction of dominance if $N_{TF,1A}$ and $N_{TF,2A}$ start well below $N_{TF,sat}/2$. 


Dominance is more sensitive to binding affinity than to differences in $N_{TF}$. Fig. 2F reflects the same conditions as Fig. 2D with $N_{TF,sat} = 100$ but with a 5-fold difference in allele-specific TF concentration, $N_{TF,1A1} = N_{TF,sat}/2$ and $N_{TF,2A2} = N_{TF,sat}/10$. Under these conditions, $A_1$ is dominant with $d = 0.17$. To facilitate comparison, the orange crosses in Fig. 2D and F share common parameter settings. Codominance is restored when binding of $A_1$ is reduced by $\sim 20\%$, becoming recessive beyond that.

In contrast, polymorphism in the B locus has no effect on the dominance of $A_1$ in the $A_1A_2 \rightarrow 1B_2B$ case (SI Appendix Fig. S2A), and only a subtle effect on the dominance of $A_1$ in the $1A_2A \rightarrow 1B_2B$ case (SI Appendix Fig. S2B).

**Detectability of dominance in the phenotype.** Fig. 3A-E shows the dominance maps of Fig. 2A-E overlaid by white opacity masks that obscure $d$ in regions where homozygotes have similar expression. Existing dominance in the $1A_2A \rightarrow BB$ case is likely to be hard to detect unless $N_{TF,sat}$ is low and alleles differ strongly (Fig. 3D), and is likely to be detectable only in loss-of-expression alleles when $N_{TF,sat}$ is high (Fig. 3E). Detectability is higher in the $A_1A_2 \rightarrow BB$ case especially when $N_{TF,sat}$ is low (Fig. 3A). As $N_{TF,sat}$ increases (Fig. 3B and C), the region of low detectability broadens in the high-expression region (low $m$; Fig. 1F and G).

**Dominance in the 3-locus pathway.** In the 3-locus pathway $AA \rightarrow BB \rightarrow CC$ (see SI Appendix), we find that dominance in $A_1$ and $A_1$ propagates to affect the expression of locus C ($\theta_C$). It is modifiable by polymorphism in $B_1$ and $B_2$, but is difficult to detect unless expression of the B locus ($\theta_B$) is low enough that $N_{TF,B}$ is well below saturation or there is significant binding mismatch in the BB$\rightarrow$CC step.

**Discussion**

We find that dominance emerges in regulatory genetic pathways due to competitive molecular interactions between transcription-factor variants as they bind to their shared promoters. Alleles with higher competitive ability are inevitably dominant with respect to their contributions to fractional occupancy. Dominance effects extend to expression of downstream loci in multi-step pathways, and polymorphism therein generates genetic background effects. However, this form of dominance is likely to be phenotypically
detectable only when TF concentrations or binding strengths are in the range where gene expression levels differ measurably between homozygotes. We discuss each of these properties and their implications.

**Binding dominance: a new mechanism for dominance.** Competition between transcription factors for binding to the promoter sites they regulate (eq. 2; the A_1A_2→BB interaction) represents a novel source of dominance at the molecular level. The strength of the dominance depends on the biophysical properties of the interaction between TF molecules and the promoter sites to which they bind. When TF variants differ in their binding affinities ($-\Delta G$), the variant with higher affinity is dominant (Fig. 2A-C). Dominance of the competing TF variants is also sensitive to overall TF availability for binding ($N_{TF}$; Fig. 2D and E). This is because when $N_{TF}$ is low, fractional occupancy is likewise low and there is little competition at the binding site; the allelic effects approach additivity. Conversely, at high $N_{TF}$, the tighter-binding TF variant dominates fractional occupancy, driving expression. In contrast, polymorphism at the downstream cis-regulatory site (AA→_1B_2B) cannot contribute to dominance. This is because expression of the cis-regulated gene product, or respectively the TF variant, proceeds independently for each allele and sums to produce overall expression.

Binding dominance differs from the type of dominance that arises in metabolic pathways, which we call *flux dominance,* though the mechanisms of both are rooted in the biophysics of molecular interactions. In enzymes embedded in metabolic pathways, dominant alleles have higher rates of catalysis ($k_{cat}$), thus producing a higher flux from substrate to product, and the degree of dominance is proportional to the difference in $k_{cat}$ values (3-5). Flux dominance is sensitive to substrate saturation of the enzyme (19), analogous to the way $N_{TF}$ affects the degree of binding dominance through fractional occupancy. Flux dominance doesn’t explain the effects of mutations at regulatory loci (5) because regulatory pathways don’t experience flux.

*Feedback dominance* results from cases where a gene product autoregulates its expression. Omholt et al. (7) analyzed feedback dominance using the biophysically relevant Hill (20) equation that permits serially repeated promoter-site sequences; they considered only cases that lacked polymorphism in the TF coding region. Gene products could
regulate either their own promoters (in our notation, $1A_2A \rightarrow 1A_2A$) or the promoters of an upstream TF ($1A_2A \rightarrow 1B_2B \rightarrow 1A_2A$). These pathways resemble the $1A_2A \rightarrow 1B_2B$ and $1A_2A \rightarrow 1B_2B \rightarrow CC$ (see SI Appendix) cases for which we find dominance, suggesting that feedback dominance may ultimately prove to be a special case of binding dominance. To our knowledge, the effects of polymorphism in the coding regions, thus competitive binding, on feedback dominance remain unexplored.

**Diffusion dominance** arises in network-based regulation of ontogenetic diffusion gradients, including morphogen concentrations, their diffusion and decay rates, and the threshold concentrations necessary to initiate a phenotypic response (8). Allelic variation affecting any of these components can show dominance in network output. While we have presented our model in the context of TF-promoter interactions, its principles apply broadly to interactions between any genetically determined, interacting regulatory molecules. Our simple regulatory pathways represent elements in these more complex diffusion-based networks, and we expect that dominance due to competitive binding will be inherent in them.

**Detectability and cryptic dominance.** Biophysical conditions that lead to especially high or low fractional occupancies, respectively the bottom left and top right corners of the G-P maps (Fig. 1B–J), can mask dominance because the two homozygotes have very similar phenotypes. This can occur when $m$ is similar for both alleles, or when allele-specific $N_{TF}$ is either high enough to saturate the binding site, or low enough that the binding site is rarely occupied by either allele. Even strong dominance at the level of molecular interactions can remain cryptic (e.g., compare Fig. 2B–E to Fig. 3B–E). When $N_{TF, sat}$ is high, only complete knockout $2A$ alleles will be detectable as recessive (Fig. 3E) and moderate to strong dominance will likely be undetected. Likewise, when both alleles have similar binding affinities or concentrations, the alleles will be nearly codominant (diagonals of Fig. 2A–E), but all individuals will have nearly identical phenotypes (Fig. 3A–E). There, even polymorphism will be difficult to detect without genotyping; the degree of dominance may be of little practical importance in these cases anyway. This pattern is consistent with the long-standing observation that codominance at the phenotypic level appears rare in nature (2), although not in these cases because codominance is rare *per se*, but rather that it can be
difficult to detect that the locus is polymorphic. Nevertheless, assays of allele-specific binding affinities and expression levels (17) will uncover cryptic dominance in expression.

**Effects of genetic background.** Polymorphism in the genetic background can enhance, obscure, or even reverse binding dominance. There are two types of background effects in the 2-locus regulatory interaction and several more in the 3-locus pathway (see SI Appendix). In the 2-locus pathway, dominance of coding-site (A₁, A₂) alleles at the TF locus is unaffected by polymorphism in the *cis*-regulated locus (i.e., \( d_{A1A2}^{1B2B} = d_{A1A2}^{BB} \)). However, in the \( 1A_{12}A_2 \rightarrow BB \) case, where \( N_{TF} \) and \( m \) covary, dominance of coding-site TF variants is affected by polymorphism in their promoters (Fig. 2F) and vice versa (Fig. 3F). For a given binding-affinity (A₁A₂) heterozygote, dominance modification is asymmetrical, being more effective when the promoter of the tighter-binding allele (therefore its \( N_{TF} \)) is varied (Fig. 3F). In contrast, for a given \( N_{TF} \) (₁A₂A) heterozygote, changes in binding affinities of either allele have effects of similar magnitude (Fig. 2F).

In the 3-locus pathway (SI Appendix), dominance in locus A with respect to fractional occupancy of locus B (\( \theta_b \)) can propagate down the pathway, such that A alleles can show dominance with respect to expression of locus C (\( \theta_c \)) (SI Appendix, Fig. S1). However, this will be difficult to detect unless \( N_{TF,B} \) is well below \( N_{TF,B,sat} \), which requires that \( \theta_b \) for at least one of the B alleles be particularly low. In practice, only loss-of-expression/function mutations at locus A are likely to be detectably recessive with respect to \( \theta_c \). However, dominance of ₁A or A₁ on \( \theta_c \) becomes increasingly easier to detect as \( m_{B,\theta_c} \) increases. Polymorphism at the coding site of locus B modifies that dominance only negligibly.

Flux dominance is similarly sensitive to allelic substitutions that occur up to several steps removed along a metabolic pathway (3, 5). Bagheri-Chaichian (19) show that the downstream dominance effects are sensitive to enzyme saturation at intermediate steps, much as we see in binding-site saturation in regulatory pathways (SI Appendix, Fig. S1E-G). Feedback dominance likewise shows downstream effects (7) in pathways with the structure \( 1A_{2}A \rightarrow BB \rightarrow (1A_{2}A \& CC) \), i.e., where the product of locus B co-regulates a downstream locus C as well as upstream locus A. In this case, dominance of the A₁ allele is detectable in the expression of locus C. They did not directly assess attenuation of the
signal due to saturation at intermediate steps; rather, they excluded it by considering only cases where homozygotes showed differences >25%.

Binding dominance is likely to interact with flux dominance. When alleles B₁ and B₂ code for metabolic allozymes, flux dominance of B₁ can be modified in ₁A₂A→B₁B₂ or A₁A₂→B₁B₂ interactions, provided that regulatory changes in B’s expression levels affect enzyme saturation in the three B-locus genotypes. Polymorphism in both the promoter and product site of the B locus, i.e., the ₁A₂A→₁B₁₂B₂ and A₁A₂→₁B₁₂B₂ cases, can further influence B₁’s flux dominance by further changing relative allozyme concentrations. Conversely, changes in allozyme concentration or k_cat due to variation in ₂B or B₂ may modify, mask or expose binding dominance at ₁A or A₁ when d_A is assessed using genotype-specific fluxes in the metabolic pathway.

Beyond the regulatory pathway, transcription factors interact with other molecules in the cell that may be influenced by genetic background. These include direct interactions with proteins that regulate TF availability, spurious DNA and RNA binding sites, and indirect effects of physiological conditions such as pH (17). These affect the Nₜf/Nₜf_sat ratio but have negligible effect on dominance and its detectability; the isoclines of Fig. 2D and E and the detectability gradients in Fig. 3D and E are constant with respect to this ratio. However, dominance may be modified in cases where TF variants differ in their responses to the non-specific background (i.e., A₁–A₂ cases with properties closer to the ₁A₁–₂A₂ case). For analytical convenience in this study, these non-specific binding effects are subsumed into Nₜf (see parameter reduction in SI Appendix). The unreduced model of Tulchinsky et al. (15) may be necessary in the design and interpretation of experiments.

**Empirical studies.** Consistent with the competitive binding model, cis-site heterozygotes typically show additive expression whereas trans heterozygotes commonly show dominance (13, 21-25), although some cis-site polymorphisms show patterns of dominance as well (21, 26). Our modeling suggests the possibility that unidentified polymorphism in regulatory loci upstream may be involved in at least some of these exceptions. Motifs with variable numbers of binding-site repeats in the promoter region could also potentially produce binding dominance and even overdominance, as they do in feedback dominance (7).
Mueller et al. (17) review empirical work on the biophysics of fractional occupancy in regulatory interactions. Gene expression is highly correlated with fractional occupancy of TFs on their binding sites, as our model assumes. Site-specific mutagenesis, using a variety of techniques for measuring binding affinity at primary vs. secondary (likely to be spurious background) binding sites, reveals strong differences in binding affinity among artificial promoter-region alleles (1B and 2B alleles, in our notation). Some of these techniques are themselves based on measures of competitive binding among sites. Guar et al. (27) review studies demonstrating that TF and promoter-region alleles show significant patterns of allele-specific gene expression in diverse model organisms. Nevertheless, to our knowledge, allelic variation in TF binding affinity and concentration, in diverse genetic backgrounds, with respect to its effects on competitive binding and heterozygote gene expression remain to be studied.

ACKNOWLEDGMENTS. We thank C. Babbitt for valuable comments on the manuscript.

References
1. Mendel, G. J. 1866. Versuche über Pflanzen-Hybriden. Verhandlungen der naturforschenden Vereins in Brunn 4: 3–47. English translation in Blumberg, R. B., *MendelWeb* [http://www.mendelweb.org/Mendel.html](http://www.mendelweb.org/Mendel.html).
2. Wright, S. 1934. Physiological and evolutionary theories of dominance. American Naturalist 68: 24–53.
3. Kacser, H., and J. A. Burns. 1981. The molecular basis of dominance. Genetics 97: 639–666.
4. Keightley, P. D., and H. Kacser. 1987. Dominance, pleiotropy and metabolic structure. Genetics 117: 319–329.
5. Keightley, P. D. 1996. A molecular basis for dominance and recessivity. Genetics 143: 621-625.
6. Bagheri, H. C., and G. P. Wagner. 2004. Evolution of dominance in metabolic pathways. Genetics 1713–1635. DOI: 10.1534/genetics.104.028696
7. Omholt, S. W., E. Plahte, L. Øyehaug and K. Xian. 2000. Gene regulatory networks generating the phenomena of additivity, dominance and epistasis. Genetics 155: 969–990.
8. Gilchrist M. A, and H. F. Nijhout. 2001. Nonlinear developmental processes as sources of dominance. Genetics 159: 423–432.
9. Li, Y., K. Varala, S. P. Moose and M. P. Hudson. 2012. Inheritance pattern of 24 nt siRNA clusters in Arabidopsis hybrids is influenced by proximity to transposable elements. PLoS One 7(10): e47043.

10. Bond, D. M., and D. C. Baulcombe. 2014. Small RNAs and heritable epigenetic modification in plants. Trends in Cell Biology 24: 100–107.

11. Stupar, R. M., and N. M. Springer. 2006. Cis-transcriptional variation in maize inbred lines B71 and Mo17 leads to additive expression patterns in the F1 hybrid. Genetics 173: 2199–2210.

12. Hughes, K. A., et al. 2006. Segregating variation in the transcriptome: cis regulation and additivity of effects. Genetics 173: 1347–1355.

13. Wray, G. A. 2007. The evolutionary significance of cis-regulatory mutations. Nature Genetics 8: 206–216.

14. Gerland, U., J. D. Moroz, and T. Hwa. 2002. Physical constraints and functional characteristics of transcription factor-DNA interaction. PNAS 99: 12015–12020. (www.pnas.org/cgi/doi/10.1073/pnas.192693599)

15. Tulchinsky, A. Y., N. A. Johnson and A. H. Porter, 2014. Hybrid incompatibility arises in a sequence-based bioenergetic model of transcription factor binding. Genetics 198: 1155–1166.

16. Khatri, B. S., and R. A. Goldstein. 2015. A course-grained model of sequence evolution and the population size dependence of the speciation rate. J. Theor. Bio. 378: 56–64.

17. Mueller, F., T. J. Stasevich, D. Maza and J. D. McNally. 2013. Quantifying transcription factor kinetics: at work or at play? Critical Reviews in Biochemistry and Molecular Biology 48: 492-514. DOI: 10.3109/10409238.2013.833891

18. Wolfram Research, Inc. 2015. Mathematica version 10.3. Champaign IL.

19. Bagheri-Chaichian, H., J. Hermisson, J. R. Vainsys and G. P. Wagner. 2003. Effect of epistasis on phenotypic robustness in metabolic pathways. Math. Biosci. 184: 27–51.

20. Hill, A. V. 1910. The possible effect of the aggregation of the molecules of hemoglobin. J. Physiol. 40: IV–VIII.

21. Guo, M., et al. 2008. Genome-wide allele-specific expression analysis using massively parallel signature sequencing (MPSS™) reveals cis- and trans-effects of gene expression in maize hybrid meristem tissue. Plant Molecular Biology 66: 551–563.

22. Tirosh, I., S. Reikhav., N. Segal, Y. Assia, and N. Barkai. 2010. Chromatin regulators as capacitors of interspecies variations in gene expression. Molecular Systems Biology 6: 435.

23. Zhang, X., a. J. Cal, and J. O. Borevitz. 2011. Genetic architecture of regulatory variation in Arabidopsis thaliana. Genome Res 21: 725–733.

24. Gruber JD, Vogel K, Kalay G, Wittkopp PJ. 2012. Contrasting properties of gene-specific regulatory, coding, and copy number mutations in Saccharomyces cerevisiae: Frequency, effects, and dominance. PLoS Genet 8: e1002497.
25. Meiklejohn, C. D., J. D. Coolon, D. L. Hartl and P. J Wittkopp. 2014. The roles of cis- and trans-regulation in the evolution of regulatory incompatibilities and sexually dimorphic gene expression. Genome Research 24: 84–95.

26. Lemos B, Araripe LO, Fontanillas P, Hartl DL. 2008a. Dominance and the evolutionary accumulation of cis- and trans-effects on gene expression. Proc Natl Acad Sci 105: 14471–14476.

27. Guar, U., K. Li, S. Mai and G Liu. 2013. Research progress in allele-specific expression and its regulatory mechanisms. Journal of Applied Genetics 54: 271–283.
Figure legends

**Fig. 1.** Genotype-phenotype maps in the diploid regulatory pathway with competitive transcription factor (TF) binding. (A) Regulatory model: Locus A codes for the TF regulating locus B. Locus A can vary at its promoter (alleles $A_1$ & $A_2$), the coding region (alleles $A_1$ & $A_2$) or both; locus B varies only at the promoter ($B_1$ & $B_2$). The concentrations of the TF alleles ($N_{TF.1A}$ & $N_{TF.2A}$) are determined by their promoter sequences. Subscripts are dropped for homozygotes. (B-J) Genotype-phenotype maps, shown as density plots of fractional occupancy ($\theta$), equivalent to phenotype in this model ($\theta = P$). Rows: Three saturating TF concentrations ($N_{TF.sat}$) at maximal binding ($m_{A1,B} = m_{A2,B} = 0$). (B-D) Effects of allelic variation in TF concentration, scaled to $N_{TF.sat}$. (E-G) Effects of allelic variation in the TF coding region expressed as mismatch ($m$) with a homozygous cis-site promoter BB. (H-J) Effects of allele-specific variation in the cis-site and a homozygous TF. Isoclines throughout represent intervals of 0.1 and the black isocline represents $\theta = 0.5$.

**Fig. 2.** Genotype-dominance maps, shown as density plots. Dominance ($d$) is with respect to the $A_1$ allele; $A_1$ is dominant in the blue region and recessive in the red. (A-C) Dominance as a function of the degree of mismatch ($m$) between a homozygous cis site and competing TF-coding alleles $A_1$ and $A_2$, for three saturating TF concentrations ($N_{TF.sat}$); the tighter-binding allele (low $m$) is dominant. The circle in (B) has the same $m$ and $N_{TF}$ values as the circle in Fig. 3F. (D, E) Dominance as a function of expression level of the two TF alleles $A_1$ and $A_2$, expressed as a fraction of the saturating TF concentration ($N_{TF.sat}$). TF concentration is saturating ($N_{TF.1A} + N_{TF.2A} \geq N_{TF.sat}$) above the dotted diagonal line. The TF with greater expression is dominant. (F) Dominance as a function of the degree of mismatch in the heterozygous genetic background where TF alleles differ in concentration, for the case where $N_{TF.1A1} = N_{TF.sat}/2$ and $N_{TF.2A2} = N_{TF.sat}/10$. The orange crosses in this and panel D have the same $m$ and $N_{TF}$ values. Isoclines throughout represent intervals of 0.1 and the thicker white isocline denotes $d = 0.5$.

**Fig. 3.** Genotype-dominance maps, shown as density plots. (A-E) Detectability of the dominance patterns in corresponding panels of Fig. 2A-E, represented by white opacity masks. Detectability increases as opacity decreases. (F) Effect of allele-specific concentration in TF in a heterozygous genetic background, where the TF’s coding region is heterozygous ($m_{A1,B}=0.1$, $m_{A2,B}=0.01$).
The circle has the same $m$ and $N_{TF} \text{sat}$ values as the circle in Fig. 2B. Isoclines throughout represent intervals of 0.1 and the thicker white isocline denotes $d = 0.5$. 

$m_{2A_{23}B} = 0.2, \; N_{TF \text{ sat}} = 100$.)
Figures:

Fig. 1:

Fig. 2:

Fig. 3:
**Supporting information**

**Parameter reduction in the biophysical model.** The biophysical model we analyze is a parameter-reduced version of the model in Tulchinsky et al. (15), developed from models of transcription-factor (TF) binding in the statistical physics literature (14). The haploid version of that model characterizes fractional occupancy of the TF on the promoter site it regulates as

\[ \theta = \frac{N'_{TF}}{N'_{TF} + \exp[-\Delta G + E_{diff}]} \]  

[S1]

where \( \theta \) is the fractional occupancy, \( N'_{TF} \) is the number of TF molecules, \( -\Delta G \) is the free energy of association between a TF molecule and promoter site, and \( E_{diff} \) is the difference between the free energy of association between a TF molecule to its primary binding site and its local environmental background, which may include the non-specific binding to the genomic background as well as inhibitors and other molecules in the nuclear matrix (17).

When \( E_{diff} < 0 \), the background is more attractive and fewer TF’s are available for gene regulation; when \( E_{diff} > 0 \), the target site is more attractive. Non-specific binding reduces the number of TF molecules in solution, making fewer available to interact with the specific binding site. We combine the \( E_{diff} \) parameter and their \( N'_{TF} \) into a single TF-availability term using \( N_{TF} = N'_{TF} \exp(-E_{diff}) \), where \( N_{TF} \) is the number of unencumbered TF molecules available for regulatory interactions, such that

\[ \theta = \frac{N_{TF}}{N_{TF} + \exp[-\Delta G]} \]  

[S2]

Gerland et al. (14) estimated that \( E_{diff} = \sim 0 \) or a little less, so in practice \( N_{TF} = \sim N'_{TF} \) unless \( N'_{TF} \) is very small.

The bioenergetic model represents the interacting TF molecules and promoter sequence as strings of bits, where binding decreases with \( m' \), the number of mismatching bits. The second parameter modification we use is define a fractional mismatch parameter \( m = m'/n \), where \( n \) is the bitstring length. Therefore, our \( -\Delta G_1 \) is equivalent to \( -n \Delta G_1 \) of Tulchinsky et al. (15). For resolution in our density plots, we treat \( n \) as an arbitrarily large, finite integer. Reducing \( n \) would increase their pixilation by averaging over blocks of area \( 1/n^2 \), without affecting the conclusions.
Propagation of dominance and genetic background effects in 3-locus pathways.

We show in the body of the article that dominance of $A_1$ and $A_2$ occurs with respect to expression of locus $B$ ($\theta_b$). In a longer pathway, where locus $B$ drives expression of locus $C$ ($\theta_c$), we expect locus $A$’s dominance to propagate, such that it is detectable with respect to $\theta_c$. Here we show the extent and limitations of that propagation.

Fig. S1A shows the 3-locus pathway. Locus $B$ codes for a TF that binds to the promoter of locus $C$, such that there are two regulatory steps, $A \rightarrow B$ and $B \rightarrow C$. The promoter and product sites of locus $B$ are assumed not to recombine, such that the double-heterozygous $B$ genotype is $B_1B_2$. Competitive binding of the $A$ alleles onto the two $B$ alleles proceeds independently, creating two fractional occupancy terms, $\theta_{B_1}$ and $\theta_{B_2}$, based on eq. 2. Expression of the $B$ alleles yields separate $N_{TF,B_1}$ and $N_{TF,B_2}$ values, which we calculate as $N_{TF,B_1} = \theta_{B_1}N_{TF,sat}/2$ and $N_{TF,B_2} = \theta_{B_2}N_{TF,sat}/2$, such that maximal expression of the $B$ locus yields $N_{TF,sat}$. Eqs. 2 and 3, with appropriate subscripts, are used to calculate $\theta_c$. For simplicity we assume $N_{TF,sat}$ is the same for both regulatory steps.

Results

Genotype-phenotype map: The G-P map of the $A_2A\rightarrow BB\rightarrow CC$ case with $N_{TF,sat} = 10$ (Fig. S1B) is a steeper version of the $A_2A\rightarrow BB$ map (Fig. 1B), such that expression of locus $C$ is nearly maximal unless $N_{TF,A}$ is very low for both $A$ alleles. Higher values of $N_{TF,sat}$ (not shown) have such steep GP maps at low $N_{TF,A}$ that only virtual double-knockout $A_2A$ genotypes are able to block C-locus expression. The G-P map for $A_1A_2\rightarrow BB\rightarrow CC$ (Fig S1C shows the $N_{TF,sat} = 10$ case) takes the same form as the $A_1A_2\rightarrow BB$ map (Fig. 1E), but greater mismatch is required for an equivalent reduction of $\theta_c$. At higher $N_{TF,sat}$ (not shown), the region of low expression becomes increasingly confined to the top right corner, where $m_{A \rightarrow B}$ is high for both $A$ alleles; the shape plateaus and squares off as it does for the $A_1A_2\rightarrow BB$ maps (Fig. 1E-G).
Propagating dominance in fractional occupancy to downstream loci.

Using a three-locus linear pathway (Fig. S1A), we assessed dominance of the $A_1$ and $A$ sites with respect to expression of locus $C$ ($\theta_C$). Both showed dominance. In the $A_1A_2\rightarrow BB\rightarrow CC$ case, the transition from dominant to recessive lies parallel to the $m_{A_1\rightarrow A_2} = m_{A_2\rightarrow A_1}$ diagonal (Fig. S1D), and increasing $N_{TF, sat}$ steepens the transition (Fig. S2F). Dominance in the $A_2A\rightarrow BB\rightarrow CC$ case is so similar to that of the $A_2A\rightarrow BB$ case of Fig. 3D that a separate figure is unnecessary.

Although this dominance in fractional occupancy is inevitable at the molecular level, it will be difficult to detect in the phenotype unless $\theta_C$ is low, i.e., when $m_{BC} = 0$ and $N_{TF, sat}$ is low (Figs. 4F and G).

Dominance of $A$ with respect to $\theta_C$ can be modified by allelic variation elsewhere in the pathway. In the $A_1A_2\rightarrow B_1B_2\rightarrow CC$ case, $\theta_C$ is reduced relative to the $A_1A_2\rightarrow BB\rightarrow CC$ case. This simultaneously weakens dominance provided that $m_{B_1\rightarrow C}$ and $m_{B_2\rightarrow C}$ are both high, and makes that dominance more detectable (Fig. S2B). In contrast, dominance in the $A_2A\rightarrow B_1B_2\rightarrow CC$ case (Fig. S2E) is unaffected relative to the $A_2A\rightarrow BB\rightarrow CC$ case (and therefore relative to the $A_2A\rightarrow BB$ of Fig. 3D); the primary effect is to reduce its detectability.

Variation in $B$’s promoter has little or no modifying effect. In the $A_2A\rightarrow B_1B_2\rightarrow CC$ case, polymorphism in the B-locus promoter reduces the dominance only negligibly even when detectability is high (Fig. S2C), and it has no effect in the $A_1A_2\rightarrow B_1B_2\rightarrow CC$ case (not shown). The effect of variation in the C-locus promoter is negligible ($A_1A_2\rightarrow BB\rightarrow C_2C$) or absent ($A_1A_2\rightarrow BB\rightarrow C_2C$) (not shown).

Nevertheless, detection of dominance of $A$ alleles with respect to $\theta_C$ is likely to be difficult under some conditions. In the $A_1A_2\rightarrow BB\rightarrow CC$ case with maximal binding in the $B\rightarrow C$ interaction ($m_{BC} = 0$) and $N_{TF, sat} = 10$, dominance becomes detectable only when binding for one $A$ allele is low (e.g., high $m_{A_2\rightarrow B}$; Fig. S1E). At high $N_{TF, sat}$ (Fig. S1F), only very weakly binding alleles are detectably recessive. The effect is somewhat more extreme in the $A_2A\rightarrow BB\rightarrow CC$ case (Fig. S1G), where only knockout alleles are detectably recessive even at low $N_{TF, sat}$. However, in all cases, detectability increases with $m_{B\rightarrow C}$ because $\theta_C$ drops below
the level of saturation and expression differences between A-allele homozygotes become detectable.

Discussion
In the 3-locus pathway, dominance in locus A with respect to fractional occupancy of locus B ($\theta_B$) can propagate down the pathway, such that A alleles can show dominance with respect to expression of locus C ($\theta_C$) (Fig. S1). However, this is more difficult to detect unless $\theta_B$ for at least one of the B alleles is particularly low. This is because $\theta_B$ determines $N_{TF,B}$, and $N_{TF,B}$ of at least one B allele must be well below saturation in order for A1’s dominance to be detectable in $\theta_C$ (Fig. S1D and E). For $\theta_B$ to be low, binding affinity and/or $N_{TF}/N_{TF,sat}$ must be fairly low at the promoters of both A alleles, especially when $N_{TF,A,sat}$ is high, and in practice, only loss-of-expression/function mutations at locus A are likely to be detectably recessive with respect to $\theta_C$. However, dominance of A1 or A1 on $\theta_C$ becomes increasingly easier to detect as $m_{B,A,C}$ increases (Fig. S2E). Nevertheless, polymorphism at the coding site of locus B or the promoter of locus C modifies the degree of that dominance only negligibly (Fig. S2E) if at all.
Figure legends

**Fig. S1.** Downstream effects of dominance in the 3-locus pathway: dominance of locus A with respect to expression at locus C. (A) The pathway. (B-C) Genotype-phenotype maps with $N_{TF.sat} = 10$. (B) $1_A2_A \rightarrow BB \rightarrow CC$, with $m_{A_2B} = m_{B_2C} = 0$. (C) $1_A2_A \rightarrow BB \rightarrow CC$, with $m_{B_2C} = 0$. (D-F) Genotype-dominance maps of $1_A2_A \rightarrow BB \rightarrow CC$, with $m_{B_2C} = 0$. (D) $N_{TF.sat} = 10$. (E) Detectability at $N_{TF.sat} = 10$ using an opacity overlay as in Fig. 3. (F) Same, with $N_{TF.sat} = 100$. Isoclines throughout represent intervals of 0.1.

**Fig. S2.** Downstream effects of dominance in the 3-locus pathway: dominance of locus A with respect to expression at locus C, overlaid by opacity masks that hide regions with low detectability of dominance. (A-B) Genotype-dominance maps of $1_A2_A \rightarrow B_1B_2 \rightarrow CC$. (A) $m_{A_1B_1} vs. m_{A_2B_2}; N_{TF.sat} = 10; m_{B_1C} = 0; m_{B_2C} = 0.9$. (B) $m_{A_1B_1} vs. m_{A_2B_2}; N_{TF.sat} = 1000; m_{B_1C} = 0.5; m_{B_2C} = 0.5$. (C-D) Genotype-dominance maps of $1_A2_A \rightarrow B_1B_2 \rightarrow CC$, with $N_{TF.1A} = 5$, $N_{TF.2A} = 0.05 \ast 5 = 0.25$, $m_{B_2C} = 0$. (C) $m_{A_2B_1} vs. m_{A_2B_2}; N_{TF.sat} = 10; (D) m_{A_2B_1} vs. m_{A_2B_2}; N_{TF.sat} = 100$. (E) Genotype-dominance map of $1_A2_A \rightarrow B_1B_2 \rightarrow CC; N_{TF.1A} vs. N_{TF.2A}; N_{TF.sat} = 10; m_{B_1C} = 0; m_{B_2C} = 0.9$. (F) Genotype-dominance map of $1_A2_A \rightarrow BB \rightarrow CC$, with $m_{B_2C} = 0$ and $N_{TF.sat} = 100$. Isoclines throughout represent intervals of 0.1.
Supplemental Figures:

Fig. S1:

Fig. S2: