Limits to DNA Fingerprinting

Amid the great excitement about the application of a new technique of molecular biology to problems of behavioral ecology, a voice of caution begins to enter

WHEN 4 YEARS AGO Alec Jeffreys hit upon the technique of DNA fingerprinting he knew it would make a splash, and it did. By providing a genetic version of the whorls on the fingertips, Jeffreys' technique soon became established as a new and valuable forensic tool, being used variously in the identification of criminals and their victims, often from very small amounts of tissue. And in courts of law of a different kind, DNA fingerprint evidence has been successfully adduced to resolve cases of disputed genetic relationship, often involving paternity. As predicted by Jeffreys, a geneticist at the University of Leicester, England, the impact of DNA fingerprinting has indeed been dramatic.

But that impact has gone beyond courts of law and is poised to transform another endeavor in which establishing genetic identity is often crucial, that of behavioral ecology. "Until DNA fingerprinting came along our measures of reproductive success were a bit sloppy," says Nick Davis, a behavioral ecologist at Cambridge University. "Now we have an opportunity to answer some important questions about how animal behavior maps onto reproductive success."

Davis, like scores of behavioral ecologists in Europe and the United States, has eagerly grasped the DNA fingerprinting technique as a way of providing information that just cannot be obtained reliably from field observations: namely, pinning down paternity and maternity in different types of mating systems. "DNA fingerprinting is *the* most useful technique to have been introduced into population biology," says William Amos, also of Cambridge University.

So great is the interest in applying this tool of molecular biology to behavioral issues, and so tenuous is the average behavioral ecologist's grasp of the practicalities involved in the technique, that Amos and fellow Cambridge geneticist Josephine Pemberton recently established a newsletter, *Fingerprint News*, to help participants keep abreast of developments (see box).

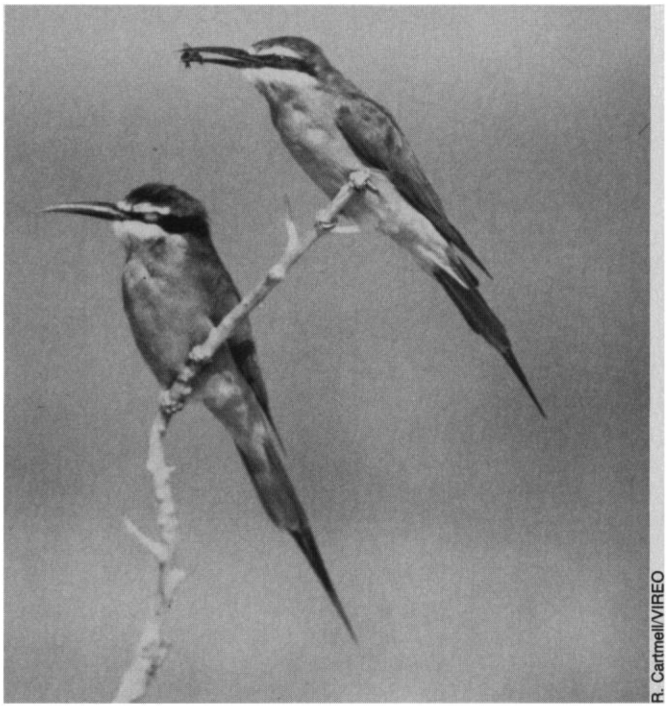
In the world of behavioral ecology, reproductive success—that is, the number of offspring an individual successfully contributes to the next generation—is a key parameter. A reproductive success slightly greater than average, and an individual's genes will come to dominate future generations; slightly less than average, and evolutionary oblivion awaits. Biologists therefore need to know to what degree an animal's behavior is tuned to achieving maximum reproductive success. And you cannot answer that without an accurate count of the offspring produced by particular individuals.

"In the absence of a technique such as DNA fingerprinting, behavioral ecologists had to make certain assumptions in calculating reproductive success," Davis told *Science*. "We assumed, for example, that if we found a pair of birds looking after a brood of chicks that all those chicks belonged to that male and that female." In this case of monogamy, reproductive success would be determined by counting the chicks associated with the adult birds, throughout a lifetime. Simple, or so it was assumed.

In other types of mating systems—polygyny, polyandry, and polygynandry, in which several males share several females—things can become a little more complicated. But by astute observation of individuals' behavior it has been possible—or so it seemed—to make reasonable estimates of numbers of offspring produced by each individual. The idea is to try to match high reproductive success with some kind of behavioral characteristic, such as fighting ability, display activity, sneaky matings, and so on.

One behavior that has long intrigued biologists, and is very interesting in the context of Darwinian evolution, is altruism: one individual, at some cost to itself, enhances another's reproductive success, for instance in helping to rear offspring. Such behavior makes Darwinian sense only if donor and recipient are related, because then the donor effectively has a genetic share of the offspring and is thereby indirectly increasing its own fitness. The phenomenon goes by the name of kin selection.

Altruism is relatively common in the animal world, but one good example, currently being studied by John Krebbs and his colleagues at Oxford University, occurs in bee-eaters, birds in which a mated pair often receives help from several unmated individuals. "One question we are interested in," explains Krebbs, "is whether the amount of effort a helper gives matches the degree of


R. Cartmell/VIREO

Bee-eaters: *Altruism is an important aspect in the social lives of these colony-living birds, with several individuals helping to provision the young of a mated pair.*

genetic relationship to the mated pair." According to kin selection theory, the more closely related a helper is to the beneficiaries, the more effort it should devote to the task.

These, then, are the two principal areas in which DNA fingerprinting is being recruited by behavioral ecologists: in identifying the parentage of offspring; and in determining the degree of relatedness among individuals in a social group. How well does the technique meet the task required of it?

Judging by the number of field workers who are currently engaging the help of molecular biologists, or are establishing molecular biological expertise in their own laboratories, the promise of the technique is perceived to be great. "It's definitely a hot area," says Michael Lynch, a population geneticist at the University of Illinois. "But it seems to me to be far less useful than most people believe. It is a real bandwagon and a lot of people will be getting data, but will not know what to do with it."

Lynch's note of caution is the result of a detailed study of the genetics of the fingerprinting technique and of the data derived from it. Yes, he says, DNA fingerprinting is more precise for paternity exclusion than, say, classic protein polymorphisms. But much further than that you cannot go with confidence. "Attempts to ascertain relatedness by means of DNA fingerprinting should proceed with caution," he says.

One problem with the technique is that genetically it is a bit of a black box. Scattered throughout the genomes of many species are clusters—called minisatellites—of tandemly repeated sequences, the core sequence of which is rather small and common to them all. If an individual's genome is now chopped up with restriction enzymes, run on a gel to separate the fragments according to size, and then treated with radioactive core sequence, a ladder-like pattern of bands is detectable.

If all of the tandem repeats in one individual were identical with those in other individuals, then the band pattern would be common to all. In fact, for various reasons of cellular genetics, the size of each tandem repeat can vary considerably among individuals: many of these loci have 20 or more variants. It is this variability that can generate a near-unique pattern—the DNA fingerprint—for each individual.

"When you look at a band pattern all you are seeing is an array of DNA fragments of different sizes," explains Lynch. "You don't know where the DNA came from or what it is. Similar sized fragments in two different gels may represent the same piece of DNA, which would indicate some degree of relatedness, or two different pieces of DNA that just happen to be the same size, in which case no relatedness would be implied." The result of all this is that two completely unrelated individuals will share a significant proportion of their bands—some 20 to 40%—simply by chance. This represents "noise" in the system.

Moreover, because many DNA fragments are rather small, many of them run off the gel, "leaving one with an incomplete picture of the minisatellite family," says Lynch. One more complicating factor is that some of the tandem-repeat loci may be linked, "and therefore should not be treated as independent estimators of relatedness." If two individuals share 45% of their bands, therefore, it might mean that they are half-siblings (25% genetic relationship, that is 45 − 20); or it might mean that they are completely unrelated, the overlap being the result of the various complicating factors, the noise.

Lynch declares himself to be "disheartened that, because of the noise in the system, you usually can't go much further than very close genetic relatedness, such as parent-offspring." Even full sibling relationship might sometimes be too uncertain to be called, he says. Jeffreys agrees that Lynch is "substantially correct, if a little too cautious on some points."

In addition to these potential theoretical constraints, there are issues of practicality too, not least of which is that "the DNA fingerprint technique is extremely fiddly, even for a competent molecular biologist,"

Check on *Fingerprint News*

It was a combination of the sign of the times and a Cambridge tradition: "As is customary for such ambitious schemes, the idea came to us in a pub," explain Cambridge University geneticists Bill Amos and Josephine Pemberton. "We were in the Clarendon Arms, Cambridge, eating chili con carne. To us, DNA fingerprinting seemed to be a field crying out for a newsletter." And a newsletter there soon was, the first issue of which appeared in January this year.

Even for molecular biologists the technique is not straightforward or easily repeatable, and so, says Amos, "for novices it was full of potential problems, in collection and preparation of samples, preparation and running of gels, making and using hot probes." There was, he realized, "a great need to swap ideas, a forum for technical hints, for keeping track of references, and for keeping in touch with who was doing what." The newsletter, called *Fingerprint News*, is not just for behavioral ecologists—the real novices in the game—but for all who are involved in using and developing the technique and related techniques and might therefore benefit from being part of an information network. "It seemed to us that communications between labs were generally poor," note Amos and Pemberton.

With four issues planned a year, *Fingerprint News* will carry a mix of grapevine news, miniarticles, and bibliography. In the January issue you can learn about drying reptile blood for DNA extraction or ponder the problem of getting material suitable for DNA extraction from otter droppings, quaintly called spraints. Later issues will contain more articles, but, to the uninitiated, this one is fascinating for the diversity of creatures in which fingerprinting is being or is shortly to be applied: they range from gray seals to vultures, mongooses to marine mussels, aphids to salmon. The work is being done in seven European countries, Japan, and the United States.

One issue that is sure to be addressed in future issues is the limits of conventional (that is, multilocus) fingerprinting and ways of getting around them. It is already clear that although identifying close relationships is feasible in many cases, the power of the technique decays rapidly as the genetic distance increases (see main story). Looking for loci of greater variability is one route, but it carries the drawback that greater variability also implies a high mutation rate, which can obfuscate relatedness. Another route is identifying and isolating single, hypervariable loci, which can then be treated as conventional genetic markers, albeit with many times the normal information content. The benefits are that normal statistical techniques can be applied to such loci (they cannot be applied to multilocus fingerprints), which greatly aids in identifying relatedness. But the drawback is that you would have to generate the system anew for each species, as opposed to the multilocus system, in which the same probes can be used in different species. That represents a great deal of very technical molecular biology even for accomplished practitioners.

The marriage between behavioral ecology and molecular biology is still in its honeymoon stages, and signs of complete consummation are eagerly awaited. ■ **R.L.**

says Amos. This is one reason why, in spite of the tremendous interest in using the technique, very little is yet in print. Terry Burke, of the University of Leicester, also points out that it is dangerous to assume that the band pattern produced by the technique is in fact a fingerprint, that most of the bands represent independently inherited loci.

"You have to demonstrate you have a true fingerprint with each new species you look at," says Burke, "and this involves a lot of work, with several crosses." So far, he notes, data demonstrating true fingerprints have been published only for human, dog, cat, mouse, and the house sparrow, although some unpublished data exist, including that for the European bee-eater.

In spite of these caveats, many researchers are pressing ahead, and results are beginning to come into the literature. For instance, Burke and his colleague M. W. Bruford have already demonstrated that about 20% of house sparrow chicks in the nests of apparently monogamous pairs are fathered by other males. The female is deceived on occasion too, when another female lays an egg in her nest, a trick known as intraspecific parasitism. In both cases, the reproductive success of the adults on the nest is lower than would have been calculated from field observations—unless they have been plying similar tricks elsewhere.

Davis, in collaboration with Burke, is soon to publish data on the hedge sparrow, whose mating system is very much more complicated. The results give a clearer insight into true reproductive success in this species and demonstrate that the birds' behavior is finely tuned to that parameter, even though the fit is not perfect.

These investigations rely on the ability of fingerprint data to identify parent-offspring relationships, which is not very controversial. In their work with the bee-eaters, Krebbs and his colleagues stray into uncertain territory, that of detecting more distant relationships. "In our population we have about a 20 to 30% sharing of bands by chance, which means that we are fairly confident of identifying full siblings," says Krebbs. For their purposes, this represents an acceptable level of background noise. "First cousins, with 12.5% genetic relationship, would be more difficult to detect, but we would not expect a significant degree of help at the nest with this distant a relationship."

The clash between high expectations and pedestrian reality is imminent in the joint DNA fingerprinting/behavioral ecology endeavor. Some problems will be solved readily, others will not, but overall it does represent major progress. ■ **Roger Lewin**

Drug-Resistant Strains of AIDS Virus Found

The emergence of AZT-resistant strains of the AIDS virus in patients treated with the drug has serious implications for treating AIDS and preventing its spread

The Burroughs Wellcome Co. announced last week that prolonged treatment with AZT, the only drug now approved for combating AIDS infections, can lead to the emergence of drug-resistant strains of the AIDS virus. The appearance of the AZT-resistant strains was not associated with any marked decline in the patients' conditions, and clinicians say that AIDS patients who are taking the drug do not need to change their treatment regimens. The new findings, which will be published in the 31 March issue of *Science*, nonetheless have serious implications for efforts to treat AIDS and prevent its spread.

The AIDS virus becomes progressively more resistant to AZT as time goes on.

For one, the findings point up the need to develop new drugs for AIDS therapy. "If it [AZT resistance] turns out to have a clinically relevant correlate, we will have to develop alternatives, or use drug combinations," Anthony Fauci, the director of the National Institute of Allergy and Infectious Diseases, said in an interview with *Science*. Several potential AIDS drugs are being evaluated in clinical trials, but are not yet widely available to patients. The people taking AZT currently number in the thousands, perhaps in excess of 20,000, according to a spokeswoman for the Burroughs Wellcome Co.

Moreover, as many as 1.5 million people in the United States may have been infected by the AIDS virus, but have not yet developed the full-blown immunodeficiency syndrome. Clinical trials to determine whether AZT can delay or prevent the development of AIDS have been started. The discovery of the drug-resistant virus variants raises the possibility that, even if the progression of the disease can be postponed, the virus that ultimately produces symptoms might not be so readily controlled by AZT. Also worrisome is the possibility that the resistant AIDS virus variants might be transmitted to more people.

The AZT-resistant viruses were identified by Brendan Larder and Graham Darby of Wellcome Research Laboratories in Kent, England, and Douglas Richman of the University of California, San Diego, and the San Diego Veterans Administration Medical Center. The researchers obtained isolates of the AIDS virus, which goes by the scientific name of human immunodeficiency virus 1 (HIV-1), from patients who had been taking AZT for varied lengths of time up to 30 months and from patients who had never received the drug.

The isolates from 5 of the 15 patients who had been on the drug for more than 6 months were markedly—as much as 100 times—more resistant to the growth inhibitory effects of AZT than isolates from untreated patients and from those who had taken the drug for less than 6 months, Larder says. Moreover, two or more sequential isolates had been obtained from a few patients, and these showed that the AIDS virus becomes progressively more resistant to AZT as time goes on. The way in which the AIDS virus acquires the resistance is currently unknown.

At present, there is no direct evidence linking the development of AZT resistance to a worsening of the patients' symptoms. The patients producing the resistant HIV-1 variants did not, for example, show increased blood concentrations of the viral antigen p24. This suggests that virus reproduction had not gone up in the patients.

Clinicians often find, however, that the condition of AIDS patients begins to deteriorate within 6 to 18 months after they begin taking the drug. "The drug is clearly effective. The responses in many people are dramatic, but they are short-lived," says Jerome Groopman of New England Deaconess Hospital in Boston. "It's terribly important to know what the biological basis of the clinical progression is." The development of AZT resistance is one possible cause, but not the only one.

Larder points out that the current study,