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Recombinant inbred strain panels: a tool for systems genetics

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SYSTEMS BIOLOGY IS "the study of the interactions between the components of biological systems and how these interactions give rise to the function and behavior of that system" (ref. Wikipedia). Ideally the practice of systems biology is a cyclical process that alternates between stages of mathematical modeling and experimentation. A model is a quantitative description of the system. By solving the model, the "forward problem," we can make predictions about the behavior of the system that can be verified experimentally. Based on the outcome of the experiment, the model may be refined and the cycle begins again. In essence, systems biology is an approach to solving an "inverse problem": given observations of a system in response to perturbations we attempt to deduce or infer the functional properties of the system. Inverse problem are notoriously hard to solve, and the key to success is to choose a set of perturbations that provides good discrimination among alternative models. When the perturbations in a systems biology experiment are genetic variants, we refer to the approach as systems genetics. Llamas et al. (2), in this issue of *Physiological Genomics,* provide us with a simple but compelling demonstration of systems genetics in practice.

For sake of illustration, consider a simplified model of the "system" of blood pressure regulation. In our initial model, blood pressure (BP) is the product of cardiac output (CO) and peripheral resistance (PR). Thus BP = CO * PR. We know that genetic factors affect blood pressure and would like to extend the simple model by adding genetic effects on cardiac output, peripheral resistance, or both. We are able to measure blood pressure and cardiac output but cannot measure both on the same animal. Direct measurements of peripheral resistance are not available to us. Fortunately we have access to a panel of recombinant inbred (RI) mouse strains with a wide range of blood pressures. We measure blood pressure and cardiac output on different, but genetically identical, animals and study the relationships of the measured traits at the level of the strain means. Moreover, the RI panel has been densely genotyped and extensively phenotyped by other researchers, opening the possibility of extending our "system" to include other measured traits. We can also map the genetic loci [quantitative trait loci (QTL)] associated with blood pressure, at no extra cost.

Suppose that we have mapped two QTL. Both are associated with blood pressure but only one QTL (Q1) has an effect on cardiac output. According to our model, the other QTL must act on blood pressure through the peripheral resistance pathway. The model is illustrated graphically in Fig. 1. The estimated allelic effects provide quantitative parameters with which one can make predictions. The effect of a "B" genotype at Q1 is to reduce cardiac output by a factor of 0.9 relative to the "A" genotype, with a corresponding decrease in blood pressure. The "B" genotype at Q2, acting through its effect on peripheral resistance, increases blood pressure by 33%. The model gives the following quantitative predictions for the two-locus genetic system (Table 1).

We can confirm (or not) that the parental strains of the RI panel have the predicted blood pressures, but what about the other combinations? Certainly they exist in the RI panel, but these are the same animals that we used to estimate the model, and thus they do not provide an independent test of our prediction. Again we are fortunate to have access to a set of chromosome substitution lines (3) between the parental strains A and B. Our prediction is that B.A_Q1 will have blood pressure lower than the low parental strain and that B.A_Q2 will have higher blood pressure than the high parental strain. This example illustrates the phenomenon of transgressive segregation that is often seen in QTL studies. In this case, the A allele at Q1 is associated with high blood pressure despite the fact that it derives from the parental strain with lower blood pressure.

Next we carry out the experiment. If we are able to validate our predictions there is some small comfort to be had in the consistency between our model and the data. But if we are lucky, our prediction will fail, and this will force us to reconsider our initial model. For example, suppose that the cardiac output has increased by 10% as predicted in the B.A_Q1 animals, but their blood pressure is only 120, similar to the B strain. One plausible explanation is a feedback loop. Perhaps high blood pressure induces a physiological mechanism that lowers peripheral resistance to maintain a normal blood pressure. Homeostatic mechanisms are common features of biological systems. This would lead to us modify our model (Fig. 1) and to devise a new experiment, perhaps using genetic perturbations, to test the new model.

As in our simple example, Llamas et al. (2) used an RI strain panel to address a question about the relationship between two quantities, cardiomyocyte size (CMS) and left ventricular mass (LVM). Very roughly, we can think of LVM as the product of CMS and number of cardiomyocytes plus a bit of other stuff that makes up the left ventricle of the heart. Both LVM and CMS are measurable quantities, but it is not possible to measure both on the same animal.

Llamas et al. (2) demonstrate how complex trait genetics can yield to careful statistical analysis. They find that the CMS-associated QTL are sex specific and that males from reciprocal F1 crosses are quite different. Further analysis reveals that this Y chromosome effect is mediated by epistatic interactions with two different autosomal loci. To achieve this insight, they employed an interacting covariate in their genome scan analysis. The result is a rich and accurate quantitative model of the genetic architecture of CMS. Next, they utilize a complementary resource, reciprocal B.AY and A.BY consomic strains, to confirm the Y chromosome effect.

The LVM trait presents some interesting twists as well. The single, major QTL for LVM has a paradoxical effect. The parent with lower LVM contributes the high allele. The paradox is easily explained. The allele effects in my fictional model

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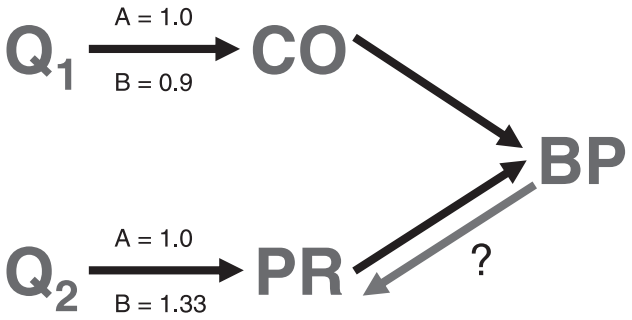

Fig. 1. A graphical model of blood pressure (BP) regulation showing BP as the product of cardiac output (CO) and peripheral resistance (PR) and two QTL (Q1 and Q2) with allelic effects as indicated.

of blood pressure regulation are similar to the QTL effects estimated by Llamas et al. (2). The observation of a transgressive QTL tells us something about the system. Another system component, genetic or physiological, is present and acting to counter the direct effect of this QTL. There is something that is still perplexing about the system. We expect LVM to be correlated with CMS and it is (Fig. 2). It follows that the QTL with direct effects on CMS should have an indirect effect on LVM. This isn't the case. The CMS QTL are quite distinct from the LVM QTL, and there is not even a hint of shared effect to be seen in the genome scans [see Fig. 2 in Llamas et al. (2)]. Llamas et al. have demonstrated that two distinct pathways are involved in the genetic regulation of LVM, but not everything is neatly tied up. Perhaps we could look at CMS and LVM in the reciprocal consomic lines B.A13 and A.B13, the site of the LVM QTL, to obtain additional information about this system.

RI strain panels provide an abundance of natural genetic variants that can serve as perturbations for systems genetics studies. Natural genetic variation is qualitatively different from engineered variation. Most natural variants are not loss of function. They tend to be subtle changes in regulation or amino acid substitutions that are compatible with normal function. Yet the phenotypic diversity that arises from mixing mouse genomes can be extreme and unpredictable. Experiments that simultaneously perturb multiple factors can be much more

Table 1. *Two-locus genetic system*

Q1	Q2	BP
A	B	90
A	A	100
B	B	120
B	A	133

Q1, quantitative trait locus (QTL) 1; Q2, QTL2; BP, blood pressure.

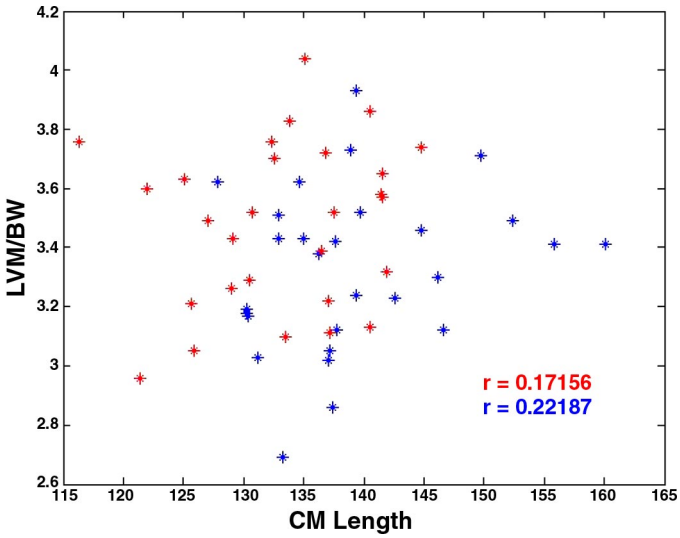

Fig. 2. Scatter plot of cardiomyocyte (CM) length by normalized left ventricular mass (LVM), data from Llamas et al. (2). Red and blue points indicate female and male data, respectively. BW, body weight.

efficient than approaches that perturb one factor at a time, but they still require a reasonable sample size to achieve effective results. Despite the success exemplified by Llamas et al. (2), existing RI mouse strain panels are too small to tackle the complex systems of genetic and physiological regulation that underlie most common disease phenotypes. In addition, existing RI panels capture only a fraction of the genetic diversity that exists in mice and there are substantial blind spots due to shared ancestry among strains (4). Larger and more diverse RI strain panels are being developed (1), and these will enable genetic experiments on the grander scale that we typically associate with systems biology. RI strain panels can provide insights into biological processes and mechanisms that go well beyond the mapping of QTL. This proof-of-principle study provides a glimpse into what systems genetics can achieve.

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