Supplementary Text S2: Effects – interacting, independent or otherwise

A key issue in defining and interpreting genetic interaction – epistasis – is understanding what is meant by the 'effect' of a locus, and what is meant by 'independent' effects of several loci. These concepts were first introduced in genetics by Bateson et al. [1] who described the concept of a character (phenotype) produced by the meeting of two distinct genetic factors, without using the specific terms 'interaction', 'epistasis', 'epistacy' or 'epistatic'. Subsequently, Bateson [2] used the term 'interaction' to describes this concept in the situation where one factor is not visible unless the other is also present, and the term 'epistatic' [3] [4] to describe this concept in the context of one factor preventing another from manifesting its effect. This terminology may perhaps originate from an earlier paper by Gadow [5] who used the term 'epistasis' in the context of arrested development in lizards, citing a German paper by Eimer [6] as the origin of the term.

The Batesonian concept of epistasis can be described in relation to tables such as the one shown below:

		Locus C		
	Genotype	c/c	c/C	C/C
Locus B	b/b	White	Brown	Brown
	b/B	Black	Brown	Brown
	B/B	Black	Brown	Brown

This table shows the coat colour in mice that results from a specific combination of two genetic factors. Note that here there is a clear (prior) understanding that the 'baseline' (reference point) genotype is the wild-type combination (b/b, c/c) which displays a phenotype of no colour (i.e. white), and that the effect of allele B at locus B is to change the color to black, while the effect of allele C at locus C is to change the colour to brown. Therefore, the modifying alleles at the different loci not only have different 'effects' but they also lead to different phenotype manifestations (black/brown) – meaning that which locus is operating can be determined directly by looking at the phenotype. This situation is perhaps somewhat analagous to consideration of biochemical interactions between proteins, where the function of each protein differs and has been well-established *a priori*.

Given well-defined effects such as these, the obvious question is what happens when modifiying

alleles at both loci are present. One might speculate as to what one might *expect* to happen if the alleles continued to act 'independently' – would the coat colour perhaps be mottled? In the table above we see that this does not happen; the alleles at locus C take precedence and locus C is said to be epistatic to locus B (or, more precisely, allele *C* at locus C is said to be epistatic to allele *B* at locus B).

Things became confused when Fisher [7] used the terms 'epistacy' and 'epistatic' to describe an apparently rather different concept, defined in terms of linear effects on a quantitative trait, much closer to the concept of statistical interaction described in Box 1. Indeed, R.C. Punnet pointed out this apparent difference in concept in his review of Fisher's paper [8]. Subsequently, the terms 'epistasis', 'epistacy', 'epistasy' or 'epistatic' seem to have been used more-or-less interchangably, but with potentially different implied meanings. In the quantitative genetics literature [9] (and more recently the human complex genetic disease literature) the usage seems to have mostly stemmed from Fisher's definition i.e. a statistical interaction signifying departure from linear effects with respect to prediction of a trait outcome, whereas biologists and biochemists have mostly used functional definitions closer in form to Batesonian epistasis.

The classical quantitative genetics formulation takes several different forms depending on the reference point and inbred line in question [9] [10] [11]; one common form is the F_∞ model shown below:

		Locus C		
	Genotype	c/c	c/C	C/C
Locus B	b/b	$\mu - a_b - a_c$	$\mu - a_b + d_c$	$\mu - a_b + a_c$
	b/B	$\mu + d_b - a_c$	$\mu + d_b + d_c + i_{dd}$	$\mu + d_b + a_c + i_{da}$
	B/B	$\mu + a_b - a_c$	$\mu + a_b + d_c + i_{ad}$	$\mu + a_b + a_c + i_{aa}$

This table shows the expected quantitative trait value for each genotype combination. In human genetics, rather than tabulating expected quantitative trait values, one might tabulate the expected log-odds or penetrance values as described in Supplementary Text S1. For simple Mendelian disorders, one would anticipate that the pentrances values should all be either 0 or 1, leading to penetrance tables such as:

		Locus C		
	Genotype	c/c	c/C	C/C
	b/b	0	0	1
Locus B	b/B	0	0	1
	B/B	1	1	1

The table above has classically been considered to represent a heterogeneity or non-epistatic model [12] (since one can aquire the disease through having the high-risk genotype at either or both loci) but note that this interpretation depends crucially on what we consider the 'effect' of each locus to be [13]. Although a 0/1 penetrance classification might seem at first sight to be similar to a categorical phenotype (as in the mouse coat colour example), in fact it is not completely equivalent since risk alleles at the two loci do not lead to different phenotype manifestations and so it is not clear which locus is actually 'causing' the phenotype; in a sense, for each cell, it is the genotype combination at both loci that 'causes' the disease. In practice, for complex diseases we do not expect to see pentrances values of 0 or 1, rather we expect a continuum of disease risks leading to penetrance tables such as:

		Locus C		
	Genotype	c/c	c/C	C/C
	b/b	0.1	0.2	0.2
Locus B	b/B	0.3	0.4	0.4
	B/B	0.3	0.4	0.4

Here, whether or not the loci 'interact' depends on what one defines the 'effect' of each locus to be. If one defines the 'effect' of a risk genotype at locus B to be the addition of a term 0.2 to the baseline pentrance, and the 'effect' of a risk genotype at locus C to be the addition of a term 0.1 to the baseline pentrance, then the loci above do not interact. If one defines the 'effect' of a risk genotype at locus B to be the multiplication of the baseline pentrance by a factor of 3, and the 'effect' of a risk genotype at locus C to be the multiplication of the baseline pentrance by a factor of 2, then the loci do interact (the non-interactive model would have values 0.6 instead of 0.4 in the table above). If one defined the 'effect' of a risk genotype at locus B to be the *conferring* of a penetrance value of 0.2 and the 'effect' of a risk genotype at locus B to be the *conferring* of a penetrance value of 0.3 then it is unclear what

the non-interactive model should be - perhaps the conferring of an average penetrance value of 0.25 instead of 0.4 in the relevant cells of the above table? Hence, depending on our definition of 'effect', and what we expect to observe if the effects operate 'independently', we may come to different conclusions concerning the presence or absence of interaction between the loci.

The relationship between linear statistical models for outcomes as observed in a population and 'effects' in terms of possible underlying biological causal mechanisms has been debated extensively in the epidemiological literature [14] [15] [16]. Of particular interest in this debate is the sufficient cause framework [17] [18] [19], in which it may be postulated that certain 'causes' of an outcome (e.g. disease) participate together in the same causal mechanism (resulting in so-called 'synergism'). Although departure from additivity with respect to a linear model defined on the absolute risk (as opposed to the log-odds) scale can, in some situations, allow one to conclude the presence of interaction or synergism in the sufficient cause sense [20], the assumptions and conditions required for this conclusion to hold are quite restrictive. It has been shown that, even if the assumptions of no unmeasured confounding and correct specification of the statistical model are met, interaction terms in statistical models do not, in fact, in general correspond to interaction or synergism in the sufficient cause sense [20].

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