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The larger problem of large samples

In the article by S. Kunte and A. P. Gore[1], the conventional statistical wisdom has been challenged more significantly than what readily meets the eye. Firstly, there is a definite problem in simply increasing the sample size beyond certain reasonable limits and the reason for these limits is actually counterintuitive. Secondly, the test criterion itself needs to be modified if the models have to be validated. What is important to note here is the intrinsic limitations of statistical tests and that, in the true Fisherian tradition, statistics is good only in so far as it helps the observer to get at some usable information. Herbert A. Simon comments in his book *Models of Discovery*[2] that one is forced to think by convention that laws like $F=ma$ are discovered simply by accumulating some observations, more the merrier, and that some statistical analysis would readily yield the answer. He further states that statistics often behaves perversely and tends to reject perfectly good, usable hypotheses, particularly when they are approximate. One possible cause is the over ambitious observer who accumulates far too many samples for his own good and applies a criterion that buckles under the weight of the data. Simon warns that modest data collection is better for approximate and yet good models!

That real data do not come from analytically tractable compact models is known only too well to the field worker. The National Sample Survey Organization data bases, much of the census-related data, the meteorological data, the National Nutrition Monitoring Bureau data collected from the nodal agency of the National Institute of Nutrition and many other data bases have two problems: firstly, these often qualify as the large data that Kunte and Gore warn us about, and secondly, these data come with marked sampling bias by way of distributions on the ordinate such as income, age, etc. as characteristic of real data. A little algebra is enough to show that correlations are badly mauled by the nature of the distribution in these data bases. Discovery of relationships or causes is severely hampered often by these peculiarities of the natural data bases as well as the problem of large samples and there are no simple usable criteria yet to help the investigators. Since major data bases cost money and are generally collected with the avowed purpose of policy decisions, limitations in analyses would severely hamper more than just statistics. Kunte and Gore have made a small and yet significant beginning and one hopes to see a better definition of the large sample problem and its better resolution in due course.

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RESEARCH NEWS

Eye of newt and tail (or limb?) of frog

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Most researchers who try to understand how organisms develop struggle to design an experiment that might contribute significantly to improving our understanding of the events in development or raise pertinent questions; as opposed to merely generating interesting data. Most fail. In such a situation, it is very refreshing to see a paper from Bhubaneswar that describes elegant experiments that require others in the field to sit up and think before they clone their next gene or order their next bag of restriction enzymes. In a recent issue of *Nature*, Mohanty-Hejmadi, Dutta and Mahapatra[1] describe results that the first homeotic transformation mediated through vitamin A. They show that tadpoles with amputated tails often regenerate limbs in place of the tail when they are grown in vitamin A.

To appreciate the results of Mohanty-Hejmadi *et al.* fully, we must examine the background material on axial patterning in vertebrates. The effect of substances such as vitamin A and retinoic acid (RA) is related to the role

of the so-called homeotic genes. Homeotic transformations result in an organ or a specific part of it being replaced by another organ or the corresponding part. Such changes have been best studied in the fruit fly *Drosophila melanogaster*. Experiments in *Drosophila* resulted in the cloning of the first homeotic genes. The *Drosophila* homeotic genes were found to contain a motif called the homeobox, which codes for a polypeptide sequence that is now called the homeodomain (see ref. 2 for a review). Subsequent experiments showed that the homeodomain was present in genes in many species[3]. Of particular interest are the *Hox* genes of vertebrates which are organized in a manner similar to the *Antennapedia* and *bithorax* gene complexes of *Drosophila*. The genes of these two complexes are organized on the chromosome in a manner similar to their pattern of expression along the body axis. Collectively the *bithorax* and the *Antennapedia* complexes are referred to as HOM-C, or homeotic complex[4].

Vitamin A has long been known to have effects on regenerating limbs of vertebrates[5]. Retinoic acid, an analogue of vitamin A, has also been shown to have dramatic effects on the polarity of the regenerating limb in vertebrates[6]. The vertebrate limb bud is known to have, at its posterior margin, 'polarizing activity' (ZPA, zone of polarizing activity): transplantation of cells containing this 'activity' to an anterior position results in a mirror-image pattern of limb formation[7]. One way in which this can happen is by release by the cells in the ZPA of a morphogen whose concentration is graded along the body and highest at the site of release. It was later shown that RA can mimic the activity of the ZPA[8] and that cells in the ZPA had high levels of RA. These experiments suggested that RA may be a classical 'morphogen'. RA has also been shown to be present in other regions undergoing morphogenetic activity[9]. The genes that encode the RA receptor have been cloned and shown to be expressed in developing limbs, further lending substance to the view that RA is a morphogen[10]. However, other experiments suggest another possibility: that RA may not be the morphogen but may cause a ZPA cell to become capable of inducing polarizing activity[11]. While this question needs to be resolved, it is clear that one effect, direct or indirect, of different levels of RA can be correlated with differential activation of the *Hox* genes. In mammals the HOM-C genes are present in four clusters, *Hox1–Hox4*. The genes of the *Hox-4* complex are expressed during limb development. In a review of vertebrate limb development Duboule[12] suggests how regeneration in the vertebrate limb can be understood. RA treatment could result in resetting the positional values in the regenerating blastema and a change in the expression pattern of the *Hox* genes. This is very plausible in terms of what is known about the effects of both RA and the expression pattern of the *Hox4* genes. Mohanty-Hejmadi *et al.* report that when tadpoles with amputated tail stumps are grown in vitamin A limb development is seen. Commenting enthusiastically on their paper, Eddie de Robertis (who has studied *Xenopus Hox* genes in detail[13]) says: 'One could imagine that when the tadpoles are grown in vitamin A the regeneration blastema becomes posteriorized, perhaps by the activation of posterior homeobox genes by retinoids. When additional non-posteriorized cells are recruited into the regenerating tail bud, apposition of cells of different positional values will result. This will frequently lead to intercalation of cells of intermediary positional values[14]. If the intercalated positional values include those of the region of the body from which hind limbs arise, this could explain the supernumerary limbs that are observed.'

The Mohanty-Hejmadi *et al.* paper is pioneering. It links the role of retinoids as possible regulators of *Hox* genes to a clear homeotic phenotype, that of development of limbs where a tail should have been. Many experimental possibilities are now open. For example, the authors suggest that vitamin A could cause the activation of *Hox* genes. This can be tested using mRNA *in situ* hybridization methods or antibody-labelling experiments. Mohanti-Hejmadi *et al.*'s experiments also suggest that similar effects could be demonstrated in other systems where regeneration has been well studied.

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