Butterflies give genetics a new dimension

Cabbage white butterflies breeding in a CSIRO laboratory have opened up a can of genetic worms. The unexpected behaviour of butterfly genes was uncovered by Mr Neil Gilbert of the CSIRO Division of Entomology in Canberra. A mathematician turned population biologist, Mr Gilbert sought to test some of the basic assumptions of quantitative genetics. His controversial conclusion is that genetic variation, far from being random, is restricted within fixed patterns.

When Gregor Mendel studied his peas, he demonstrated that whether a plant is tall or short does not affect the colour — red or white — of its flowers. This 'independence' of genes has been applied to quantitative genetics, which seeks to understand how genes control characteristics that vary in quantity, instead of taking on one of two possible values (red or white, for example). Characteristics of primary ecological importance, such as body size, vary continuously. Such quantitative (non-Mendelian) characters are controlled by a collection of genes acting together.

Independent gene effects?

Until now it has been generally assumed that, even though these genes acted collectively, each acted so that its effect on the value of an inherited character was statistically independent of that of other genes. The final result was, to a first approximation, the sum total of the influences of each separate gene. The butterflies have refuted this supposition.

Further, they have shown that, within one population, certain unfavourable patterns of genetic variation are forbidden, and this exclusion is dictated at the genetic level. In other words, some overriding supervision of genetic variation takes place.

The new experimental results have not come as a total surprise, since evidence had been mounting that genes were not acting as independently as had been assumed.

If it were true that genes always produce independent effects, then natural selection would operate independently on different genes, and it should favour all those genes that maximise an individual's fitness to the environment. As a result, the population should become virtually homogeneous, possessing very little genetic variation.

Scientists were therefore startled when, in 1966, electrophoresis revealed very large amounts of genetic variation in wild populations. However, theoreticians argued that the variation was random, with no selective importance. The alternative — to suggest that genetic variation is deliberately maintained by some mechanism or other — necessarily implicates a methodical kind of genetic interaction.

But clearer signs of something unexpected going on have recently emerged in the findings of a number of scientists that variations in certain characteristics of wild populations are not random. American researchers have been comparing life-history traits — larval growth rates, fecundity, hatching rate, and so on — of different insect populations from different localities. They have found that various traits are genetically correlated, and that the average values of different traits match the ecological dictates of the local environment.

Mr Gilbert's work has gone one step further and shown that the patterns he has observed in the butterflies are imposed genetically, rather than by some physiological factor (although in the final analysis, the two approaches must merge).

A classical paradox

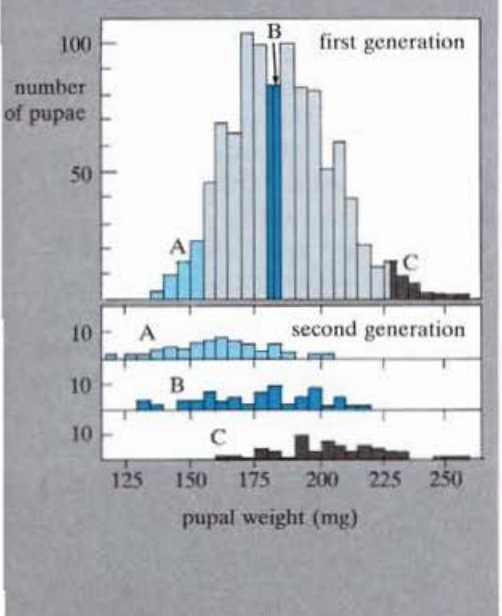
The starting point was to reflect on the fact that, in every species of insect so far tested, large females lay more eggs than small ones. The same is true of birds, reptiles, and fish. Combine that fact with our present concept of natural selection, and we are drawn to conclude that all these species should get bigger and bigger, world without end. So why don't they?

The classical theory answers either that there is no genetic variation for size and fecundity, or that the advantage conferred on larger females is exactly cancelled by greater mortality at some stage of the life cycle. The answers are compelling, since these creatures clearly don't become increasingly large.

But Mr Gilbert wanted to put the theory to the test, and he undertook experiments with cabbage white butterflies. This species reproduces quickly and is easily bred in the laboratory, and it's easy to follow all stages of its life cycle under field conditions. You can easily monitor a butterfly's genetic endowment for size, too: you simply weigh the pupa, because there's a direct link between the weight of the pupa and the

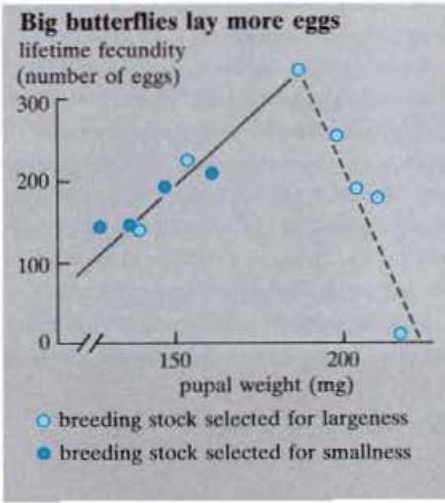
A population of cabbage white butterflies shows a distribution in their pupal weight (top). If the groups A, B, and C are selected according to size, and bred, then their progeny inherit their size accordingly (small pupae hatch into small butterflies; large pupae into large ones).

Pupal weight is an inherited characteristic



Large pupae grow into large butterflies, and generally lay more eggs. Since mortality is no greater in large butterflies than in small ones, why doesn't the whole population, over several generations, become larger and larger? Part of the answer comes from noticing that, through an internal genetic process, the fecundity falls drastically beyond 200 mg (dotted line). Even so, if individual fitness were paramount (classical genetics), the population would shift to the 200 mg mark. The true population average is about 170 mg, and the limit is at 240 mg.

Big butterflies lay more eggs



Size is an inherited characteristic.

ultimate size of the butterfly that emerges from it. Counting the number of eggs the butterfly lays gives the fecundity.

The first alternative of the classical theory was soon ruled out: a strong genetic influence on the size of a butterfly emerged. The eggs of larger butterflies hatched into larger specimens, on average, than did eggs of smaller butterflies, and the difference persisted in later generations.

Surprisingly, the second alternative was also clearly wrong. Mr Gilbert found that under field conditions — either in large outdoor cages (where necessary) or in the open field — mortality was certainly no greater among larger animals; if anything, larger specimens survived rather better than smaller ones at every stage of the life cycle.

According to the classical theory this is an impossible situation, and it took some years of experiments to find out what was going on.

He found that, after several generations of intensive selection for greater size (only the largest specimens were allowed to breed), the butterflies ran into a 'brick wall' — a selective limit that was impossible to pass. It proved genetically impossible to obtain butterflies appreciably larger than the largest individuals encountered in the wild.

Actually, such limits have been known to plant and animal breeders, but were not thought to be a vital aspect of the way populations function. It was easy enough to believe that the plant or animal had reached a size whereat some vital physiological balance had been disrupted, or that selection had exhausted all the available genetic variation.

But establishing the limit doesn't by itself solve the problem: why doesn't the whole butterfly population move up to the limit? Something else must be going on. It turns out that when butterflies approach the limit they begin to suffer a severe loss of fecundity (the number of eggs they lay), and the eggs can become infertile as well.

For the common good

Mr Gilbert has no idea why a butterfly carrying genes for extreme size lays many fewer eggs than a butterfly of the same size carrying genes for normal size. But that is how average size, and fecundity, of butterflies is stabilised. The stabilisation balances one genetic influence (that of increased fecundity of larger phenotypes) against another (loss of fecundity for very large genotypes). It does not balance greater individual fecundity against greater mortality.

Yet the population does not end up consisting solely of butterflies with peak fecundity. There is some genetic mechanism that maintains a range of sizes around the population mean.

This means, Mr Gilbert concludes, that the genetic influences on fecundity cannot act independently; instead, a higher-level genetic blueprint applies that benefits the population as a whole.

Here we contradict the 'central dogma' of population biology — that individual advantage takes precedence over the welfare of the population as a whole. For most characters, the two coincide: what is best for one individual is best for all. But several attributes would confer an advantage on the individual, or its progeny, at the expense of other members of the population.

Increased fecundity is one of them, and others include cannibalism, and switching from sexual reproduction to parthenogenesis (virgin birth). Although these options offer an immediate advantage to any individual (or her progeny), they do

A new understanding in ecological genetics

One may wonder how some butterflies managed to reveal features that many decades of work by population geneticists have not uncovered.

The difficulty has been in trying to see the ecological consequences of particular genetic variations. For simplicity, geneticists have used readily identifiable inherited characteristics — such as shell patterns in snails, and wing patterns in butterflies. But then it is extremely difficult to identify and measure the ecological effects: what difference does it make to a moth whether its wings are white or grey?

Even in the famous case in England where the peppered moth *Biston betularia* changed from white to grey in the midst of increasing industrial soot falling on the countryside, results are not clear-cut. Higher predation of predominantly white moths, by birds, is insufficient to explain the observed changes in gene frequency.

Electrophoresis allows us to measure genetic variation, but does not tell us what that variation does ecologically. Rather than start with easy genetics and run into an ecological barrier, Mr Gilbert chose another approach. He studied characters of known ecological significance, even though the genetics aren't so simple.

Fecundity, for example, isn't a simple Mendelian characteristic: it isn't limited to a small number of possible alternatives (like red or white flowers), but ranges over continuously variable values.

Since the underlying genetics of non-Mendelian characteristics are hard to trace, they land researchers in the morass of 'quantitative genetics', which permits no detailed genetical analysis unless you make the simplifying assumption that gene effects are independent.

The incentive to allow that assumption is strong, for otherwise theoreticians find that much of their evolutionary theory goes down the drain.

The big advantage of cabbage white butterflies is that each stage of the life cycle can be followed under field conditions, and fecundity is one ecologically vital inherited characteristic that can be easily measured.

Mr Gilbert has put years of work into his experiments, which were done not only at the Entomology Division in Canberra but also at the Division of Wildlife and Rangelands Research, Canberra; University of British Columbia and Agriculture Canada Research Station, Vancouver; and Imperial College, Silwood Park, England. Professor Rhondda Jones, John Leighton, Sheryl MacFarlane, Dr Vince Nealis, and Julie Roberts have all helped. Every essential piece of evidence has been duplicated to be quite sure.

The picture of genetic variation in wild populations that emerges is not the one you read in the textbooks. As Mr Gilbert says, 'Who do you believe: Professors of genetics or a bunch of butterflies?' As usual, Nature is more complex than we thought, and it's impossible to find out how a complex works by examining its parts in isolation.

Wielding a butterfly net, Julie Roberts catches butterflies in one of the outdoor cages used in the experiments.

not usually benefit the whole population. These strategies are only invoked in Nature under extreme conditions when a widespread benefit can result.

For example, aphids turn to parthenogenesis — all become females and reproduce without mating — when food is abundant. In this way the whole population can expand more rapidly than when burdened down by (temporarily unnecessary) males.

Yet there must be an advantage in sexual reproduction, and it is obviously something connected with maintaining the population's genetic diversity. A parthenogenetic population would quickly come to comprise individuals of one genotype only — that with the greatest possible fecundity. Sex maintains a continuing mixture of types throughout the generations; and it subordinates individual advantage to the welfare of all.

So to reiterate the main point so far: average size and fecundity of butterflies are stabilised because rare individuals — those with genes for very large size — have abysmally low fecundity. Control of average fecundity is achieved purely internally, by genetics, without any need to call on outside ecological forces.

No random variation

But the butterflies have another unexpected feature. An organised pattern of genetic expression ensures that only ecologically valuable types of genetic variation can occur. That is, genetic variation within a population is not random.

Four possible kinds of genetic variation can affect a butterfly's size. At any one temperature, the caterpillars may develop quickly or slowly, and their growth rate may be either slow or fast. But changes in temperature affect development and growth in different ways. In theory, you can select for slow or quick development at any temperature, but it turns out that at high temperatures (26–28°C) you get no response: the genetic variation for development rate is expressed only at lower temperatures — about 15–20°C.

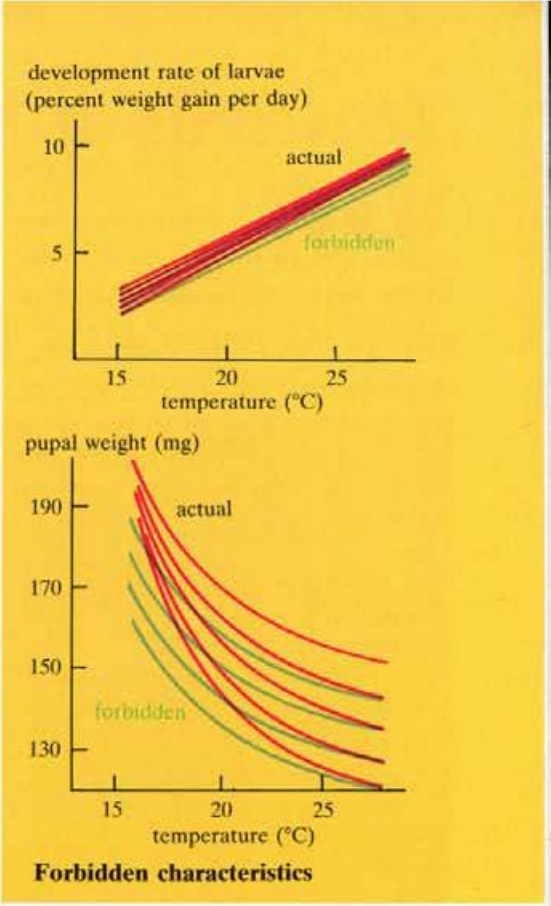
Conversely, there is plenty of genetic variation for growth rate at high temperatures, but none at low. The variation is restricted to the patterns shown in the graphs. Although the missing combinations are physiologically possible, apparently they are not ecologically necessary.

In the first generation of each season, the advance wave of hatching eggs must face erratic spring-time temperatures. If the eggs develop too quickly and hatch too early, then there's a chance a cold snap will kill them. On the other hand, if temperatures stay mild, early hatching provides a number of benefits: first bite of the young cabbage; lesser numbers of predators; and not being eaten by caterpillars of cannibalistic tendencies. These hazards have been checked out in Canberra back gardens. The observed pattern of variation maximises the chance that some caterpillars survive.

We are led to conclude that the dimensions of genetic variation are themselves under some kind of genetic control. Genetic variation is not a free-for-all. Genes are well organised in a sensible network, and resist any selection that attempts to impose characteristics that go against the ecological optimum for the population. This may not surprise anyone who has compared a kangaroo with a magpie, but here it works within a single species.

(Some theoreticians — for example, Professor John Maynard Smith of the University of Sussex — have very recently speculated that some such restricted pattern might occur, but they produced no direct evidence in support.)

It follows that any evolutionary shift calls not just for changes in the frequency of occurrence of particular genes, but for a wholesale movement of the total gene pattern. This can be seen as a possible basis for 'punctuated evolution', the theory that currently attempts to interpret sudden evolutionary shifts. The Japanese race of this same cabbage white species is much larger than the Australian one. To transform one race into the other, you would have to alter the whole pattern of variation.



Forbidden characteristics

Each line represents a different genotype. Neither physiology nor classical genetics offers any reason why the lines should not be parallel — allowing wide variation in larval development rate at high temperature and variation in pupal weight at low temperature. But these variations, which make poor sense ecologically, are ruled out by some previously unrecognised genetic effect.

This work has married genetics and ecology, a long-sought aim of population ecologists. The butterflies have decisively contradicted some basic assumptions of the classical theory of population genetics. But then, it must be acknowledged that as yet we have no alternative theory to put in its place.

Andrew Bell

More about the topic

Control of fecundity in *Pieris rapae*. I. The problem. II. Differential effects of temperature. III. Synthesis. IV. Patterns of variation and their ecological consequences. N. Gilbert. *Journal of Animal Ecology*, 1984, **53**, 581–609; 1986, **55**, 317–29.

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