Genetic Flip-Flop without an Accompanying Change in Linkage Disequilibrium

In a recent issue of the *American Journal of Human Genetics*, Lin et al.[1] showed that a change in linkage disequilibrium (LD) between a causal (B) and the observed (A) variants can lead to a reversal of genetic effect between two studies (a flip-flop). In this report, we show that a flip-flop can occur without a change in LD, even under simple noninteractive models. Further, we examine interactive models that allow for a flip-flop to take place under linkage equilibrium (LE). We provide specific conditions for the form of A and B interaction that permits a zero LD flip-flop, examine the behavior of allelic variance in the case of quantitative traits, and discuss potential implications of the findings for association mapping.

First, we consider a binary trait, $\mathcal{Y}:\{\mathcal{Y}_D,\mathcal{Y}_N\}$, where $\mathcal{Y}_D$ indicates a condition, such as the presence of disease. As in Lin et al.,[1] we will assume that the trait is influenced by two genetic variants, A:$\{A_1,A_2\}$ (observed locus) and B:$\{B_1,B_2\}$ (unobserved locus), and that there is no confounding. For simplicity, we disregard dominance effects, thereby allowing an essentially haploid treatment of the problem.[2] The two-locus penetrance values are given by $\mathbf{M}$ and population frequencies of the haplotypes are given by $\mathbf{P}$ (see Table 1). This uses the notation $\mu_{A_iB_j} = \Pr(\mathcal{Y}_D|A_i,B_j)$ for the entries of $\mathbf{M}$. Among those with a particular allele, the expected proportion of individuals who will develop the condition is

$$\mu_{A_1} = \Pr(\mathcal{Y}_D|A_1) = \frac{\mu_{A_1B_1}p_{A_1B_1} + \mu_{A_1B_2}p_{A_1B_2}}{p_{A_1B_1} + p_{A_1B_2}} \tag{1}$$

and

$$\mu_{A_2} = \Pr(\mathcal{Y}_D|A_2) = \frac{\mu_{A_2B_1}p_{A_2B_1} + \mu_{A_2B_2}p_{A_2B_2}}{p_{A_2B_1} + p_{A_2B_2}}. \tag{2}$$

The relative risk is

$$RR = \frac{\mu_{A_1}}{\mu_{A_2}} = \frac{(\mu_{A_1B_1}p_{A_1B_1} + \mu_{A_1B_2}p_{A_1B_2})(p_{A_2B_1} + p_{A_2B_2})}{(\mu_{A_2B_1}p_{A_2B_1} + \mu_{A_2B_2}p_{A_2B_2})(p_{A_1B_1} + p_{A_1B_2})}. \tag{3}$$

The RR remains the same if $\mathbf{M}$ is multiplied by a constant. A flip-flop takes place whenever there is a change in sign of $\mu_{A_1} - \mu_{A_2}$. For a given penetrance configuration, $\mathbf{M}$, a flip-flop point is defined by a combination of haplotype frequencies such that $\mu_{A_1} = \mu_{A_2}$ (or equivalently, RR = 1).

Flip-Flop under a Constant LD

The simplest flip-flop case is the "proxy model," in which the locus A has no functional significance but is related to the locus B only via LD. The penetrance array for the proxy model has the form $\mathbf{M} = \{x, y, x, y\}$. In this case, it is necessary that the LD sign should change for a flip-flop to occur, as can be shown by writing the haplotype frequencies in terms of the LD coefficient (D) and solving $\mu_{A_1} = \mu_{A_2}$ for D. This calculation results in $D = 0$; thus, the proxy model most closely corresponds to the findings of Lin et al.[1] Equations 1 and 2 show that for a given penetrance configuration, allelic effects are functions of haplotype frequencies. Thus, the driving force of flip-flip is a change in haplotype frequencies. There are multiple frequency configurations that can result in the same LD, and a flip-flop is not necessarily accompanied by a change in the LD. As an example, consider a simple noninteractive penetrance model of additive contributions by the two loci, $\kappa_{A_1} = 0.2$, $\kappa_{A_2} = 0.3$, $\kappa_{B_1} = 0.4$, and $\kappa_{B_2} = 0.1$, with haplotype effects given by $\mathbf{M} = \{\kappa_{A_1} + \kappa_{B_1}, \kappa_{A_1} + \kappa_{B_2}, \kappa_{A_2} + \kappa_{B_1}, \kappa_{A_2} + \kappa_{B_2}\}$. In this model, a flip-flop is possible without a change in LD. When $\mathbf{P} = \{0.075, 0.01, 0.25, 0.665\}$, the association is positive; $\mu_{A_1} = 0.56$ versus $\mu_{A_2} = 0.48$. A switch of population frequencies to $\mathbf{P} = \{0.075, 0.25, 0.01, 0.665\}$ leads to a flip-flop as follows: $\mu_{A_1} = 0.37$ versus $\mu_{A_2} = 0.40$. In both cases, the LD is the same: $r_{AB} = 0.36$, $D' = 0.83$, $D = 0.05$, where the correlation r_{AB} and D' are the usual LD standardizations.[3,4]

Zero LD Flip-Flop

The population haplotype frequencies under LE are given by $\mathbf{P} = \{p_{A_1}p_{B_1}, p_{A_1}p_{B_2}, p_{A_2}p_{B_1}, p_{A_2}p_{B_2}\}$, where $p_{A_2} = 1 - p_{A_1}$ and $p_{B_2} = 1 - p_{B_1}$. The penetrance values and the relative risk at the observed locus are given by

$$\mu_{A_1} \overset{\text{LD}=0}{=} \mu_{A_1B_2} + p_{B_1}(\mu_{A_1B_1} - \mu_{A_1B_2}), \tag{4}$$

$$\mu_{A_2} \overset{\text{LD}=0}{=} \mu_{A_2B_2} + p_{B_1}(\mu_{A_2B_1} - \mu_{A_2B_2}), \tag{5}$$

and

$$\text{RR} \overset{\text{LD}=0}{=} \frac{\mu_{A_1B_2} + p_{B_1}(\mu_{A_1B_1} - \mu_{A_1B_2})}{\mu_{A_2B_2} + p_{B_1}(\mu_{A_2B_1} - \mu_{A_2B_2})}. \tag{6}$$

Note that these quantities do not depend on the frequencies of the observed locus, A. Whether any particular $\mathbf{M}$ is permitting a flip-flop under LE is determined by the solution of RR = 1 for p_{B_1} (which defines the flip-flop point). This value is given by

$$p_{B_1}^{(\text{RR}=1)} = \frac{1}{1 + \frac{\mu_{A_2B_1} - \mu_{A_1B_1}}{\mu_{A_1B_2} - \mu_{A_2B_2}}}. \tag{7}$$

For an effect reversal, this value has to be inside the (0, 1) interval. An equivalent condition is $\text{sign}(\mu_{A_2B_1} - \mu_{A_1B_1}) = \text{sign}(\mu_{A_1B_2} - \mu_{A_2B_2})$. The condition implies that the effect of A_1 has to be reversed when the background of B is switched

Table 1. Two-Locus Penetrance Values and Population Frequencies of the Haplotypes

Haplotype	P	M
A_1B_1	$p_{A_1B_1}$	$\mu_{A_1B_1}$
A_1B_2	$p_{A_1B_2}$	$\mu_{A_1B_2}$
A_2B_1	$p_{A_2B_1}$	$\mu_{A_2B_1}$
A_2B_2	$p_{A_2B_2}$	$\mu_{A_2B_2}$

from B_1 to B_2. Two examples of such penetrance configurations are shown in Table 2, in which the values (δ_1, δ_2) of the same sign can be considered to be deviations from the "base values" (x, y). The $\mathbf{M}_2$, with some constraints on δ_1, δ_2, is an example of a "yin-yang" model, in which dissimilar haplotypes have similar susceptibilities. This model has been considered in several publications in the context of association mapping.[5–8]

The relations for the binary phenotype discussed above remain the same for the case when $\mathcal{Y}$ is quantitative. For example, the μ_{A_1},μ_{A_2} become the conditional expected values $\mu_{A_1} = E(\mathcal{Y}|A_1)$ and $\mu_{A_2} = E(\mathcal{Y}|A_2)$. These are given by the same formulas as before. The "relative risk" becomes the ratio of the two allelic means. An additional quantity of interest in the case of a quantitative trait is the allele-specific variance:

$$V_{A_1} = \frac{V_{A_1B_1}p_{A_1B_1} + V_{A_1B_2}p_{A_1B_2}}{p_{A_1B_1} + p_{A_1B_2}} + \frac{p_{A_1B_1}(\mu_{A_1B_1} - \mu_{A_1})^2 + p_{A_1B_2}(\mu_{A_1B_2} - \mu_{A_1})^2}{p_{A_1B_1} + p_{A_1B_2}} \quad (8)$$

where $V_{A_iB_j} = \mathrm{Var}\ (\mathcal{Y}|A_iB_j)$, with a similar expression for A_2. Assuming a common underlying variance, $\sigma^2 = V_{A_1B_1} = V_{A_1B_2} = V_{A_2B_1} = V_{A_2B_2}$, and LE, the allele-specific variances become:

$$V_{A_1} \overset{LD=0}{=} \sigma^2 + p_{B_1}(1 - p_{B_1})(\mu_{A_1B_1} - \mu_{A_1B_2})^2$$
$$V_{A_2} \overset{LD=0}{=} \sigma^2 + p_{B_1}(1 - p_{B_1})(\mu_{A_2B_1} - \mu_{A_2B_2})^2$$

As a function of p_{B_1}, the ratio of the variances under LE reaches the maximum or the minimum at $p_{B_1} = 0.5$. As a function of the joint effects of A and B, the variances V_{A_1} and V_{A_2} are unequal as long as $(\mu_{A_1B_1} - \mu_{A_1B_2})^2 \neq (\mu_{A_2B_1} - \mu_{A_2B_2})^2$. This condition excludes models in which A is a nonfunctional locus. Thus, under LE, $V_{A_1} \neq V_{A_2}$ requires that the A is not merely a proxy for the B but has a functional involvement. This argument assumes that there is no confounding; this is the same assumption that we would make when comparing allelic effects. Under

Table 2. Two Examples of Penetrance Configurations

Haplotype	$\mathbf{M}_1$	$\mathbf{M}_2$
A_1B_1	x	x
A_1B_2	y	y
A_2B_1	$x + \delta_1$	$y \pm \delta_1$
A_2B_2	$y - \delta_2$	$x \pm \delta_2$

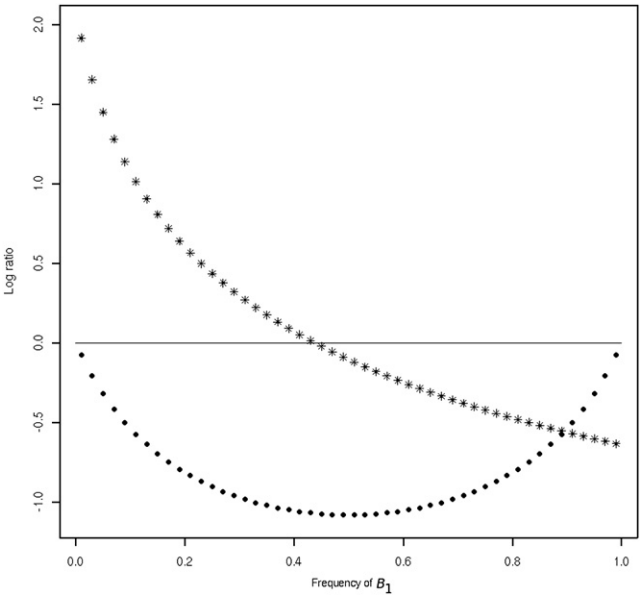

Figure 1. Values for $\ln(\mu_{A_1}/\mu_{A_2})$ and $\ln(V_{A_1}/V_{A_2})$ for a Zero-LD Model

Values for $\ln(\mu_{A_1}/\mu_{A_2})$ are indicated by the line of asterisks, and values for $\ln(V_{A_1}/V_{A_2})$ are indicated by the line of filled black dots. ($\mathbf{M}$) = 10 × {0.5, 0.4, 0.95, 0.05} and $\sigma^2 = 10$.

LE, the ratio V_{A_1}/V_{A_2} approaches 1 as p_{B_1} approaches either 0 or 1. Figure 1 illustrates the behavior of the ratio of allelic means and allelic variances at the observed locus, under LE, as a function of the unobserved allele frequency p_{B_1}. The variance contrast is strongest around the flip-flop point where the mean effect of A cannot be detected.

The flip-flop condition under LE between the studied and the unobserved variants has important implications outside of observational studies. Suppose that A_1, A_2 are levels of a factor under investigation, and these levels might be introduced in an interventional study on a random background of an unobserved B. A possible scenario is a study of efficacy of a drug A_1 on a condition in the presence of genetic effects of the locus B. In an interventional study, a possible correlation between A and B is removed via randomization. Suppose an effect of A_1 is claimed by a randomized study. Our analysis shows that in the presence of population heterogeneity with respect to the p_{B_1}, there might be an effect reversal in a different study. Some studies could report that there is no effect of A at all, despite the importance of A at specific categories of B.

In the case of a quantitative trait, the allele-specific variances can be compared, in addition to the usual comparison of the means. In both types of comparisons, μ_{A_1} versus μ_{A_2} and V_{A_1} versus V_{A_2}, the unknown factor, B, can either be genetic or environmental. According to our analysis, in the absence of correlation between the A and the B, the allelic variances are unequal only in the presence of a functional involvement of the A. In this regard, interpretation of a comparison of the allelic variances is similar to interpretation that follows from the usual comparison of the allelic means. Rejection of the hypothesis $H_0 : V_{A_1} = V_{A_2}$ leads to

a similar claim as does the rejection of the hypothesis $H_0 : \mu_{A_1} = \mu_{A_2}$; yet, the variance contrast might be substantial when the usual mean difference is undetectable. In either case, one can claim that the locus *A* is either directly associated with the trait or that it is a marker associated via correlation with an unobserved factor, *B*. When potential confounding due to population stratification is not an issue, the latter case leads to a standard claim that there is a nearby causal locus *B* correlated with the marker *A* via LD.

A practical question remains: How do we distinguish a genuine flip-flop from a statistical artifact? Our analysis shows that the underlying mechanism of a flip-flop is a change in the *AB* haplotype frequencies or, in the case of a zero-LD flip-flop, in the allele frequencies of *B* between populations. Examples can be constructed where both the allele frequency of the observed variant as well as the population prevalence of the trait ($\mathbf{M} \cdot \mathbf{P}$) remain the same across populations, despite the flip-flop. Nevertheless, these are contrived situations that take place only at specific values of the four haplotype frequencies. Thus, a flip-flop is usually accompanied by a change in the population prevalence and in the case of a nonzero LD, by a change in the frequency of the observed variant as well. There would be a higher confidence that the flip-flop is genuine in those cases where studied populations are of distinct ancestry, with evidence of allele-frequency differences at many loci. In addition, we suggest that in the case of a quantitative trait, the allelic-variance contrast can be examined. This contrast can be informative even at the flip-flop point, where no allelic effect can be detected. If normality of the trait can be assumed, the variance contrast provides an independent evidence that the studied variant has a genetic involvement, either as a LD proxy for causal variation or as a part of a functional unit. A significant allelic-variance contrast in both samples that exhibit a flip-flop may serve as an additional evidence for a genuine genetic association. Statistical tests for comparison of allelic and haplotypic variances will be detailed in a subsequent paper.

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Response to Zaykin and Shibata

Opposite directions of association of the same allele with disease in different populations (i.e., the flip-flop phenomenon) complicate the interpretation of association findings. We recently reported that variation in linkage disequilibrium (LD) or interlocus correlation in the context of multilocus effects may lead to flip-flop associations.[1] In the current issue of the *Journal* Zaykin and Shibata report that the flip-flop phenomenon may also be observed when there is constant LD, even without interactive multilocus effects, or when there is no LD for certain interactive disease models.

Zaykin and Shibata show how a flip-flop can occur in the case of constant LD with an example in which the frequencies of two haplotypes (i.e., A_1B_2 and A_2B_1) are switched in two populations, resulting in the same level of LD, but a reversal of the effect of allele A_1 in the two populations. This occurs because the effect of A_1 is a weighted sum of the haplotype effects over alleles at the B locus. The weights change in the two populations with different haplotype-frequency configurations. This example represents a special case in which haplotype frequencies differ significantly but LD remains the same. This may be the exception rather than the rule when haplotype frequencies diverge. Nevertheless, this example correctly demonstrates that it is differences in haplotype-frequency configuration, not