The Importance of Being Cross-Bred (1955)

A PRACTICAL PROBLEM

If you want a lot of mice (or rabbits or pigs or plants) all resembling each other as much as possible, how do you set about it?

For the experimental biologist this is often a very real problem. The more his animals vary among themselves, the more of them he must use in each experiment if he wants the significance of his measurements to stand out clearly. His results must, as it were, speak louder than the background noise which is made by uncontrolled variability in the responses of his animals.

More animals mean more money, not to mention more space, time, and hard work. The amounts of money may be considerable, especially in the use of animals on a mass scale to assay the potency of drugs, hormones, food factors, poisons, bacterial and virus suspensions, and so forth.

A recent survey showed that the number of mice used in Britain's laboratories in one year was 1,180,000. The cost of producing one mouse ranges between 1s and 1s 6d, so that the national expenditure on mice alone must be somewhere between £60,000 and £90,000 per annum.

BIOLOGICAL VARIATION: ITS DUAL CAUSATION

A large proportion of the variability between animals and plants of the same species is due to small differences in the environmental circumstances in which

they have grown up. But there is another component of variation, due to genetic differences between different individuals. Biological variation is in fact the resultant of the forces of nature and nurture acting jointly. Penrose has defined nature as comprising all causes acting before fertilization, and nurture as all cases acting after fertilization.

As an example of the dual causation of biological differences, mice of the DBA/1 strain have thirteen pairs of ribs, while those of the BULB/c Scott strain have fourteen. Here is a difference due to nature. But Ingalls, Avis, Curley, and Temin found that the DBA/1 rib number, stable under ordinary conditions, reacted violently to a change in early nurture. On the ninth day of pregnancy DBA/1 females were subjected for five hours to an atmosphere deficient in oxygen, similar to that encountered by Tensing and Hillary during the last 2,000 feet of their Everest climb. In response to the transient oxygen shortage over half the embryos departed in their subsequent development from the rib number normally standard for their strain.

Another strain, however, with the same standard rib number as DBA/1 gave very little response when subjected to the same treatment. Here again is a genetic difference, but one that requires an environmental factor (prenatal oxygen shortage) to bring it to light.

The dual causation of variability is even more obvious with a character which varies on a continuous scale such as human height, where both nutrition and inherited effects are plainly recognizable.

THE BASIC BALANCE-SHEET OF VARIATION

Mathematically-minded biologists have been led to express this dual causation by drawing up a balance-sheet of biological variation, as follows:

environmental variation	+	genetic variation*	=	total measurable (phenotypic) variation*

If, then, we want a uniform group of organisms, how should we set about reducing these two components of variation?

* Measured in statistical terms as the 'variance'.

ENVIRONMENTAL VARIATION

First consider environmentally caused variation. It has become part of the ABC of experimentation to standardize as far as possible the conditions of diet, temperature, etc., in which the animals are raised, and for a given experiment to use them at a standard age. It may sometimes be desirable to go further. Some characteristics have been shown to vary according to the age interval separating them from their elder brothers and sisters.

But even when we have minimized such environmental causes of variation, we still have to reckon with the other, genetic, component.

GENETIC VARIATION

A method for reducing genetic variation in sexually reproducing organisms was demonstrated as early as 1903 by Johanssen. He bred a number of lines of beans by matting each plant to itself for several generations. He then practised selection for bean size within each line and showed that it was without effect. The plants grown from the largest beans bore beans which were no bigger in average size than those borne by the plants from the smallest beans. He concluded that inbreeding had purged all the genetic variation from his lines, which he accordingly termed 'pure lines'.

It is in fact doubtful whether complete 'purity' (absence of genetic variation) can be attained by inbreeding. The design of Johanssen's experiment has been criticized by Llysenko on the ground that in each generation he selected the biggest and smallest beans, instead of the plants with the biggest and smallest average bean size. He was thus to a large extent selecting from the variation between different beans of the same plant, which in any event we should not expect to be genetically caused. But in general terms Johanssen was right. It is a simple consequence of the fact that inheritance is particulate that genetic variation decreases towards a limit the longer a line is inbred; if inheritance were blending there would be no variation after the first generation.

Here seemed the answer to the rest of the experimenter's problem. You cannot self-mate most animals, but you can brother–sister mate them in each generation

and thus evolve genetically highly uniform strains. In one branch of scientific work after another the use of highly inbred strains came to be adopted.

TWO PLUS TWO NOT EQUAL TO FOUR?

Inbred strains seemed to so obviously be an answer to a prayer that they were gladly adopted without an experimental check. It seems logical enough. Turning back to the basic balance-sheet we see an equation of the form A + B = C. By minimizing both A and B we must minimize their sum, C.

Where is the fallacy? The balance-sheet in its simplest traditional form rests on an unproved assumption: that A and B can each be varied while the other stays put. But suppose that A and B interact in some way? Suppose in particular that some methods of decreasing B (e.g. inbreeding) bring about an associated increase in A: reducing B may then leave C unaffected, or worse still actually increase it.

In terms of the balance-sheet of variation this would mean an increase of environmental variation in response to a decrease in genetic variation. For instance, one of the effects of intense inbreeding may be to make the organisms more susceptible to the small differences between the different environments in which they develop and grow up. By inbreeding we could then lose as much (or more) on the developmental swings as we gained on the genetical roundabouts.

WHAT IS THE EVIDENCE?

The earliest hint of such a possibility was given in 1930, when Livesay found that inbred strains of rats were more variable in body weight than the offspring of a cross between the strains. Such offspring are known as first-generation, or 'F1', hybrids.

Since, as we have seen, members of an inbred line resemble one another genetically very closely, they will be alike in the kind of sex cells which they produce. Moreover, members of such a line are genetically very 'pure', or in the geneticist's term 'homozygous'; so that any one member of the line produces sex cells like all the others. Hence effectively only one genetic kind of sex cell can

be got from any one inbred line. It follows that only one genetic kind of F1 offspring can be got from crossing animals from any two such lines.

We can see then that Livesay's interstrain F1 hybrids should be genetically uniform, like the parent inbred strains. Their lower total variability suggests that during their development and growth they were for some reason less affected than the inbreds by unavoidable small differences, which must exist in any colony of animals, between the environments to which different individuals were expressed.

Nine years later Emmens made a direct test of the suitability of an inbred strain of mice for the bio-assay of oestrongens. He compared the variability of their response with that shown by a random-bred mouse. Neither Livesay nor Emmens offered a feasible interpretation, and their result—perhaps for this reason—did not attract attention.

Although Hagedoorn has already in 1939 noted and discussed the phenomenon of hybrid uniformity in connexion with animal breeding, the first explicit alarm to reach the ears of experimental biologists was sounded by Mather in 1946. He mentioned the possibility that, for some characters measured in bio-assay, inbred strains might prove to be more variable than interstrain hybrids.

This has recently been experimentally tested and the effect found to be surprisingly large. McLaren and Michie measured the duration of narcosis induced by a standard dose of the anaesthetic 'Nembutal' (pentobarbitone sodium) in mice, and found that an inbred strain was 3–5 times more variable than the F1 offspring of an interstrain cross. Claringbold and Biggers in a larger and better-designed experiment found a four fold superiority of F1 hybrids in the uniformity of their response to oestrogens.

These results well illustrate the magnitude of the saving of money and effort that may become possible through correct choice of experimental material—namely a reduction of the number of animals needed by something in the region of 70 to 75 per cent. The basis of the calculation is that an n-fold increase in variability (measured as the 'variance' in statistical language) requires an n-fold increase in the number of animals which must be used to obtain a result of a given accuracy.

THE PROPER USE OF INBRED STRAINS

We are here comparing inbred strains with F1 hybrids between inbred strains. Although we have cited cases where random-bred animals proved to be more uniform than inbreds, this cannot by any means always be expected. Random-bred animals have the disadvantage that they differ from one another genetically, for which they may or may not compensate, by greater indifference to environmental causes of variation when compared with inbreds. F1 hybrids on the other hand combine the advantages of both the other types: they have the genetic uniformity of inbreds, but since they are not themselves inbred they possess, as do random-breds, a high degree of developmental stability in the face of environmental disturbances.

The use of inbred strains has therefore not been eclipsed in bio-assay. On the contrary we are now for the first time in a position to put them to their proper use—the production of interstrain F1 hybrids...

FURTHER READING

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